The fate of pollen in two morphologically contrasting buzz-pollinated *Solanum* flowers

Christian A. Vasquez-Castro [1,2]
Elodie Morel[1,3]
Bernardo Garcia-Simpson*[1,4]
Mario Vallejo-Marín[1]

[1] Department of Ecology and Genetics, Uppsala University, Uppsala, 752 36, Sweden
[2] Department of Biology, Faculty of Science, Universidad Nacional Agraria La Molina, Lima, 150 24, Peru
[3] Institute of Biology, University of Neuchâtel, 2000, Neuchâtel, Switzerland
[4] Círculo de Investigación en Taxonomía, Florística y Ecología Vegetal, Department of Biology, Faculty of Science, Universidad Nacional Agraria La Molina, Lima, 150 24, Peru

*Author for correspondence

Manuscript received _____; revision accepted _____

Running title: Pollen fates in buzz-pollinated *Solanum*

ABSTRACT

Premise of the study: Pollen transfer efficiency (PTE) and pollen deposition patterns on a pollinator's body significantly influence plant reproductive success. However, studies on pollen fates (i.e., the end point of pollen grains after being released from the anthers) in animal-pollinated species, particularly those offering pollen as the sole reward, are limited. Here, we investigated pollen fates in two nectarless, buzz-pollinated *Solanum* species with contrasting floral morphology.

Methods: We conducted experimental trials consisting of one pollen donor and four recipient flowers of *Solanum rostratum* and *S. dulcamara*, using captive *Bombus terrestris* as pollinator. After each trial, the amount of pollen remaining in the anthers, deposited on stigmas, placed on the pollinator, and on falling to the ground was assessed. From this, we estimated pollen fates and PTE, and modelled their pollen deposition curves.

Key results: We found that *S. rostratum* produced more pollen but had a more restricted pollen dispensing schedule compared to *S. dulcamara*. Although PTE was statistically similar between species (0.72% vs. 1.07%, for *S. rostratum* and *S. dulcamara,* respectively), pollen loss occurred mainly due to falling to the ground in *S. rostratum* and due to bee grooming in *S. dulcamara*, potentially explained by their different floral architectures. The pollen deposition curve of both species displayed a typical exponential decay pattern, with the first visited flowers receiving the most outcross pollen.

Conclusions: Our results suggest that PTE in pollen-rewarding, buzz pollinated species is quantitatively similar to the PTE in nectar-rewarding flowers with single pollen (PTE = 1-2%), and that different buzz-pollinated flower architectures achieve similar PTE although through different pollen-loss pathways.

Keywords: Anther cone; buzz pollination; floral morphology; heteranthery; pollen carryover; pollen transfer and deposition; *Solanum* (Solanaceae)

INTRODUCTION

Pollen transfer from anthers to stigmas is a major determinant of plant fitness (Minnaar et al., 2019; Christopher et al., 2020). Plants produce a large amount of pollen per flower (Cruden, 2000), yet very few pollen grains produced in anthers ever make it to their destination. For instance, it is estimated that 2.4% of pollen grains produced by animal-pollinated plants with pollen grains released as single units (monads) reach the stigma of conspecific individuals (Harder and Thomson, 1989; Johnson and Harder, 2023). Pollen fates can be decomposed in three phases: pollen production and presentation, pollen

transfer, and pollen germination (Minnaar et al., 2019). Among these, pollen transfer, the pathway between pollen release and pollen deposition, is highly stochastic and mediated by the behavior of pollinators in animal-pollinated plants (Thomson and Plowright, 1980; Richards et al., 2009) (Fig. 1). However, despite its importance for plant reproductive success, studies of pollen transfer are scarce and often focused on species with pollen packed in pollinia such as in orchids (Minnaar et al., 2019). The pathway followed by pollen is especially hazardous for plants giving pollen as the only reward, as floral visitors actively collect pollen grains during floral visitation and might further reduce the pool of pollen remaining for fertilizing other flowers (Luo et al., 2008; Vallejo-Marín et al., 2009).

Pollen transfer efficiency (PTE) measures the proportion of pollen removed from the anthers that successfully reaches a conspecific stigma and is therefore available for potential ovule fertilization (Johnson and Harder, 2023). In animal-pollinated species, PTE usually decreases as the amount of pollen released per visit increases (Harder and Thomson, 1989; Harder, 1990), giving plants with gradual pollen release or pollen packaging strategies a fitness advantage. The Pollen Presentation Theory (Thomson, 2006) suggests that natural selection via male fitness should favor traits that regulate pollen dispensing and/or restrict pollen removed per visit (Thomson, 2006).

In addition to PTE, a key characteristic affecting the fate of pollen and its consequences for plant fitness is the amount of pollen deposited on each stigma in a consecutive sequence of recipient flowers, i.e., the shape of its pollen deposition curve (pollen carryover curve; Thomson and Plowright 1980). Pollen deposition curves tend to show an exponential decay pattern, but most studies have found longer tails than expected under an exponential model (i.e., leptokurtic distribution) due to long dispersal events (Lertzman, 1981; Price and Waser, 1982; Morris et al., 1994; Santa-Martinez et al., 2021). The slope of the curve varies with pollinator type (Castellanos et al., 2003; Santa-Martinez et al., 2021), type of flower, and reward availability in the recipient flower (Thomson and Plowright, 1980). Deposition curves have been studied in hummingbird pollinated *Ipomopsis* and *Castilleja* (e.g., Lertzman, 1981; Price and Waser, 1982; Morris et al., 1994), and in bee-pollinated species (e.g., Thomson and Plowright, 1980, Castellanos et al., 2003; Santa-Martinez et al., 2021). These studies have shown that grooming behavior plays a crucial role in determining the steepness of the curve. However, pollen deposition curves have rarely been estimated for nectarless plants that use pollen as the only reward to floral visitors. Given that in these plants pollen serves both as a vehicle for male gametes and simultaneously as a reward to potential pollinators (Vallejo-Marín et al., 2009), pollen deposition curves could have a particularly steep slope if pollinators are efficient at grooming pollen following a visit.

Here, we characterize and compare the removal, dispersal, and transfer efficiency of pollen (pollen fates) in two species of nectarless, bee-pollinated species of *Solanum* (Solanaceae). As most of the other ~1,200 described *Solanum* species (Hilgenhof et al., 2023), the species studied here are buzz pollinated, i.e., they are pollinated by pollen-foraging bees that use vibrations to harvest pollen from their tube-like poricidal anthers (Buchmann, 1983; Vallejo-Marin and Russell, 2024). To characterise pollen fates in these two species, we exposed experimental flowers to visits by a generalist buzz-pollinating bee, *Bombus terrestris* (Apidae). The specific objectives of our research were: (1) To quantify pollen fates and pollen transfer efficiency of two *Solanum* species with contrasting size and floral architecture (*Solanum rostratum* and *S. dulcamara*), and (2) to estimate the pollen deposition curves for both species. Our study provides a starting point for establishing pollen fates in nectarless, buzz pollinated species, and to investigate how floral architecture may influence pollen removal and transfer efficiency.

MATERIALS AND METHODS

Plants

As most other *Solanum* species, the ones studied here have bisexual and nectarless flowers, with five poricidal stamens (anthers that open through small apical pores). However, the species studied here differ markedly in their stamen shape and architecture (i.e., the shape and arrangement of anthers within the flower). *Solanum dulcamara* is a perennial vine native to Eurasia and introduced into North America (Knapp et al., 2013). Its flowers are actinomorphic, relatively small (1.5-2 cm diameter) and have monomorphic anthers that are fused into an anther cone that surrounds the centrally-placed pistil (Fig. 2A) (Glover et al., 2004). In Europe, *S. dulcamara* is visited by different species of bumblebees (Free, 1970), it is self-compatible, but its mating system is unknown. Conversely, *S. rostratum* is an annual herb native to Mexico and perhaps southern USA, and introduced in disturbed habitats around the world (Zhao et al., 2013). Flowers are medium-sized (2.3-3.5 cm diameter) and have two morphologically and functionally distinct sets of stamens in the same flower (i.e., heteranthery): one set is composed of four smaller, bright yellow 'feeding' anthers centrally located, and the other set includes a single larger, yellow to brown 'pollinating' anther that is laterally displaced, opposite to the style (Fig. 2B)(Vallejo-Marín et al., 2009). *Solanum rostratum* is also enantiostylous, i.e., each individual produces flowers with the style deflected either to the right- or left-hand side; this type of enantiostyly is thought to increase

pollen export between flowers of reciprocal morphs (Jesson and Barrett, 2002). In its native and introduced range, *S. rostratum* is visited by different types of bees, including bumblebees (*Bombus spp.*) (Bowers, 1975; Solís-Montero et al., 2015). Genetic estimation of outcrossing rates, indicates that *S. rostratum* is predominantly outcrossing (t = 0.70) in both native and introduced ranges (Vallejo-Marín et al., 2013; Zhang et al., 2017). For our study, plants of *S. rostratum and S. dulcamara* were grown from field-collected seeds at the glasshouses in Uppsala University. For *S. rostratum,* we used seeds from accessions 10s71-5, 10s74, 10s77, 10s79, and 10s80 (San Miguel de Allende, Guanajuato, Mexico), and 11s182, 11s188, 11s191, 11s205, and 11s207 (Dolores, Guanajuato, Mexico). For *S. dulcamara,* we used seeds from accessions 22-UPP-1 and 22-UPP-2 collected at Uppsala. Seeds were germinated as described in Vallejo-Marín et al. (2014), grown at 16h/20°C light and 8h/16°C dark cycles, and fertilised with general purpose liquid fertiliser.

Pollinators

For evaluating pollen transfer, we used *Bombus terrestris*, a generalist, buzz-pollinating bumblebee that is widely distributed in Europe and which is commonly used to supplement pollination of soft-fruit crops. *Bombus terrestris* visits *S. dulcamara* in the native range and is similar in size and behaviour to other bumblebees visiting *S. rostratum* in the Americas (Bowers 1975; MVM, pers. obs.). We used individuals from two commercial colonies (Koppert Biological Systems, UK; and Biobest Group, Belgium). Colonies were maintained on 2 M solution of sucrose available from artificial feeders within enclosed foraging arenas (82×60×60 cm), follow Russell et al. (2017). Twice a week, bees were also provided with approximately 10g of “pollen dough” made of pulverised honeybee-collected pollen (Koppert Biological Systems) mixed with sucrose solution and placed inside the colony. The foraging arenas were kept at room temperature and illuminated with an LED ceiling panel set to a 14 h:10 h light:dark cycle. Before the experiment, we conditioned the bees to visit *Solanum* flowers by allowing them to forage freely on flowers placed in the foraging arena. This conditioning phase was never done on the same day of the experimental trials to avoid bees carrying over pollen grain collected during the conditioning phase to the experimental trials.

Experimental trials

Before each trial, fresh flowers were collected from the greenhouse each morning before experiments and carefully transported in floral foam (OASIS Floral Products, Washington, UK) within a closed plastic

146 box. Each trial consisted of one donor flower from which pollen could be removed by the bee, and four
147 recipients (R1-R4) that were experimentally prevented from releasing pollen by sealing the anther pores
148 using a small drop of PVA glue (Elmer's Products, Ohio, USA) under a stereoscopic microscope. A similar
149 amount of glue was applied just below the anther pore of the donor flowers as a sham treatment. After
150 the glue dried, we mounted each flower on a bamboo stick with Blu-Tack adhesive gum (Bostik,
151 Staffordshire, UK) and placed it in a plastic tube containing floral foam. A black plate was placed under
152 the donor flower to recover pollen falling from the flower (dislodged pollen). To control for differences
153 in pollen transfer within and between morphs of enantiostylous *S. rostratum*, we set all recipients to
154 have the reciprocal mirror-image morph of the donor flower (i.e., the left-hand side morph for all
155 recipients if the donor was a right-hand flower, and vice versa).
156 For each trial, one donor flower was placed in the centre of the foraging arena and a single bee
157 was allowed to enter the arena. Immediately after the bee landed on the donor flower, we placed the
158 first recipient flower (R1) next to it in the arena and removed the donor flower from the arena as soon
159 as the bee departed from it. We proceeded like so with the consecutive recipients (R2, R3 and R4). If
160 after 10 minutes the bee had not landed on the donor flower, we ended the trial and removed the bee.
161 All trials were video recorded using a camera (Canon EOS M50, Tokyo, Japan) placed right outside of the
162 arena. Recordings were used to calculate the duration of each flower visit. Temperature, humidity of the
163 arena and time of the day were recorded.

164 **Pollen sampling and counting**

165 After each trial, the stigmas of the four recipients were individually cut and placed on fuchsin jelly cubes
166 (32 mm3) (Kearns and Inouye, 1993). Dislodged pollen was collected from the black plate using 1-2
167 cubes of jelly, and anthers from the donor flower were cut and preserved in 1ml of 70% ethanol. Under
168 a stereoscopic microscope, we removed the pollen in the corbiculae of the bee and placed it on a jelly
169 cube and collected pollen on the body of the bee with a separate jelly cube. Each sample was stored in
170 individual 1.5 mL Eppendorf tubes. Each fuchsin jelly cube containing a stigma sample was mounted on a
171 slide and examined under a light microscope at 400x magnification for pollen grain counting. To process
172 the remaining pollen samples, 500 µL of 70% ethanol was added to tubes containing pollen from the
173 ground, corbiculae, and body. Donor anthers were subjected to sonication using an Elmasonic S40H
174 ultrasonic cleaner (Elma Schmidbauer GmbH, Singen, Germany), followed by staining pollen grains with
175 200 µL of lactoglycerol-aniline blue (Kearns and Inouye, 1993). For pollen grain counting, an aliquot was
176 taken from each tube and placed onto a hemocytometer chamber until 200 pollen grains were counted.

To estimate the number of pollen grains, we considered the combined volumes of ethanol, jelly, and aniline used in their respective cases. After the trial concluded, we estimated: (C) the number of pollen grains remaining of the donor anthers, (D) number of pollen grains that fell on the ground, (F) the number of pollen grains on the bee's corbiculae and (G) on the bee's body, and (H) the number of pollen grains deposited on the stigma of each recipient. From these variables we also calculated the following derived variables: total pollen production (A = D + F + G + H), pollen released (B = F + G + H + D), pollen collected (E = F + G + H), and pollen lost from the pollination process (I = D + F).

Statistical analyses

Proportion of pollen grains deposited on the body or the stigma were calculated from the total pollen production and from the amount of pollen released. To determine if the proportions of pollen grains deposited on body and stigmas varied between species, we first verified the assumptions of normality and homoscedasticity using the Shapiro-Wilk and Levene's tests from *stats* (R Core Team, 2023) and *car* (Fox and Weisberg, 2019) packages, respectively. These assumptions were tested using absolute values and the log transformations from both species separately. In all cases, at least one of the assumptions was not met. Thus, we compared each variable using a non-parametric Wilcoxon test (R Core Team, 2023).

Pollen deposition curves (Thomson and Plowright, 1980) were analyzed using Generalized Linear Mixed-effects Models (GLMM) with a Poisson error (Log-link) using the *glmer* function (*lme4*) (Bates et al., 2014). First, we built a model using the number of pollen grains deposited in each recipient flower was the response variable, recipient order (R1-R4), species, and visit time were considered as fixed effects, and trial number as a random effect. Second, we analyzed a similar GLMM model as above but adding the number of removed pollen grains as an offset variable. This allowed us to compare pollen deposition curves independently of the absolute number of pollen grains removed from the donor. Back transformation and visualization were conducted using the *sjPlot* package (Lüdecke, 2018). We assessed overdispersion and zero inflation using the *DHARMa* package (Hartig, 2018). All statistical analyses were done in R v.4.1.0 (R Core Team, 2021) using *RStudio* (RStudio Team, 2021).

RESULTS

Pollen fates and pollen transfer efficiency

The absolute numbers of pollen grains in each stage of pollen transfer are presented in Fig. 3. We found that *Solanum rostratum* flowers produce on average 27,59% more pollen grains per flower than *S. dulcamara* flowers (Fig. 4). However, after a single visit, a bee removed on average 37.5% of the flower's total production of pollen grains in *S. dulcamara*, and only 8.91% in a *S. rostratum*. This is equivalent to the removal of about four times as many pollen grains in *S. dulcamara* than in *S. rostratum* in absolute numbers (Table 1). From the pollen released by the flowers, between 19.12% (*S. dulcamara*) and 67.93% (*S. rostratum*) fell to the ground. In addition to the pollen wasted on the ground, bees collected in their corbiculae, on average, 65.82% of released pollen grains for *S. dulcamara* and 24.03% for *S. rostratum*. The stigmas of recipient flowers received 1 860 $\pm$ 370 (mean $\pm$ se; *S. dulcamara*) and 356 $\pm$ 93 (*S. rostratum*) pollen grains. Thus, only approximately 8.03% (*S. rostratum*) to 15.06% (*S. dulcamara*) of released pollen grains remain available for pollination (pollen on stigmas and body) (Table 1).

The pollen transfer efficiency (PTE; i.e., the percentage of released pollen grains that reach any of the recipient stigmas) was 1.07% for *S. dulcamara* and 0.72% for *S. rostratum* (Fig. 5). Both PTE and the percentage of pollen remaining on the body were not significantly different between the two species ($U = 65$, $P = 0.113$; $U=44$, $P = 0.968$, respectively). Most of the pollen was lost on the ground (67.93% in *S. rostratum*) or in the corbiculae (65.82% in *S. dulcamara*) (Fig. 5). There was a significant difference in the percentage of pollen lost in the corbiculae and the dislodged pollen between species ($U = 77$, $P = 0.008$; $U = 5$, $P < 0.001$, respectively). However, the percentage of all the pollen lost (from released pollen) was not significantly different between both species ($U = 43$ $P = 0.9$).

Pollen deposition curves

As expected, most pollen from the donor was deposited in the first recipient flower visited, strongly decreasing with the increase of recipient order (Fig. 6 and 7B; Table 2, $Z = -43.19$, $P < 0.001$ and $Z = -42.53$, $P < 0.001$, for analyses with and without using pollen released as an offset, respectively). We found a statistically significantly difference in the average number of pollen grains per recipient stigma between *Solanum* species with fewer pollen grains deposited on *S. rostratum* than in *S. dulcamara* (Fig. 6; Table 2, $Z = -3.65$, $P < 0.001$). However, our analysis of the relative rates of pollen deposition

232 (using the number of pollen grains released as an offset variable) suggests that both species deposit
233 approximately the same proportion of pollen grains (Fig. 7B; Table 2, Z = 0.01, P = 0.996). The
234 relationship between recipient order and pollen deposited differed between species, with *S. rostratum*
235 exhibiting a steeper pollen deposition curve, as indicated by the statistically significant interaction
236 between recipient order and flower species (Table 2, Z = -7.23, P < 0.001 and Z = -7.89, P < 0.001, for
237 analyses with and without using pollen released as an offset, respectively). We also found that longer
238 visits resulted in more pollen deposited per stigma (Fig. 7A; Table 2, Z = 30.24, P < 0.001 and Z = 30.02, P
239 < 0.001, with and without pollen released as an offset, respectively).

240 DISCUSSION

241 Our study's main findings can be summarised as: (1) Both buzz-pollinated species of *Solanum* studied
242 here have a similar or lower values of pollen transfer efficiency (PTE) as those reported for other non-
243 buzz pollinated plants that disperse pollen in individual pollen grain units (monads), (2) pollen
244 deposition curves follow a classic exponential decay pattern, as in other animal-pollinated plants, and
245 (3) although both *Solanum* species studied showed a similar percentage of pollen loss, the pathway to
246 this loss differs and is either lost on the ground (*S. rostratum*) or in the bees' corbiculae (*S. dulcamara*),
247 potentially reflecting the functional effects of different flower architectures.

248 Pollen transfer efficiency in buzz-pollinated flowers

249 Buzz pollinated species are thought to produce large numbers of pollen grains compared to nectar-
250 producing relatives as a mechanism to offset the costs of active pollen collection by floral visitors (Vogel,
251 1978; De Luca and Vallejo-Marín, 2013; Vallejo-Marin and Russell, 2024). The average pollen production
252 per flower is indeed high in *S. dulcamara* (>600,000) and *S. rostratum* (>1,000,000), and comparable to
253 other nectarless plant species (Vallejo-Marin et al., 2009; Vallejo-Marin et al., 2014; Vallejo-Marín et al.,
254 2022). In our experiment, visitation by a generalist, buzz-pollinating bumblebee resulted in a pollen
255 transfer efficiency (PTE) of between 0.72 and 1.07%, which is towards the lower end of the 1-2% PTE
256 range reported for other species with monads (Johnson and Harder, 2023). Pollen transfer efficiency has
257 only been rarely quantified for buzz pollinated plants, but low PTE characterizes two previously studied
258 species. *Rhexia virginica* (Melastomataceae), an herbaceous species distributed in eastern North
259 America, has a PTE of 0.92% (Supplementary material from Johnson and Harder, 2023), while
260 *Senna reticulata* a neotropical tree, has a much lower PTE (0.05%) (Supplementary material from

261 Johnson and Harder, 2023). These relatively low values of PTE in buzz pollinated species might suggest
262 that active pollen collection indeed impacts the realized efficiency of pollen transfer, and that higher
263 rates of total pollen production are selected for to ensure delivery of sufficiently high numbers of pollen
264 grains to fertilize all ovules. However, further work comparing total pollen production and PTE in buzz
265 pollinated taxa and their closest non-buzz pollinated relatives are required to establish if this is a general
266 pattern. Furthermore, PTE can vary among different species of floral visitors, and this is an area that
267 requires additional study.

268 Although both *Solanum* species studied here achieve a similar PTE to each other, the pathways
269 of pollen production, release, and loss differ (Fig. 5). Percentage of pollen released in other buzz
270 pollinated species ranges widely from 10-60% (*Rhexia virginica*: 10–43%; Larson and Barrett, 1999;
271 *Senna reticulata*: 25%; Snow and Roubik, 1987; *Erica tetralix* and *Vaccinum myrtillus:* 28–60%; Moquet
272 et al., 2017), and is similar or lower than in non-buzz-pollinated species such as *Aconitum septentrionale*
273 (44-61%; Thøstesen and Olesen, 1996), *Malus domestica* and *Prunus dulcis* (40-50%; Thomson and
274 Goodell, 2001), and *Calluna vulgaris* (≤ 88%; Moquet et al., 2017). The small-flowered *S. dulcamara*
275 produces fewer pollen grains than *S. rostratum* but releases a higher number (and percentage) of pollen
276 grains after a single visit by a bumblebee. If all pollen produced in the flower is available for removal,
277 about 11 visits would be required to empty a flower of *S. rostratum* (8.9% of total pollen removed per
278 visit), while in *S. dulcamara* fewer than three visits would be needed (37.5% pollen released per visit).
279 However, pollen inside the anther may not all be available to be removed at first, and it appears that
280 some buzz pollinated flowers present a staggered release of pollen as the flower ages (Harder and
281 Barclay, 1994; Kemp and Vallejo-Marin, 2021). The extent to which pollen becomes available
282 simultaneously or in a staggered fashion in buzz pollinated species is not well understood. Regardless of
283 the mechanism, the high rates of pollen release observed in *S. dulcamara,* characterize other small-
284 flowered, non-heterantherous taxa in *Solanum* Section *Androceras* and this has been interpreted as a
285 consequence to a shift of mating system towards more selfing (Kemp and Vallejo-Marín, 2021). The
286 mating system of *S. dulcamara* is presently unknown, but this self-compatible species sometimes sets
287 fruit autonomously (Vallejo-Marin per. obs.). In contrast, although *S. rostratum* is also self-compatible, it
288 has a high outcrossing rate (0.70-0.90) (Vallejo-Marín et al., 2013; Morra-Carrera et al., 2019) and rarely
289 sets fruits autonomously, at least in the native range. Thus, the difference in the proportion of pollen
290 removed per visit could be associated to different floral morphologies and mating systems in
291 *S. dulcamara* and *S. rostratum*. For outcrossing species visited regularly by pollinators, a more restricted

292 pollen schedule might allow them to dispense pollen more efficiently or to more flowers (Harder and
293 Thomson, 1989; Harder and Wilson, 1994; Kemp and Vallejo-Marín, 2021).
294 Although both species end up exporting similar proportion of pollen grains to stigmas, the
295 pathway to pollen loss differs: In *S. dulcamara*, pollen is mostly loss to bees collecting it and placing it in
296 their corbiculae, while in *S. rostratum,* pollen is mostly loss by falling on to the ground, meaning that this
297 removed pollen does not contribute to the fitness of either bee or plant. What is the reason for these
298 contrasting pollen fates in these two species? With only two species studied, it is difficult to generalize,
299 but in *S. dulcamara* the bees can curl their body in close proximity of the anther pores while buzzing the
300 flower. The fused anther cone of *S. dulcamara* then works as a single pollen dispensing unit that places
301 pollen grains in the ventral region of the bee where it can be groomed and placed in the corbiculae
302 (Nevard & Vallejo-Marín, 2022). In contrast, in heterantherous *S. rostratum*, the bee cannot work
303 simultaneously both feeding and pollinating anthers, and it usually focuses its attention on the feeding
304 anthers. The feeding anthers contact the bee on the ventral side, while the pollinating anthers contact
305 the bee on the dorsal side of the abdomen, where it is harder to groom by the bee. The body of the bee
306 often contacts the terminal pores of the feeding anthers, but the distance between the bee's body and
307 the terminal pore of the pollinating anther depends both on the positioning of the bee and on the
308 relative size of bee and flower. Because approximately 50% of the total pollen production in these
309 flowers resides on the single pollinating anther (Vallejo-Marin et al., 2009; Vallejo-Marin et al., 2014),
310 flower handling and bee-flower size matching can then affect significantly whether the pollen reaches
311 the bee's body or falls to the ground. Although we did not quantify this in our experiment, we expect
312 that much of the pollen loss occurring during buzz pollination in *S. rostratum* is due to mismatches
313 between the size of the bee and the flower. All else being equal, a bee of similar characteristics to the
314 ones studied here might benefit more from a single visit to a species like *S. dulcamara* than one like
315 *S. rostratum.* Of course, differences in total pollen production, and number of flowers per plant might
316 help offset the increased difficulty of gathering pollen from flowers with a more complex morphology as
317 in *S. rostratum*.

318 **Pollen deposition curves**

319 Our results demonstrate that the pollen deposition curves (i.e., the pattern of pollen deposition on the
320 stigmas of consecutive recipients) for the buzz-pollinated *Solanum* species studied follow a typical
321 exponential decay pattern, as reported for other species (Thomson & Plowright, 1980; Mitchell et al.,
322 2013; Santa-Martinez et al., 2021). Specifically, the amount of pollen deposited on stigmas decreased by

27.6% and 36.9% with each successive recipient for *S. dulcamara* and *S. rostratum*, respectively. These percentages are on the higher end of the spectrum for pollen carryover curves, which ranges from 50% to 0.5% (see Table 1 in Robertson, 1992). This indicates a relatively steep decline in deposited pollen with each successive recipient. Generally, grooming pollinators such as bees exhibit steeper deposition curves, indicating less extensive pollen carryover, compared to pollinators like hummingbirds and hawkmoths (Robertson, 1992; Castellanos, 2003; Mitchell et al., 2013). However, this pattern has not been studied in other buzz-pollinated plants, highlighting a gap in our understanding of these systems.

Pollen deposition curves are influenced by both the amount of pollen that lands on the pollinator's body and the intensity of its grooming before visiting the next flower. Buzz-pollinated *Solanum* flowers, which produce and gradually release large amounts of pollen as a reward for pollinators, tend to encourage more intense grooming behavior compared to nectar-producing, non-buzz-pollinated flowers (Harder, 1990; Johnson & Harder, 2023). This intense grooming results in steeper pollen deposition curves (Holmquist et al., 2012), potentially influencing gene flow patterns (Santa-Martinez et al., 2021). Consequently, if buzz-pollinated flowers generally exhibit steeper pollen deposition curves, they might experience more restricted gene flow compared to non-buzz-pollinated species offering other rewards, such as nectar. While insect-pollinated species often display higher levels of population genetic structure (Dellinger et al., 2022; Gamba & Muchhala, 2020; Gamba & Muchhala, 2022), the specific impact of buzz pollination on genetic structure remains underexplored

CONCLUSIONS

Empirical studies of pollen fates, although experimentally complex and time-consuming, provide an opportunity to illuminate a key process affecting the reproductive success of plants. Our study found significant differences in pollen fates between *S. dulcamara* and S*. rostratum*, highlighting the impact of floral architecture on pollen transfer, even among species visited by the same buzz-pollinating bee. Specifically, we observed distinct pollen deposition curves and pathways of pollen loss between species. *S. rostratum* primarily loses pollen through dislodging due to its restricted dispensing schedule, while *S. dulcamara* experiences significant pollen loss from bee grooming. Despite these differences, both species achieve comparable pollen transfer efficiency (PTE). These findings highlight the role of floral morphology on pollen transfer dynamics and suggest that different floral architectures can lead to similar reproductive outcomes. Future research should encompass a wider range of species with diverse

morphological traits and reward types and explore how different pollen pathways affect realized female and male fitness.

ACKNOWLEDGMENTS

We thank D. Scaccabarozzi and L. van Kolfschoten for their technical support and advice on the development of this work. We are also grateful to the Research Experience for Peruvian Undergraduates (REPU) Program and the exchange program of the University of Lausanne for allowing A.V.V, B.G.S and E.M to create this project at Uppsala Universitet. This work was partly supported by start-up funds from the Plant Ecology and Evolution program at Uppsala University, awarded to M.V.-M.

AUTHOR CONTRIBUTIONS

A.V.C., E.M., B.G.S., and M.V.M conceived the study. A.V.C., E.M. and B.G.S. conducted the experiments, collected the data and performed the analyses. A.V.C., E.M., B.G.S., and M.V.M wrote and revised the manuscript. M.V.M supervised the study and acquired funding.

DATA AVAILABILITY STATEMENT

Pollen transfer data used for the analyses and the R script will be made available upon acceptance.

LITERATURE CITED

Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software*, *67*(1), 1–48. https://doi.org/10.18637/jss.v067.i01

Bowers, K. A. (1975). The pollination ecology of Solanum rostratum (Solanaceae). American Journal of Botany, 62(6), 633-638.

Buchmann, S. L. (1983). Buzz pollination in angiosperms. *Buzz Pollination in Angiosperms.*, 73–113.

Castellanos, M. C., Wilson, P., & Thomson, J. D. (2003). Pollen transfer by hummingbirds and bumblebees, and the divergence of pollination modes in penstemon. *Evolution*, *57*(12), 2742–2752. https://doi.org/10.1111/j.0014-3820.2003.tb01516.x

Christopher, D. A., Mitchell, R. J., & Karron, J. D. (2020). Pollination intensity and paternity in flowering plants. *Annals of Botany*, *125*(1), 1-9. https://doi.org/10.1093/aob/mcz159

Cruden, R. W. (2000). Pollen grains: Why so many? In A. Dafni, M. Hesse, & E. Pacini (Eds.), *Pollen and Pollination* (pp. 143–165). Springer. https://doi.org/10.1007/978-3-7091-6306-1_8

De Luca, P. A., & Vallejo-Marín, M. (2013). What's the 'buzz' about? The ecology and evolutionary significance of buzz-pollination. *Current Opinion in Plant Biology*, *16*(4), 429–435. https://doi.org/10.1016/j.pbi.2013.05.002

Dellinger, A. S., Paun, O., Baar, J., Temsch, E. M., Fernández-Fernández, D., & Schönenberger, J. (2022). Population structure in Neotropical plants: Integrating pollination biology, topography and climatic niches. *Molecular Ecology*, *31*(8), 2264-2280. https://doi.org/10.1111/mec.16403

Fox, J., & Weisberg, S. (2019). car: Companion to Applied Regression. R package version 3.1-2.

Free, J. B. (1970). The Flower Constancy of Bumblebees. *Journal of Animal Ecology*, *39*(2), 395–402. https://doi.org/10.2307/2978

Gamba, D., & Muchhala, N. (2020). Global patterns of population genetic differentiation in seed plants. *Molecular Ecology*, 29(18), 3413–3428. https://doi.org/10.1111/mec.15575

Gamba, D., & Muchhala, N. (2023). Pollinator type strongly impacts gene flow within and among plant populations for six Neotropical species. *Ecology*, *104*(1), e3845. https://doi.org/10.1002/ecy.3845

Glover, B. J., Bunnewell, S., & Martin, C. (2004). Convergent evolution within the genus Solanum: The specialised anther cone develops through alternative pathways. *Gene*, *331*, 1–7. https://doi.org/10.1016/j.gene.2004.01.027

Harder, L. D., & Barclay, R. M. R. (1994). The functional significance of poricidal anthers and buzz pollination: controlled pollen removal from Dodecatheon. Functional Ecology, 509-517.

Harder, L. D. (1990). Behavioral responses by bumble bees to variation in pollen availability. *Oecologia*, *85*(1), 41–47. https://doi.org/10.1007/BF00317341

Harder, L. D., & Wilson, W. G. (1994). Floral evolution and male reproductive success: Optimal dispensing schedules for pollen dispersal by animal-pollinated plants. Evolutionary Ecology, 8(5), 542–559. https://doi.org/10.1007/BF01238257

Harder, L. D., & Wilson, W. G. (1998). Theoretical consequences of heterogeneous transport conditions for pollen dispersal by animals. Ecology, 79(8), 2789–2807. https://doi.org/10.1890/0012-9658(1998)079[2789:TCOHTC]2.0.CO;2

Harder, L. D., & Thomson, J. D. (1989). Evolutionary Options for Maximizing Pollen Dispersal of Animal-Pollinated Plants. *The American Naturalist*, *133*(3), 323–344. https://doi.org/10.1086/284922

Hartig, F. (2022). DHARMa: Residual Diagnostics for Hierarchical (Multi-Level / Mixed) Regression Models. R package version 0.4.6. https://CRAN.R-project.org/package=DHARMa

Hilgenhof, R., Gagnon, E., Knapp, S., Aubriot, X., Tepe, E. J., Bohs, L., ... & Särkinen, T. (2023). Morphological trait evolution in Solanum (Solanaceae): Evolutionary lability of key taxonomic characters. *Taxon*, *72*(4), 811-847. https://doi.org/10.1002/tax.12990

Holmquist, K. G., Mitchell R. J., & Karron J. D. 2012. Influence of pollinator grooming on pollen-mediated gene dispersal in Mimulus ringens (Phrymaceae). Plant Species Biology 27: 77–85. https://doi.org/10.1111/j.1442-1984.2011.00329.x

Jesson, L. K., & Barrett, S. C. H. (2002). Solving the puzzle of mirror-image flowers. *Nature*, *417*(6890), 707–707. https://doi.org/10.1038/417707a

Johnson, S. D., & Harder, L. D. (2023). The economy of pollen dispersal in flowering plants. *Proceedings of the Royal Society B: Biological Sciences*, *290*(2008), 20231148. https://doi.org/10.1098/rspb.2023.1148

Kearns, C. A., & Inouye, D. W. (1993). *Techniques for pollination biologists*. University press of Colorado.

Kemp, J. E., & Vallejo-Marín, M. (2021). Pollen dispensing schedules in buzz-pollinated plants: Experimental comparison of species with contrasting floral morphologies. *American Journal of Botany*, *108*(6), 993–1005. https://doi.org/10.1002/ajb2.1680

Knapp, S. (2013). A revision of the Dulcamaroid Clade of Solanum L. (Solanaceae). *PhytoKeys*, *22*, 1–428. https://doi.org/10.3897/phytokeys.22.4041

Larson, B. M., & Barrett, S. C. (1999). The ecology of pollen limitation in buzz-pollinated Rhexia virginica (Melastomataceae). *Journal of Ecology*, *87*(3), 371-381. https://doi.org/10.1046/j.1365-2745.1999.00362.x

Lertzman, K. P. (1981). *Pollen transfer : processes and consequences* (T). University of British Columbia. Retrieved from https://open.library.ubc.ca/collections/ubctheses/831/items/1.0095274

Lüdecke, D. (2023). sjPlot: Data Visualization for Statistics in Social Science. R package version 2.8.14, https://CRAN.R-project.org/package=sjPlot

Luo, Z., Zhang, D., & Renner, S. S. (2008). Why two kinds of stamens in buzz-pollinated flowers? Experimental support for Darwin's division-of-labour hypothesis. *Functional Ecology*, *22*(5), 794–800. https://doi.org/10.1111/j.1365-2435.2008.01444.x

Minnaar, C., Anderson, B., de Jager, M. L., & Karron, J. D. (2019). Plant–pollinator interactions along the pathway to paternity. *Annals of Botany*, *123*(2), 225–245. https://doi.org/10.1093/aob/mcy167

Mitchell, R. J., Wilson, W. G., Holmquist, K. G., & Karron, J. D. (2013). Influence of pollen transport dynamics on sire profiles and multiple paternity in flowering plants. PLoS One, 8(10), e76312. https://doi.org/10.1371/journal.pone.0076312

441 Moquet, L., Bruyère, L,. Pirard, B., Jacquemart, A. (2017). Nectar foragers contribute to the pollination
442 of buzz-pollinated plant species. American Journal of Botany, 104 (10): 1 – 13.
443 https://doi.org/10.3732/ajb.1700090
444 Mora-Carrera, E., Castañeda-Zárate, M., Fornoni, J., Boege, K. and Domínguez, C.A., 2019. On the
445 adaptive value of monomorphic versus dimorphic enantiostyly in Solanum rostratum. Annals of
446 botany, 123(1), pp.205-212. https://doi.org/10.1093/aob/mcy162
447 Morris, W. F., Price, M. V., Waser, N. M., Thomson, J. D., Thomson, B., & Stratton, D. A. (1994).
448 Systematic Increase in Pollen Carryover and Its Consequences for Geitonogamy in Plant
449 Populations. *Oikos*, *71*(3), 431–440. https://doi.org/10.2307/3545831
450 Nevard, L., & Vallejo-Marín, M. (2022). Floral orientation affects outcross-pollen deposition in buzz-
451 pollinated flowers with bilateral symmetry. *American Journal of Botany*, *109*(10), 1568–1578.
452 https://doi.org/10.1002/ajb2.16078
453 Price, M. V., & Waser, N. M. (1982). Experimental studies of pollen carryover: Hummingbirds and
454 Ipomopsis aggregata. *Oecologia*, *54*(3), 353–358. https://doi.org/10.1007/BF00380004
455 Richards, S. A., Williams, N. M., & Harder, L. D. (2009). Variation in Pollination: Causes and
456 Consequences for Plant Reproduction. *The American Naturalist*, *174*(3), 382–398.
457 https://doi.org/10.1086/603626
458 Robertson, A. W. (1992). The relationship between floral display size, pollen carryover and geitonogamy
459 in Myosotis colensoi (Kirk) Macbride (Boraginaceae). *Biological Journal of the Linnean*
460 *Society*, *46*(4), 333-349. https://doi.org/10.1111/j.1095-8312.1992.tb00868.x
461 Russell, A. L., Buchmann, S. L., & Papaj, D. R. (2017). How a generalist bee achieves high efficiency of
462 pollen collection on diverse floral resources. *Behavioral Ecology*, *28*(4), 991–1003.
463 https://doi.org/10.1093/beheco/arx058
464 Santa-Martinez, E., Cardoso Castro, C., Flick, A., Sullivan, M., Riday, H., Clayton, M. K., & Brunet, J.
465 (2021). Bee species visiting Medicago sativa differ in pollen deposition curves with consequences
466 for gene flow. *American Journal of Botany*, *108*(6), 1016–1028. https://doi.org/10.1002/ajb2.1683
467 Snow, A. A., & Roubik, D. W. (1987). Pollen Deposition and Removal by Bees Visiting Two Tree Species in
468 Panama. Biotropica, 19(1), 57–63. https://doi.org/10.2307/2388460
469 Solís-Montero, L., Vergara, C. H., & Vallejo-Marín, M. (2015). High incidence of pollen theft in natural
470 populations of a buzz-pollinated plant. *Arthropod-Plant Interactions*, *9*(6), 599–611.
471 https://doi.org/10.1007/s11829-015-9397-5

Thomson, J. D. (2006). Tactics for male reproductive success in plants: Contrasting insights of sex allocation theory and pollen presentation theory. *Integrative and Comparative Biology*, *46*(4), 390–397. https://doi.org/10.1093/icb/icj046

Thomson, J. D., & Goodell, K. (2001). Pollen Removal and Deposition by Honeybee and Bumblebee Visitors to Apple and Almond Flowers. Journal of Applied Ecology, 38(5), 1032-1044. https://www.jstor.org/stable/827241

Thomson, J. D., & Plowright, R. C. (1980). Pollen carryover, nectar rewards, and pollinator behavior with special reference to Diervilla lonicera. *Oecologia*, *46*(1), 68–74. https://doi.org/10.1007/BF00346968

Thøstesen, A. M, & Olesen J. M. (1996). Pollen Removal and Deposition by Specialist and Generalist Bumblebees in Aconitum septentrionale. Oikos, 77(1), 77–84. https://doi.org/10.2307/3545587

Vallejo-Marín, M., Manson, J. S., Thomson, J. D., & Barrett, S. C. H. (2009). Division of labour within flowers: Heteranthery, a floral strategy to reconcile contrasting pollen fates. *Journal of Evolutionary Biology*, *22*(4), 828–839. https://doi.org/10.1111/j.1420-9101.2009.01693.x

Vallejo-Marín, M., Pereira Nunes, C. E., & Russell, A. L. (2022). Anther cones increase pollen release in buzz-pollinated Solanum flowers. *Evolution*, *76*(5), 931–945. https://doi.org/10.1111/evo.14485

Vallejo-Marín, M., & Russell, A. L. (2024). Harvesting pollen with vibrations: towards an integrative understanding of the proximate and ultimate reasons for buzz pollination, *Annals of Botany*, 133(3), 379–398. https://doi.org/10.1093/aob/mcad189

Vallejo-Marín, M., Solís-Montero, L., Souto Vilaros, D., & Lee, M. Y. Q. (2013). Mating system in Mexican populations of the annual herb Solanum rostratum Dunal (Solanaceae). *Plant Biology*, *15*(6), 948–954. https://doi.org/10.1111/j.1438-8677.2012.00715.x

Vallejo-Marín, M., Walker, C., Friston-Reilly, P., Solís-Montero, L., & Igic, B. (2014). Recurrent modification of floral morphology in heterantherous Solanum reveals a parallel shift in reproductive strategy. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *369*(1649), 20130256. https://doi.org/10.1098/rstb.2013.0256

Vogel, S. (1978). Evolutionary shifts from reward to deception in pollen flowers. In A. J. Richards (Ed.), The pollination of flowers by insects (pp. 89-96). Academic Press.

Zhang, L., Yu, L., & Lou, A. (2017). No evolutionary change in the mating system of Solanum rostratum (Solanaceae) during its invasion in China. *Scientific Reports*, *7*(1), 17698. https://doi.org/10.1038/s41598-017-17881-2

503 Zhao, J., Solís-Montero, L., Lou, A., & Vallejo-Marín, M. (2013). Population Structure and Genetic
504 Diversity of Native and Invasive Populations of Solanum rostratum (Solanaceae). *PLOS ONE*, *8*(11),
505 e79807. https://doi.org/10.1371/journal.pone.0079807
506

507 # TABLES

508 **Table 1**. Estimated number of pollen grains (mean ± se) at different stages of the pollination process in
509 *Solanum dulcamara* and *S. rostratum*.

	S. dulcamara		***S. rostratum***	
	N	**Number of pollen grains**	**N**	**Number of pollen grains**
Total pollen production (A)	9	641 780 ± 92 979	10	1 065 712 ± 116 142
Pollen release phase				
Pollen released (B)	9	243 323 ± 49 331	10	86 732 ± 21 423
Remaining pollen (C)	10	378 642 ± 65 596	10	1 000 891 ± 121 491
Pollen transfer phase				
Pollen on ground/dislodged (D)	9	41 177 + 19 734	10	53 561 + 17 369
Pollen collected (E)	9	202 146 + 51 589	10	29 358 + 9 222
Pollen on the pollinator				
Pollen in corbiculae (F)	9	188 601 ± 53 757	10	24 121 ± 8 634
Pollen on body (G)	9	11 685 + 4466	10	4 892 + 2198
Pollen deposition phase				
Pollen deposited on stigmas (H)	9	1860 + 370	10	356 + 93
Pollen loss				
Pollen on corbiculae and on the ground (I)	9	229 779 + 51 778	10	81 495 + 21 477

510

511 **Table 2.** Statistical analysis of the effect of recipient order, flower species (*S. dulcamara, S. rostratum*)
512 and duration of visit (s) on the number of pollen grains deposited on stigmas. Model estimates were
513 obtained from GLMMs with trial number as a random effect and recipient order, flower species and
514 duration of visit as fixed effects.

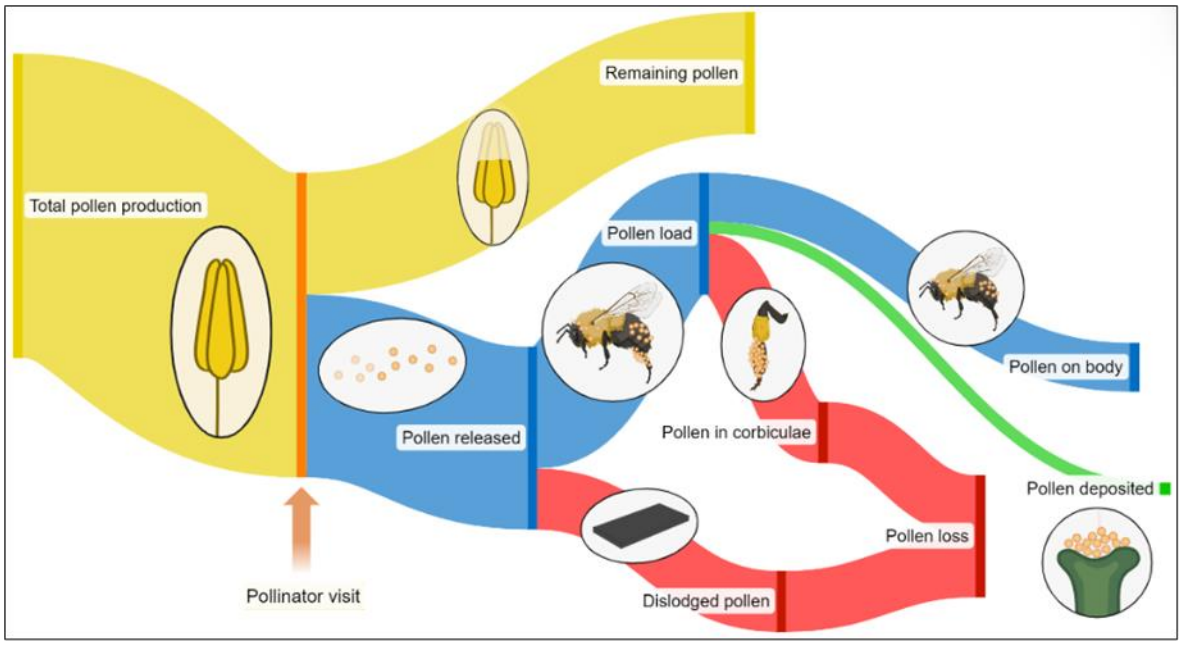
Fixed effects	**Coefficient**	**Standard error**	**Effect size (Z)**	**p-value**
(A) Pollen deposition model				
Intercept	5.801	± 0.205	28.33	<0.001
Recipient order	-0.303	± 0.007	-42.53	<0.001
Flower species (*S. rostratum*)	-1.053	± 0.289	-3.65	<0.001
Log (duration of visit (s))	0.324	± 0.011	30.02	<0.001
Recipient order: flower species (*S. rostratum*)	-0.147	± 0.019	-7.89	<0.001
(B) Pollen deposition model with offset variable				
Intercept	-6.248	± 0.317	-19.67	<0.001
Recipient order	-0.323	± 0.007	-43.19	<0.001
Flower species (*S. rostratum)*	0.002	± 0.449	0.01	0.996
Log (duration of visit (s))	0.332	± 0.011	30.24	<0.001
Recipient order: flower species (*S. rostratum*)	-0.138	± 0.019	-7.24	<0.002

515

516 # FIGURE LEGENDS

517 **Figure 1**. Pathways depicting the fates of pollen in a bee pollinated species such as the ones studied
518 here. The diagram shows pollen contained inside the anthers (yellow); pollen released that can be
519 potentially transferred to conspecific stigmas (blue); pollen lost from the fertilization process, including
520 dislodged pollen that falls to the ground and pollen stored by the bee (e.g., in corbiculae) and thus
521 unable to contribute to fertilization (red); and pollen deposited on stigmas (green).
522 **Figure 2**. (A) Flower of *S. dulcamara*, arrow indicates fused anthers (anther cone). (B) Flower of *S.*
523 *rostratum*, arrows indicate the feeding (above) and pollinating anthers (below). Photo credits: M.
524 Vallejo-Marin.
525 **Figure 3**. Diagram showing the fates of pollen of (A) *S. rostratum* and (B) *S. dulcamara*. The number of
526 pollen grains for each stage of the pollen dispersal process is specified (mean ± SE).
527 **Figure 4.** Percentage (from the total pollen production) of pollen in different paths after one visit on a
528 donor flower in *S. dulcamara* and *S. rostratum*.
529 **Figure 5.** Percentage (from the amount of pollen released of pollen) in different paths after one visit on
530 a donor flower in *S. dulcamara* and *S. rostratum*.
531 **Figure 6.** Number of pollen grains deposited on recipient flower stigmas as influenced by flower species
532 and recipient order. Plotted lines indicate estimated means and shaded regions indicate standard errors.
533 **Figure 7.** Number of pollen grains deposited on recipient flower stigmas as influenced by (A) duration of
534 the visit in seconds, and (B) flower species and recipient order with released pollen as offset variable.
535 Plotted lines indicate estimated means and shaded regions indicate standard

536 # FIGURES

537 

538 **Figure 1**. Pathways depicting the fates of pollen in a bee pollinated species such as the ones studied
539 here. The diagram shows pollen contained inside the anthers (yellow); pollen released that can be
540 potentially transferred to conspecific stigmas (blue); pollen lost from the fertilization process, including
541 dislodged pollen that falls to the ground and pollen stored by the bee (e.g., in corbiculae) and thus
542 unable to contribute to fertilization (red); and pollen deposited on stigmas (green).

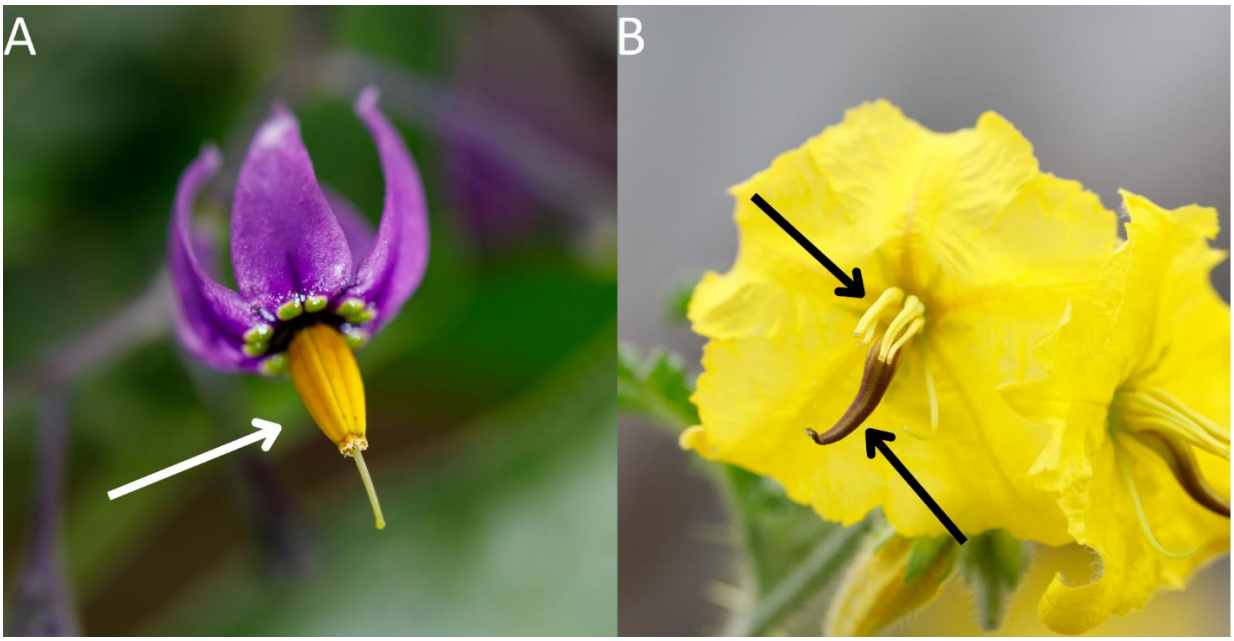


Figure 2. (A) Flower of *S. dulcamara*, arrow indicates fused anthers (anther cone). (B) Flower of *S. rostratum*, arrows indicate the feeding (above) and pollinating anthers (below). Photo credits: M. Vallejo-Marin.

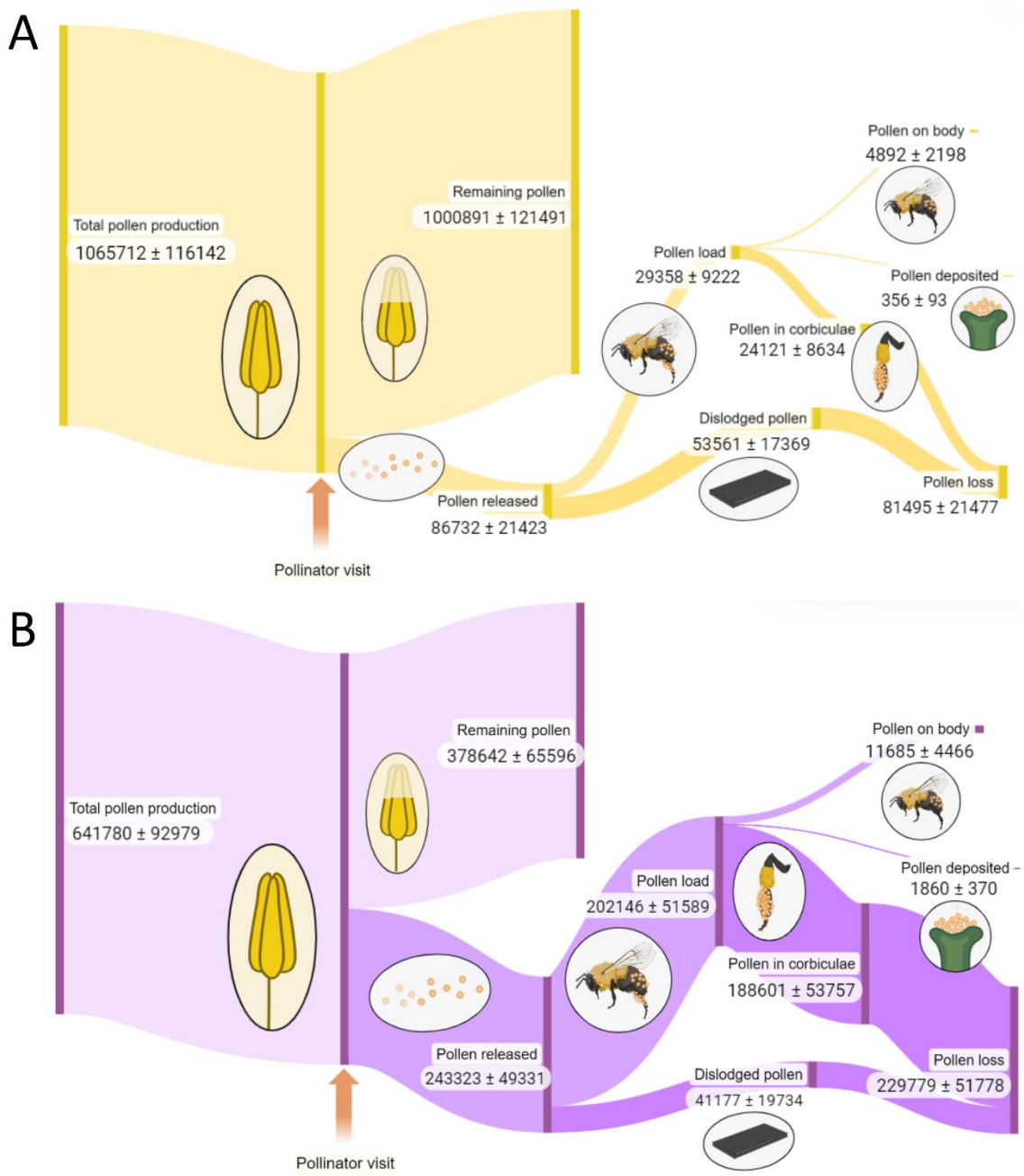


Figure 3. Diagram showing the fates of pollen of (A) *S. rostratum* and (B) *S. dulcamara*. The number of pollen grains for each stage of the pollen dispersal process is specified (mean ± SE).

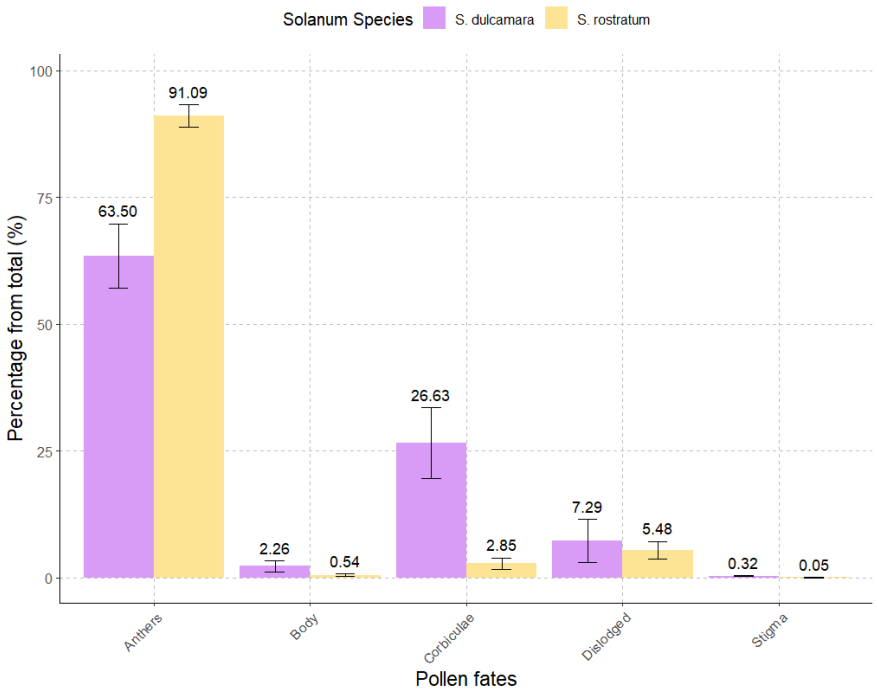


Figure 4. Percentage (from the total pollen production) of pollen in different paths after one visit on a donor flower in *S. dulcamara* and *S. rostratum*.

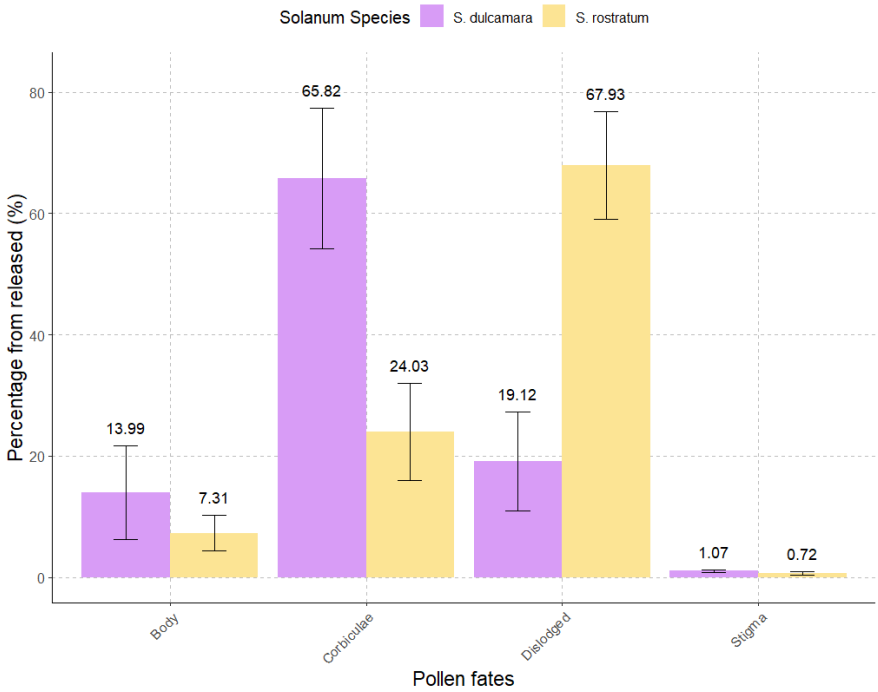


Figure 5. Percentage (from the amount of pollen released of pollen) in different paths after one visit on a donor flower in *S. dulcamara* and *S. rostratum*.

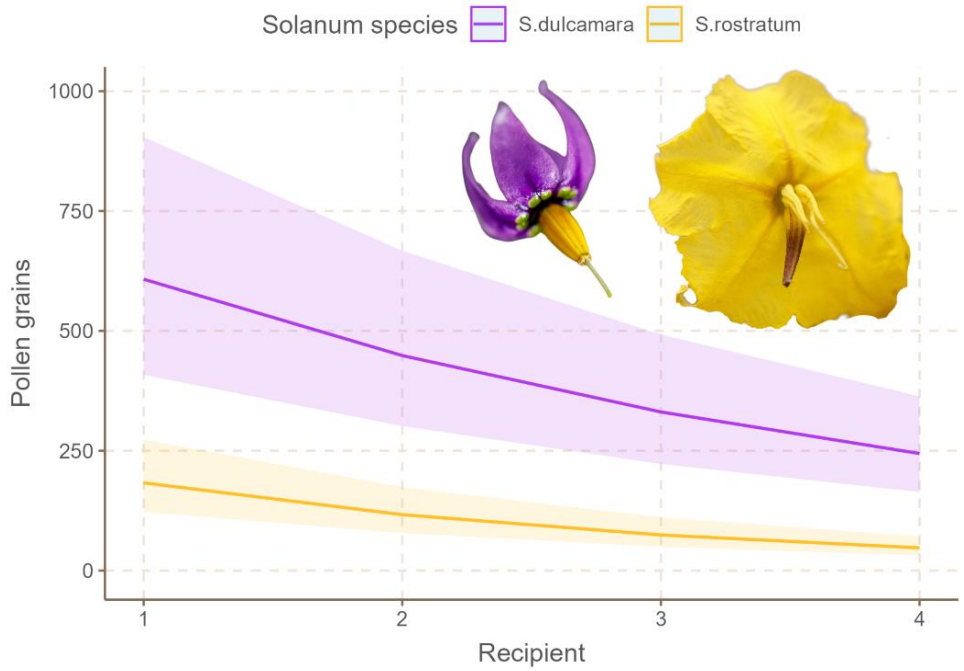


Figure 6. Number of pollen grains deposited on recipient flower stigmas as influenced by flower species and recipient order. Plotted lines indicate estimated means and shaded regions indicate standard errors.

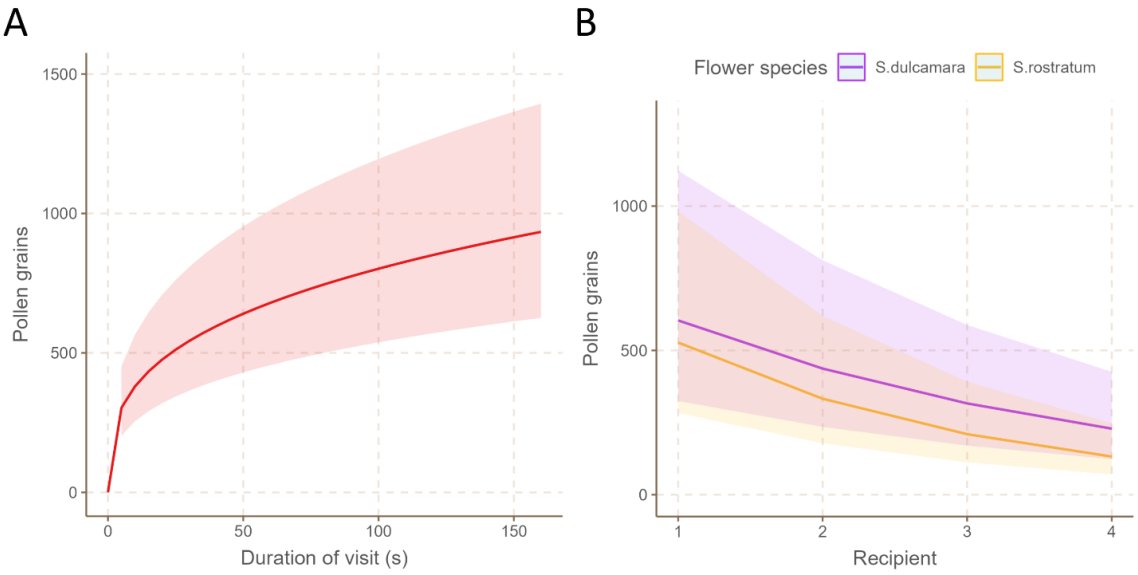


Figure 7. Number of pollen grains deposited on recipient flower stigmas as influenced by (A) duration of the visit in seconds, and (B) flower species and recipient order with released pollen as offset variable. Plotted lines indicate estimated means and shaded regions indicate standard errors.