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ORIGINAL ARTICLE

Intra-individual variation in pollen availability: an experimental analysis of its impact on plant–pollinator interactions

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- **Background and Aims** Intra-individual variation in the quantity and quality of floral rewards can affect pollinator foraging and plant fitness. Few studies have examined how this variation affects pollinator behaviour, particularly in pollen-rewarding plants.
- **Methods** We quantified intra-individual variation in pollen production in two nectarless species (*Solanum sisymbriifolium* and *S. rostratum*), in which pollen is concealed inside poricidal anthers. Then, for one of these species (*S. rostratum*), we manipulated pollen availability to create plants with increased intra-individual variation (three flowers with high, natural levels and no pollen) or plants with natural levels of variation (three flowers with natural pollen levels). We exposed both treatments simultaneously to bumblebees (*Bombus terrestris*), recording visitation and buzzing behaviour.
- **Key Results** Natural intra-individual variation in pollen production was high, with 46–58 % of the variation attributable to differences among flowers of the same plant, and coefficients of variation of 14–23 %. Analysis of foraging patterns capturing >2500 visits and 9700 buzzing events showed that bumblebees performed fewer and shorter buzzing visits on pollen-less flowers, whereas they were more likely to remain on a plant after visiting higher-reward flowers, potentially reducing outcrossing opportunities. These flower-level responses did not scale up to the plant level. Contrary to our expectations and to previous work in nectar-rewarding systems, plants in both variation treatments received visits in similar quantity and quality.
- **Conclusions** These findings suggest that *B. terrestris* can detect and respond to differences among flowers in pollen quantity despite high intra-individual variation, but that there is no plant-level effect. Given that bees respond to individual flowers rather than integrating information at the plant level, the behavioural patterns documented here might help plants to buffer fitness consequences of intra-individual variation in pollen rewards, which we show is substantial even at the level of pollen production and is likely to be exacerbated following visitation.

Key words: bees, *Bombus terrestris*, buzz pollination, intra-individual variation, pollen rewards, *Solanum*.

INTRODUCTION

Flowering plants are modular organisms characterized by the reiteration of organs and structures, including leaves and flowers, in the same individual (White, 1979; Herrera, 2017). The recurrence of flowers in the same individual allows plants to deploy plastic and diverse reproductive strategies (Barrett, 2010). For instance, different flowers in the same plant can express different sexual functions (e.g. monoecy, andromonoecy and gynomonoecy) (Renner, 2014), present mirror-imaged flowers that promote outcrossing (Jesson *et al.*, 2003), or be integrated into compound inflorescences composed of multiple floral types, such as in the capitula of Asteraceae or the cyathia of Euphorbiaceae. However, probably the most ubiquitous form of intra-individual floral variation occurs when plants produce multiple flowers that appear morphologically similar yet differ in ways that are important to pollinators, including the quantity and quality of floral rewards. Intra-individual variation in floral rewards can influence the pattern of pollinator visitation and thus plant fitness (Herrera, 2009; Harder *et al.*, 2019). Most previous work has focused on nectar rewards, and we know comparatively little about how intra-individual variation in pollen affects plant–pollinator interactions, even though pollen is an older and phylogenetically widespread reward used by pollinators, from beetles to bees.

Plants with poricidal flowers keep pollen inside tubular structures (usually enlarged, modified anthers that open via small apical pores or slits) and often use pollen as the main reward for floral visitors (Buchmann, 1983). In these plants, the amount of pollen in the flower is visually cryptic: flowers can appear full of pollen even when completely empty. This feature enables plants to decouple the amount of reward remaining in a flower from its visual appearance, forcing pollinators either to use non-visual cues or to sample pollen directly to assess its availability. Many species with poricidal flowers are nectarless and buzz pollinated. During buzz pollination, bees produce vibrations (floral buzzes) with their indirect flight muscles to remove pollen (De Luca and Vallejo-Marín, 2013; Vallejo-Marín, 2019; Vallejo-Marín and Russell, 2024). Bees need to expend a significant amount of energy to produce these floral vibrations, as recently demonstrated by Rossi *et al.* (2026). As predicted by pollen presentation theory, the flowers of buzz-pollinated plants can function as effective pollen-dispensing mechanisms, requiring considerable buzzing effort (e.g. buzzing time) to release pollen gradually (Vallejo-Marín and Lundgren, 2026). Buzz-pollinated plants thus represent an excellent system to study the general phenomenon, in which key information about floral reward becomes available only after close interaction with the flower and considerable foraging effort by the pollinator. Buzz pollinators visiting plants with multiple flowers should encounter substantial uncertainty in pollen rewards within a single foraging bout, because both variation in pollen production and previous pollinator visitation should inject intra-individual variation in this non-replenishable floral reward.

Because pollen reward is visually cryptic and can be assessed only after deploying energetically costly behaviours, this sets up a foraging scenario in which incomplete information and high sampling costs are unavoidable. The ability of pollinators to detect and respond to intra-individual variation could therefore have important consequences for both foraging behaviour and the efficiency of pollen transfer. Buzz-pollinating bees have been shown to respond to lower pollen availability by reducing the length of their visits and the number of their buzzes (Buchmann and Cane, 1989), and perhaps even by adjusting the mechanical properties of the floral buzzes themselves (Switzer *et al.*, 2019). These changes in behaviour probably reflect dynamic assessment of the amount of pollen being removed through buzzing, because previous work has not detected consistent changes in volatile cues in anthers where pollen has been removed (Moore *et al.*, 2024), and visual cues, such as bite marks left in anthers (bee kisses), are useful only for subsequent visitors. In most bees, collected pollen is transported externally (e.g. in corbiculae or scopae), although some bees also ingest pollen during a floral visit (Danforth *et al.*, 2019; Mayberry *et al.*, 2024). Thus, bees could assess pollen returns by assessing externally loaded pollen loads or, potentially, via gustatory mechanisms (Thorp, 2000; Mayberry *et al.*, 2024). Although the mechanism behind dynamic pollen assessment during buzz pollination remains unknown, work to date suggests that bees possess the sensory capacity to detect pollen availability in visually cryptic pollen-rewarding plants.

To the extent that bees can detect pollen rewards in buzz-pollinated plants, differences in reward levels among flowers could influence visitation patterns. For example, they could affect how long a visitor stays in a flower and how much pollen it attempts to extract from it. Differences in pollen rewards could also affect whether a pollinator visits other flowers in the same plant (resulting in geitonogamous visitation) or moves to a different plant (exogamous visitation). According to optimal foraging theory (OFT), foraging decisions should maximize currencies that act as proxy of fitness, such as the long-term rate of energy gain when collecting nectar (Pyke, 2019; Pyke and Starr, 2021). In pollen collection, defining a currency is more difficult, but it appears to be a function of the amount of pollen collected relative to energetic costs (Rasheed and Harder, 1997*a*, *b*). Importantly, unlike nectar, pollen within a flower is a non-replenishable reward that cannot be regained once removed, adding variation among flowers with different histories of visitation. Variation in the quantity or quality of floral rewards introduces a risk of encountering low-quality flowers. In these conditions, pollinators might develop risk-sensitive strategies, such as risk aversion, and might favour more predictable floral resources over more variable ones (Harder and Real, 1987; Cartar and Dill, 1990; Biernaskie *et al.*, 2002; Novinger *et al.*, 2025). In plants with multiple flowers, an ecologically relevant level to define a resource patch is the individual or inflorescence. In non-clonal plants with discrete spatial distribution, pollinators need to travel shorter distances to move to flowers in the same inflorescence or individual than among plants (Charnov and Orians, 1973). The capacity of bees to detect and respond to intra-individual variation in pollen rewards could affect visitation and pollen transfer in buzz-pollinated plants. Pollen transfer is linked directly to both male (pollen export) and female (pollen receipt) fitness components (Minnaar *et al.*, 2019), and changes in pollinator behaviour in response to intra-individual variation can therefore affect natural selection on plant traits (Herrera, 2009; Paglia *et al.*, 2023).

Empirical evidence of risk-aversion behaviour in plant–pollinator systems comes almost exclusively from studies of artificial flowers mimicking nectar-producing systems. In both hummingbirds (*Selasphorous rufus*) and bumblebees (*Bombus flavifrons*), increased intra-individual variability in nectar volume triggers risk-averse behaviour that causes individuals to be more likely to leave inflorescences earlier despite similar average rewards (Biernaskie *et al.*, 2002). The authors suggest that this early departure might reduce geitonogamy, although this was not compared formally in their study. Interestingly, the reverse effect on visitation was observed in a recent study of increased nectar variation in artificial flowers visited by a different species of bumblebee (*Bombus impatiens*). Maldonado *et al.* (2023) found that more variable inflorescences received more visits than less variable inflorescences. Moreover, they did not find significant changes in the relative proportion of geitonogamous to exogamous visits, suggesting that although visitation per flower increases, it does not change the relative opportunities for outcross pollen import. The increased rate of visitation observed in their study contradicts the simple prediction that bees always adopt a risk-aversion strategy, as suggested by Biernaskie *et al.* (2002). Instead, the results from Maldonado *et al.* (2023) might suggest that bees respond disproportionally to high-rewarding flowers even at the risk of encountering low-rewarding ones in the same inflorescence. These studies illustrate that intra-individual variation can shape pollinator foraging decisions in multiple ways and might itself be a trait under selection. However, current empirical evidence of the effects of intra-individual variation on pollinator foraging is focused on nectar rewards, and more empirical studies are needed to generalize these findings and extend them to pollen-rewarding systems.

In the present study, we test how intra-individual variation in pollen availability influences pollinator behaviour using a system in which the amount of pollen is visually cryptic and where sampling pollen requires an energetically costly and specialized behaviour. Owing to the scarcity of studies explicitly quantifying intra-individual variation in pollen production in buzz-pollinated plants, we begin by estimating this in two species in the genus *Solanum* (Solanaceae). The magnitude of intra-individual variation sets the baseline of uncertainty in pollen rewards that pollinators will experience when foraging. We address three questions. First, what is the level of intra- versus inter-individual variation in the amount of pollen produced by flowers of two buzz-pollinated species (*S. sisymbriifolium* and *S. rostratum*)? Here, we also distinguish true biological variation from variation in pollen estimation attributable to measurement error (i.e. variation between experimental replicates of the same flower). Second, to what extent can bumblebees (*Bombus terrestris*) detect and respond to variation in pollen availability per flower? Based on previous studies, we expected that bees could indeed detect pollen availability and adjust their on-flower visitation behaviour. Specifically, we expected that flowers with more pollen should receive more or longer visits and more or longer floral buzzes than flowers with fewer pollen grains. Third, do behavioural responses at the flower level translate to overall differences in visitation in plants with increased intra-individual variation? We hypothesized that, to the extent that *B. terrestris* adopts a risk-aversion strategy, plants with higher intra-individual variation should receive fewer visits, and that bees should be more likely to move to a different plant (exogamous visit) than when visiting plants with lower intra-individual variation. If, instead, bees favour above-average flower rewards even at the risk of encountering lower-than-average ones, then we predict that plants with higher intra-individual variation should receive more visits, and that bees might be more likely to stay in the same individual in consecutive visits (i.e. higher geitonogamy). Our experimental approach to study pollinator behaviour uses natural flowers, where pollen access is manipulated to create natural and increased intra-individual variance treatments, and audio- and video-recorded visits by workers of *B. terrestris* in laboratory conditions, which allows a high-level description of foraging behaviour.

MATERIALS AND METHODS

Briefly, our general approach consisted of quantifying intra- and inter-individual variation in pollen production in two buzz-pollinated species (*Solanum sisymbriifolium* and *S. rostratum*) and assessing the response of buzz pollinators (*Bombus terrestris*) to experimentally induced variation in one species (*S. rostratum*). Below, we describe the study system and approach in detail.

Study species

To assess the level of intra- versus inter-individual variation in pollen production, we used two species of *Solanum* subg. *Leptostemonum* (Solanaceae): *S. sisymbriifolium* Lam. and *S. rostratum* Dunal. Like other species of *Solanum* and most buzz-pollinated plants, the flowers of these two species are nectarless, buzz pollinated and have five poricidal anthers. *Solanum sisymbriifolium* (sticky nightshade) is a herbaceous perennial, with white flowers and red fruits covered with prickles and glandular trichomes (Vorontsova and Knapp, 2014). It is native to South America, although it has been introduced to North America, Europe and Asia (Layek *et al.*, 2025). It has a cymose inflorescence composed of 6–50 actinomorphic, pentamerous flowers, 35–45 mm long, initially white and changing to pale purple, and five loosely held yellow anthers similar in both size and colour, opening through an apical pore (Vorontsova and Knapp, 2014). A single inflorescence of this species usually has two to five open flowers. When a bumblebee visits *S. sisymbriifolium*, it can grab a single or multiple anthers, bite the base of the anthers, and curl its body around the apical pore while producing vibrations with its thoracic muscles to extract pollen.

The second species studied here, *S. rostratum*, is an annual plant, with yellow flowers and fruits that dry inside the accrescent spiny calyx and remain attached to the plant after maturation. It is native to Mexico, and perhaps the southern USA, although it is now found throughout the Americas, Europe, Oceania and Asia, sometimes becoming invasive (Zhao *et al.*, 2013; Zhang and Lou, 2015; Vega-Polanco *et al.*, 2023). In both native and introduced ranges, it is visited by a diverse array of bee species, including *Bombus* (Bowers, 1975; Solís-Montero *et al.*, 2015; Zhang and Lou, 2015). The inflorescences are lateral cymes composed of 4–12 partly zygomorphic flowers with a rotate corolla (20–40 mm in diameter). In a single inflorescence, usually two to four flowers are open simultaneously, but a large plant can have multiple inflorescences in bloom at the same time. The flowers of *S. rostratum* are heterantherous (Todd, 1882), and a single flower produces two morphologically and functionally distinct types of stamens (Vallejo-Marín *et al.*, 2009; Barrett, 2021). One set of stamens is composed of four centrally positioned yellow anthers, and the second set consists of a single, larger anther that has a characteristic S-shape and sometimes differs in colour, becoming dark red or brown, especially towards the tip of the stamen (Vallejo-Marín *et al.*, 2009) (Fig. 1). All anthers open through a pair of small apical pores, each ~100–150 μm in diameter (Kemp and Vallejo-Marín, 2021). As suspected by Darwin (Darwin, 1881) and proposed by Fritz Müller (Müller, 1881) in a letter to his brother, the two stamen types have different functions (Vallejo-Marín *et al.*, 2009). The four shorter anthers serve as the focus of attention for visiting bee pollinators, which usually bite the

Fig. 1. Study system and schematic diagram of the experimental set-up. (A) Worker of *Bombus terrestris* approaching an unmanipulated flower of *Solanum rostratum* during a pre-experiment training phase. The four feeding anthers are presented centrally in the flower, whereas the larger, dark-coloured pollinating anther is displaced away from the centre to either the right or left of the axis of symmetry, and opposite to the style. Both right-hand (pictured) and left-hand side flowers are produced in the same individual. (B) Top view of the octagonal arrangement of the eight experimental plants inside a 60 cm × 60 cm × 60 cm flight arena. Each plant was placed at a vertex of the octagon, and plants displayed three flowers each. Both natural and increased intra-individual variation treatments were included in each trial and their position randomized in each trial. A dual microphone recorder was placed at the centre of the octagon to record floral buzzing events. A video camera was placed above the arena with a full view of all flowers to record visitation.

base of the anthers while vibrating their body and removing pollen from all anthers. These four stamens are thus called feeding anthers (FA). In contrast, the large anther is not as involved in pollinator attraction and is rarely buzzed, instead playing a disproportionate role in fertilization. Experimental work has shown the pollen grains of the large anther are more likely to reach the stigma of other flowers, in part owing to the fact that this specialized pollinating anther (PA) places pollen in a location on the body of the bee that is both harder to clean and more likely to contact the correspondingly placed stigma (Vallejo-Marín *et al.*, 2009). In *S. rostratum*, anther dimorphism within the flower is associated with a division of labour of stamens between feeding and fertilization functions, similarly to what has been shown in other heterantherous species (Luo *et al.*, 2009; Mesquita-Neto *et al.*, 2017; Papaj *et al.*, 2017; Dellinger *et al.*, 2019; Barrett, 2021). Although anther dimorphism might have other functional explanations in other taxa (Kay *et al.*, 2020; Waterman *et al.*, 2025), the two types of stamens in *S. rostratum* appear to have clearly different roles, owing, in part, to the way in which they interact with bee pollinators. Recent work has shown that floral morphological traits, including the size of the anthers, show considerable intra-individual variation, and that at least part of this variation is genetically based and heritable (Parraguirre *et al.*, 2025).

Plant material

For *S. rostratum*, we used field-collected seeds from two populations (San Miguel de Allende, Queretaro, accessions 10s71 and 10s74; and Dolores, Hidalgo, Mexico, accession 11s188). Seeds of *S. sisymbriifolium* were obtained from a commercial supplier (Chiltern Seeds, UK, accession 24-1180M). Seeds were planted on 8 May 2024, and germinated after treatment with 2000 ppm of gibberellic acid (GA3, Sigma-Aldrich, Dorset, UK) using the protocol described by Vallejo-Marín *et al.* (2014). After germination, seedlings were planted in 2–3 L pots with 3:1 growing medium compost (SWHorto, Hammenhög, Sweden):expanded clay lightweight aggregate (Leca, Linköping, Sweden) under a 16 h:8 h cycle of light:dark photoperiod and 20 °C:16 °C temperature. Liquid fertilizer was applied weekly.

Estimating variation in pollen production

To estimate variation in pollen production, we sampled ten flowers from 21 individuals of *S. sisymbriifolium* ($n = 210$ flowers; one accession) and six flowers from each of 12 individuals of *S. rostratum* ($n = 72$ flowers; six from accession 10s71, and three each from accessions 10s74 and 11s188). We chose recently opened flowers and sampled different inflorescences in each plant as available. Thus, our samples included flowers at different positions in the inflorescence and from multiple inflorescences per individual. For each species, flowers were sampled over a period of ~1–3 days.

The anthers of each flower were collected and stored in 1.5 mL microcentrifuge tubes with 1000 μL of 70 % ethanol and refrigerated until pollen counting. In *S. sisymbriifolium*, all five anthers were collected in a single tube. In *S. rostratum*, we collected the feeding and pollinating anthers separately. Pollen was extracted from the stored anthers using an Elmasonic S40H ultrasonic cleaner (Elma Schmidbauer GmbH, Singen, Germany) running for 5 min for *S. sisymbriifolium* and 30 min for *S. rostratum*. Pollen counting and volumetric size estimation was performed using a particle counter (Multisizer 4e, Beckman Coulter Life Sciences, Indiana, USA). Briefly, a known volume of the pollen sample was placed in 10 mL of Isoton II electrolyte solution. The volume of pollen sample used was chosen to yield a concentration of particles between 3 and 7 %, as recommended in the manual for the instrument. To assess the measurement error in estimating the number of pollen grains using the particle counter, we conducted pollen counts in quadruplicate for *S. sisymbriifolium*. At least four replicates were performed for each sample of this species (210 × 4; ~840 samples in total). Pollen grain size (diameter) was estimated from the volumetric data generated by the particle counter by assuming a spherical pollen shape. We measured particle sizes using 400 bins between 10 and 40 µm (*S. sisymbriifolium*) or between 10 and 28 µm (*S. rostratum*). Following preliminary analysis of pollen grain size variation, particles between 15 and 33 µm (*S. sisymbriifolium*) or between 16 and 22 µm (*S. rostratum*) were classified as pollen grains. Pollen grain size had a unimodal distribution for *S. rostratum* but was bimodal for *S. sisymbriifolium* owing to the presence of smaller inviable pollen (Kearns and Inouye, 1993; Layek *et al.*, 2025). Thus, for *S. sisymbriifolium*, pollen viability was estimated for each sample by calculating the proportion of pollen grains corresponding to viable pollen size (diameter >22.5 µm) divided by the total number of pollen grains counted.

Creating intra-individual variation experimentally

To assess the response of bumblebees to intra-individual variation in pollen availability, we used *S. rostratum*. We created an inflorescence by placing together three flowers (from the same inflorescence if possible) in a 50 mL tube filled with water-soaked floral foam (Oasis, etc.). The poricidal anthers of buzz-pollinated flowers prevent the option of adding pollen to anthers without destroying them. Thus, we artificially generated differences in intra-individual variation in pollen availability by differentially reducing the amount of available pollen among flowers of a single inflorescence. The pollen availability in each inflorescence was manipulated by using water-based vinyl glue (PVA) to seal the apical pore of some of the anthers, thus effectively preventing pollen from being expelled from the blocked anthers. PVA is a compound that becomes transparent when dried and does not affect the behaviour of pollinators (Buchmann and Cane, 1989; Vallejo-Marín *et al.*, 2009). Using this approach, we created two treatments: increased intra-individual variation and natural intra-individual variation. In the increased intra-individual variation treatment, we blocked the pores all four feeding anthers of one flower, two of four feeding anthers of the second flower, and none of the feeding anthers of the third flower. In the natural intra-individual variation treatment, we blocked two of the four feeding anthers of all three flowers. Thus, both the increased and natural treatments had, on average, half of the feeding anthers blocked, but the increased treatment had a higher coefficient of variation (CV) than the natural treatment (ΔCV = 81.6 % higher). In both treatments, the pollinating anther was also blocked. All non-blocked anthers also received a small drop of glue on the side of the anther as a sham treatment.

Bumblebees

We used two colonies of *Bombus terrestris* L. (Biobest, Belgium). *Bombus terrestris* is a generalist bumblebee that readily buzz pollinates flowers in both natural and laboratory conditions. It has been used for laboratory experiments on bee behaviour for four decades (Ghisbain *et al.*, 2025) and, more recently, as a model for buzz-pollination studies (Vallejo-Marín, 2022). Although not native to the distribution of *S. rostratum* and *S. sisymbriifolium*, this medium-sized bumblebee resembles the size and behaviour of other native bumblebee visitors (Bowers, 1975; Solís-Montero *et al.*, 2015) and quickly learns to manipulate flowers of buzz-pollinated plants (De Luca *et al.*, 2013; Morgan *et al.*, 2016). For our experiment, bumblebee colonies were provided with *ad libitum* access to artificial ‘nectar’ (Biobest) within their colony. In addition, we provided a 2 M sucrose solution in feeders outside the colony to encourage foraging. Twice a week, the bumblebees received ~10 g of a pollen mass composed of honeybee-collected pollen, pulverized and mixed with the sucrose solution.

Responses of bumblebees to intra-individual variation in pollen availability

We presented arrays of eight inflorescences to individual bumblebees in a flight arena (60 cm × 60 cm × 60 cm) illuminated with an LED panel. For a detailed description of the flight arena, see Vallejo-Marín and Lundgren (2026). In each array, we had four natural variation and four increased variation inflorescences. For each trial, we randomly placed inflorescences in the vertices of an octagon at the centre of the arena to minimize any positional effects (Fig. 1) and allowed a single bumblebee to enter the arena. A dual microphone recorder (Zoom H4n Pro, Sound Service GmbH, Berlin, Germany) was placed at the centre of the octagon, with microphones placed at a 45° angle relative to the ground to register flight and buzz-pollination sounds. The audio was recorded in stereo at a sampling frequency of 44.1 kHz and stored in uncompressed WAV format. In addition, we video recorded each trial using an ACE2 5MP camera and f6mm lens (Basler AG, Ahrensburg, Germany) placed at the top of the box, providing a bird’s-eye view capable of capturing activity on all eight plants simultaneously.

Prior to the experiments, the bumblebee colonies were trained in an identical replica of the experimental arena (training arena). This training consisted of replicating the experimental arena design, with the same octagonal arrangement of plants, a microphone in the centre and a camera at the top, to accustom the colony to this arrangement. During this phase, the bumblebees could freely enter and exit the flight box, allowing them not only to become accustomed to the experimental environment but also to learn how to manipulate and vibrate the *S. rostratum* flowers. This conditioning training phase was repeated through the duration of the experiment but never coincided with the experimental trial days.

For each trial, a single bumblebee was allowed to visit the floral array freely. The entire trial was audio and video recorded, and an observer also recorded floral visitation. A trial was ended after ~20 min or when the bee failed to visit a flower for >5 min, whichever occurred first. A total of 30 trials (each with a different bee) were conducted: 16 from colony 1 and 14 from colony 2. We recorded only visits with at least one floral buzzing, because the bee only very rarely landed and departed without buzzing. All visits with buzzing were recorded even if the bee afterwards remained inactive for a while on the flower, which also occurred rarely, before leaving to the next flower.

Bee behaviour

The following variables were recorded: visit duration (time elapsed between the moment when the bumblebee landed on a flower and when it departed); number and duration of buzzes; and total bout length (time interval between the first and the last buzz within a foraging bout). All duration data were obtained from the combined audio and video recordings and benchmarked with our observations. Videos were analysed using BORIS (Behavioural Observation Research Interactive Software; Friard and Gamba, 2016). Visiting events were initially extracted from the video, then matched with the buzzes identified in the audio analysis (see below), thereby creating a temporal 'window' of visits, within which each buzz was assessed. To ensure that all visits were properly accounted for, video recordings were cross-checked against the real-time notes taken during each foraging bout.

Audio analysis. We used the audio recordings as a mechanism to time the duration of floral buzzing events precisely (Vallejo-Marín and Lundgren, 2026). Audio files were analysed in R v.4.5.2 (R Core Team, 2025), with the packages *seewave* (Sueur *et al.*, 2008) and *tuneR* (Ligges *et al.*, 2023). Audio files were imported into R and filtered using a 150 Hz high-pass filter using *seewave:fir* to reduce background noise. This cut-off frequency was chosen after a preliminary inspection of the spectrograms in Audacity v.3.0.2 (Audacity, 2025) and the statistical environment, where this range was identified as non-biological (ventilation fan) noise. Next, each audio file was segmented into 30 s blocks to facilitate analysis and to exclude segments containing noise or temporary disturbances, such as flights too close to the microphone that clipped the signal or masked floral buzzing.

We developed a semi-automatic method for identifying and distinguishing floral buzzes and flight buzzes in the audio recordings. Although floral buzzes have a higher fundamental frequency than flight buzzes (1.2 to 2 times higher; De Luca *et al.*, 2019), background noise complicates automatic identification based on fundamental frequency alone. Therefore, we took advantage of the differences in harmonic structure between flight and floral buzzes. Floral buzzes have more and higher harmonics in comparison to flight sounds (Buchmann *et al.*, 1977; Pritchard and Vallejo-Marín, 2020; Arvelos *et al.*, 2025). For each buzz event, we calculated the relative energy contained in the higher frequencies (1000–10 000 Hz) using *seewave:meanspec* and divided it by the energy in the lower frequencies (<1000 Hz). The ratio, hereafter 'harmonic ratio', provides a quantitative measure to distinguish flight and floral buzzes. Floral buzzes have higher harmonic ratios than flight sounds. To select a threshold value for the harmonic ratio, we plotted the natural logarithm of the buzz ratio for each trial, which yielded a clearly bimodal distribution. Based on this plot, we manually selected a threshold value ([e.g. $\log_e(5)$] to separate each modal peak, corresponding to either a floral buzz or a flight buzz. The histograms and threshold values for each trial are presented in the Supplementary Data (Fig. S1). To confirm the accuracy of our semi-automatic harmonic ratio classification, we selected three trials and manually classified buzzing events as pollination, flight or undefined, by playing the audio in Audacity and visually examining the corresponding spectrograms. The automatic classification correctly identified pollination buzzes in 97.11, 95.00 and 96.62 % of the cases for each of the three trials, respectively.

In our experimental design, the natural variation treatment is linked to always having two blocked anthers in all three flowers, and thus we classified flower treatments into four types: (A) flowers with no blocked anthers; (B) flowers with two blocked feeding anthers; (C) flowers with all four feeding anthers blocked (these first three flower treatments occurred in the increased variation inflorescence treatment); and (D) flowers with two blocked anthers in the natural variation inflorescence treatment. If there is an interaction between plant and flower treatment, we expect to see differences between flower classes B and D.

Hierarchical analysis of foraging behaviour. Because pollinator visitation involves both immediate behavioural decisions during a floral visit and cumulative interactions over multiple visits to a flower and/or multiple flowers in a plant, we aggregated variables at the visit, flower and plant levels. Visit-level measurements quantify the immediate behaviours expressed during each flower visit and thus provide a mechanistic insight into how bees allocate efforts within a single decision event. Flower-level measurements integrate behaviours across all visits to a flower and enable us to capture the total visitation and buzzing effort to a floral phenotype. These variables are related to pollen export and deposition, informing us of the functional consequences of pollinator behaviour for the plant. Finally, plant-level measurements summarize the cumulative foraging activity of bees across all flowers in a plant. This provides insights into how plant-level traits, such as the level of intra-individual variation in pollen availability, affect the overall pollinator visitation environment. By measuring and analysing bee behaviour at these three nested scales, we can capture both the fine-scale mechanistic decisions of visiting bees and their emerging functional effects.

The hierarchical behavioural and functional variables we analysed are described next and shown in Table 1.

At the visit level, we focused on the instantaneous buzzing effort by measuring the duration of individual buzzes. For each visit, we calculated the weighted mean of buzz duration (VDB) by dividing total buzzing time per visit by the number of buzzes. In addition, we also classified each visit depending on their pollen export potential: geitonogamous visits (visits towards a flower in the same individual; GV) and exogamous visits (visits towards a flower in a different individual; EV).

Table 1. Variables used to describe the pattern of visitation.

Level	Variable	Abbreviation	Description and units
(1) Per visit	Duration of an individual buzz	VDB	Mean duration of an individual buzz (s)
	Geitonogamous visits	GV	Visits to a flower in the same individual
	Exogamous visits	EV	Visits to a flower in a different individual
(2) Per flower	Total number of visits	FVN	Number of visits to a given flower
	Total visit length	FVL	Sum of visit length across all visits to a given flower (s)
	Total buzzing time	FTB	Sum of buzzing time across all buzzes of a given flower (s)
	Proportion of exogamous visits	FEV	Proportion of exogamous visits [EV/(EV + GV)]
(3) Per plant	Total number of buzzes	PNB	Sum of all buzzes to a given plant
	Number of visits	PNV	Number of visits to a given plant
	Total visit length	PVL	Sum of all visit length to a plant (s)
	Total buzzing time	PTB	Sum of all buzzes to a given plant (s)
	Proportion of exogamous visits	PEV	Proportion of exogamous visits [EV/(EV + GV)]

Geitonogamous visits can lead to transfer of self-pollen, whereas exogamous visits can represent instances of outcross pollen export. We chose to classify visit quality focusing on pollen export rather than pollen receipt (Maldonado *et al.*, 2023), because we expected that the characteristics of the flower being visited would determine whether the bee stays on the same plant (leading to a geitonogamous visit) or moves to the next one (leading to an exogamous visit).

At the flower level, we aggregated visit data for each flower by calculating the sum of the visit lengths across all visits to a given flower (FVL), the sum of time spent buzzing in that flower (FTB) and the number of visits to that flower (FVN). We also calculated the proportion of exogamous visits [exogamous/(exogamous + geitonogamous); FEV].

At the plant level, for each plant we aggregated visit data and estimated the total number of buzzes (PNB), total number of visits (PVN), total visitation time (PVL) and total time spent buzzing (PTB) for each plant. We also calculated the proportion of exogamous visits (PEV).

Statistical analyses

Pollen production. To partition the variance in pollen production within and between individuals, we fitted linear mixed-effects models using *lme4* (Bates *et al.*, 2014). Each species was analysed in a separate model. For *S. sisymbriifolium* we fitted the model:

$$pollen_{ijk}^{sisymb} = \mu + u_i + v_{ij} + \varepsilon_{ijk},$$

where *pollen* is the number of estimated pollen grains in individual i, flower j, replicate k, μ is the intercept, u_i and v_{ij} are the random effects associated with individual and flower, respectively, and ε_{ijk} is the residual error. The random effects and error are modelled as:

$$u_i \sim \mathrm{N}(0, \sigma^2_{\text{individual}}),$$

$$v_i \sim \mathrm{N}(0, \sigma^2_{\text{flower}}),$$

$$u_i \sim \mathrm{N}(0, \sigma^2_{\text{residual}}).$$

Where N denotes the normal distribution. Given that we had multiple replicates for each flower in *S. sisymbriifolium*, we calculated the intraclass correlation (ICC) as a measure of the biological variation in pollen production after removing measurement error: $\mathrm{ICC}_{\text{flower}} = \frac{\sigma^2_{\text{flower}}}{\sigma^2_{\text{flower}} + \sigma^2_{\text{residual}}}$.

For *S. rostratum*, we combined pollen from feeding and pollinating anthers into one variable of total pollen per flower. Given that we measured only one replicate per flower for this species, the fitted model was:

$$pollen_{ijk}^{rostratum} = \mu + u_i + \varepsilon_{ijk},$$

where the residual error contains both variation between flowers of the same individual and measurement error. As a standardized measure of intra-individual variation (Herrera, 2009), we also calculated the coefficient of variation associated with flowers within individuals as: $\mathrm{CV} = 100 \times (S_{\text{flower}}/\bar{x})$, where the mean per individual ($\bar{x}$) and standard deviation among flowers of the same individual (S_{flower}) were calculated from the single (*S. rostratum*) or averaged (*S. sisymbriifolium*) values for each flower. Finally, we estimated Pearson's product–moment correlations between the CV and either pollen production or the proportion of viable pollen.

Hierarchical statistical models of behaviour. For timed variables (VDB, FVL, FTB, PVL and PTB), we fitted a generalized linear mixed-effects model (GLMM) with a Gamma distribution and a log-link function using the package *glmmTMB* (Brooks *et al.*, 2017) in R. A Gamma distribution was selected to account for the right skew of duration data. For visit- and flower-level data, the dispersion parameter was allowed to vary among flower treatments. For visit-level analyses (VDB), flower treatment was used as a fixed effect, and flower, plant and bee identity (which was equivalent to trial identity because each bee was used once) were used as random effects (random intercepts). For flower-level analyses (FVL and FTB), flower treatment was used as a fixed effect, and plant and bee identity were included as random effects. For plant-level analyses (PVL, PDB and PTB), plant treatment was used as a fixed effect, and only bee identity was included as a random effect. For count variables (FVN, PNB and PNV), we used a GLMM with a negative binomial distribution using the *nbinom2* option in *glmmTMB* and the same fixed and random effect structure as above. Model assumptions were checked using *DHARMa* (Hartig, 2021). Marginal means were calculated with *emmeans* (Lenth, 2021), and Tukey's tests were used to assess pairwise differences between flower types. All analyses were performed in R v.4.5.2 (R Core Team, 2025).

RESULTS

Intra-individual variation in pollen production

Both plant species showed considerable intra-individual variation in pollen production among flowers. In *S. sisymbriifolium*, a flower produced an average of 417 796 ± 9880 (mean ± s.e.; $n = 210$ flowers) pollen grains (Supplementary Data Fig. S2). Intra-individual variation explained 46.5 % of the variation in the number of pollen grains ($\text{ICC}_{\text{flower}}$; $n = 10$ flowers measured per individual on average), and among-individual variation explained a slightly higher proportion of the variance (50.8 %, $n = 21$ individuals). In contrast, we found that variation between replicate measurements of the same flower explained a relatively small fraction of the variation (2.6 %, $n = 4.35$ replicates per flower). The intra-individual CV in pollen production for *S. sisymbriifolium* (using mean values per flower and calculated for each individual) was 22.99 ± 1.80 % ($n = 21$). We found no correlation between the CV in pollen production and either the total amount of pollen per flower (Pearson's $r = -0.288$, $n = 21$, $P = 0.205$) or the proportion of viable pollen ($r = -0.094$, $n = 21$, $P = 0.685$). The mode pollen size for viable pollen 26.98 µm and for inviable pollen grains 18.024 µm ($n = 3\,145\,930$ pollen grains).

In *S. rostratum*, a flower produced on average 843 507 ± 20 923 pollen grains (mean ± s.e.; $n = 71$ flowers) (Supplementary Data Fig. S2). Of these, the four feeding anthers contained collectively 34.8 % of the pollen grains in a flower, and the single pollinating anther produced 65.2 % (see Supplementary Data Fig. S2 for a breakdown of relative allocation in each flower). Thus, on a per anther basis, the pollinating anther produced nearly 7.5 times more pollen than a feeding anther. In contrast to *S. sisymbriifolium*, pollen grain size was unimodal, suggesting a negligible amount of inviable pollen in the studied plants of *S. rostratum*. The average pollen grain diameter in the feeding anthers of *S. rostratum* was highly similar (19.02 ± 0.65 µm, $n = 336\,618$ pollen grains) to the size of pollen in the pollinating anther (18.86 ± 0.69 µm, $n = 628\,038$). Among-individual variation explained 41.44 % of the variance in pollen production per flower. The remaining 58.56 % of the variance in pollen production can be attributed to the combined effects of intra-individual variation and measurement error. The intra-individual CV for total pollen per flower was 14.32 ± 1.63 % ($n = 12$). We did not detect a correlation between CV and the number of pollen grains ($r = -0.359$, $n = 12$, $P = 0.251$).

Behavioural responses to pollen availability

Over ∼9 h of recorded observations across the experiment, we captured 9732 individual buzzes, distributed in 2583 floral visits by 30 individual bees (16 from colony 1 and 14 from colony 2; Fig. 2). Throughout the experiment, 97.5 % (234/240) of plants were visited at least once, and 85.4 % (615/720) of flowers received at least one visit.

Visit-level response. Bees applied significantly shorter buzzes to flowers in which all anthers were blocked (flower treatment C) compared with all other flowers (Fig. 3; Table 2). Surprisingly, buzz duration did not increase significantly for flowers with no blocked feeding anthers (treatment A) compared with the duration of buzzes observed in flowers with two blocked feeding anthers (treatments B and D). Bees also applied buzzes of similar duration to flowers with two blocked anthers, regardless of whether they occurred in the increased (treatment B) or natural (treatment D) variation plants (Fig. 3; Table 2). The mean individual buzz duration across the experiment was 0.573 ± 0.007 s ($n = 2583$ visits; median = 0.483 s). A bee produced 3.77 ± 0.106 buzzes in the average visit, which lasted 7.59 ± 0.346 s from landing to departing from a flower.

Flower-level responses. Flower treatment significantly influenced the pattern of visitation of buzz-pollinating bumblebees. Bees conducted fewer visits and spent less time in flowers in which all feeding anthers were blocked (treatment C; Fig. 4). The number of visits was similar across flowers with either no blocked feeding anthers or with only two feeding anthers blocked, regardless of whether they occurred in the natural or increased intra-individual variation plants (Fig. 4A). In contrast, we found that the total time spent visiting flowers with no blocked feeding anthers (treatment A) was higher than the visit length to flowers with two blocked anthers (Fig. 4B). The buzzing time spent by a bee on a flower also depended on the pollen availability treatment (Fig. 4C). Bees buzzed for less time on flowers with all feeding anthers blocked. Flowers with only two blocked anthers were buzzed for the same amount of time regardless of whether they occurred in the natural or increased variation plants. However, flowers with no blocked anthers were buzzed for longer than flowers with two blocked anthers in the natural variation plants (Fig. 4C).

We detected a significant effect of flower treatment on the probability that a bee moves to a flower in a different plant (exogamous visit) rather than to a flower in the same individual (geitonogamous visit) (Fig. 4D). The proportion of exogamous visits was significantly lower after visiting a flower in which no feeding anthers were blocked, in comparison to all other flower treatments. In contrast, whether a flower had two or all feeding anthers blocked did not change the probability that a bee moves to a different plant (Fig. 4D). In general, visiting a flower with at least some of the feeding anthers blocked was associated with a higher probability to move to a different plant.

Plant-level responses. In contrast to the responses observed at the visit and flower levels, we did not detect any significant effect of plant-level treatments (natural versus

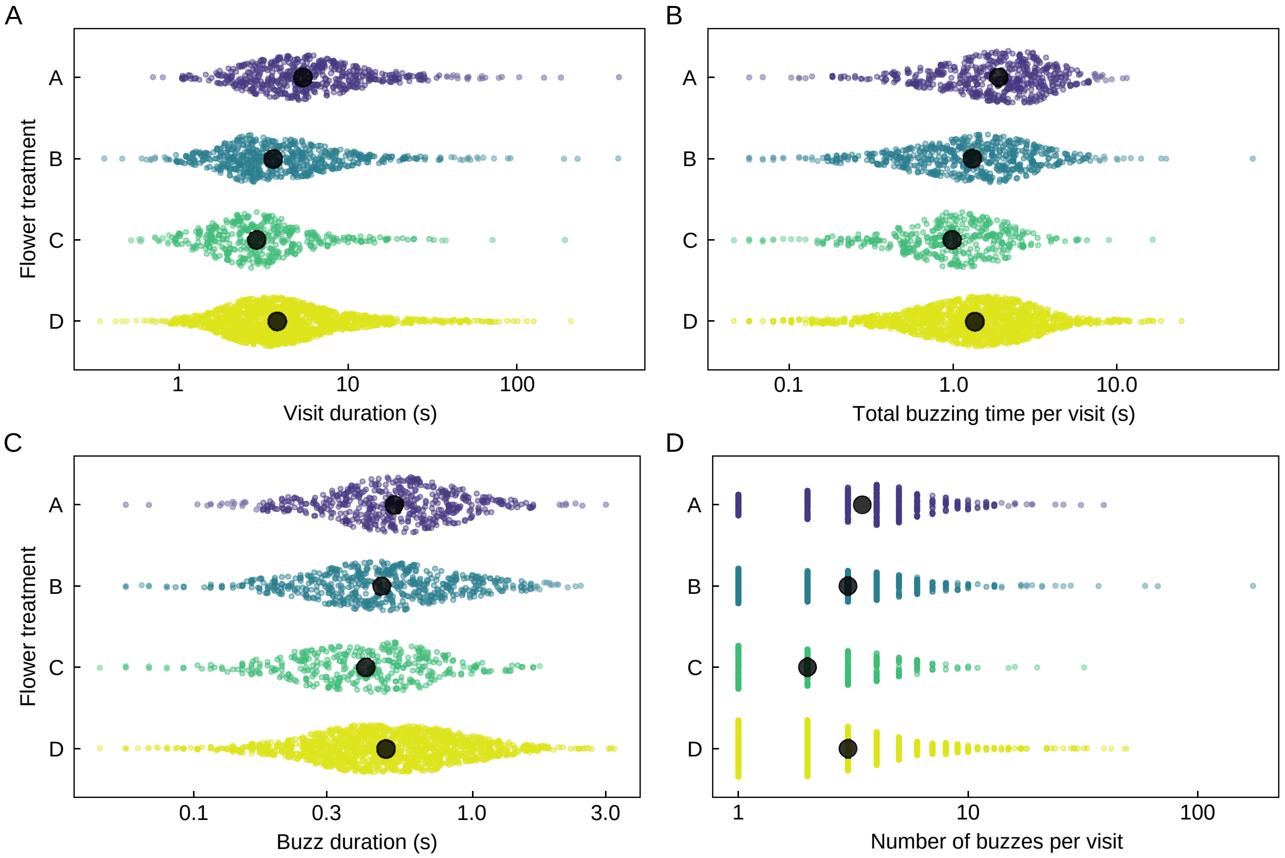

FIG. 2. Empirical distribution of behavioural traits expressed by 30 *Bombus terrestris* workers visiting experimental *Solanum rostratum* flowers differing in pollen availability. Each point represents a single visit ($N = 2583$). (A) Visit duration. (B) Total buzzing time per visit. (C) Individual buzz duration. (D) Number of buzzes per visit. Flower treatments: A = no blocked feeding anthers; B and D = two blocked feeding anthers; and C = all four feeding anthers blocked. All flowers had the pollinating anther also blocked. Flower treatments A–C occurred in natural intra-individual variation plants, and treatment D in increased intra-individual variation plants. Larger black points indicate median values. Notice the $\log_{10}$ scale.

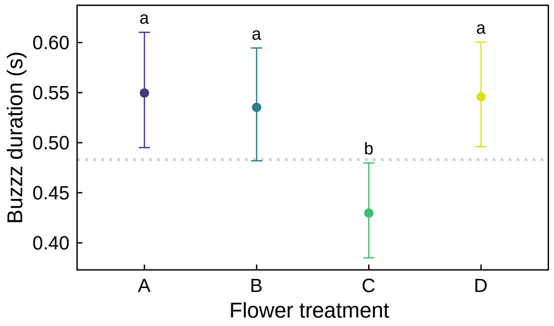

FIG. 3. Visit-level behavioural response of buzz-pollinating *Bombus terrestris* (measured as the duration of an individual buzz) to encountering flowers with different pollen availability. The symbols represent estimated marginal means derived from a mixed-effects model, and error bars indicate 95 % confidence intervals, fitted using a gamma distribution and log-link and back transformed to the original units. The dotted line represents the median buzz duration. Flower treatments are as follows: A = no blocked feeding anthers; B and D = two blocked feeding anthers; and C = all four feeding anthers blocked. All flowers had the pollinating anther also blocked. Flower treatments A–C occurred in natural intra-individual variation plants, and treatment D in increased intra-individual variation plants. Different small-cap letters denote statistically significant differences assessed with pairwise Tukey's tests. $n = 2583$ visits, summarizing 9732 individual pollination buzzes by 30 bees.

increased intra-individual variation) in any of the measured behavioural traits. Bees performed similar number of visits and number of buzzes across treatments (Table 2). Total visit length and total buzzing time did not differ statistically between plant-level treatments, although the increased intra-individual variation treatment had marginally significantly longer visitation time (Table 2). Finally, the proportion of exogamous visits was similar in each treatment, with both types of plants receiving more exogamous than geitonogamous transitions, on average (Table 2).

DISCUSSION

Our study yields three main results: (1) there is substantial intra-individual variation in pollen rewards; (2) bees can detect and respond to pollen availability at the flower level even in visually cryptic systems that require considerable energetic expenditure to assess reward level; and (3) bees do not exploit plant-level information on intra-individual variability of pollen rewards to shape their foraging patterns. We show substantial intra-individual variation in primary pollen production (46–58 % of the total variance) in two nectarless plants, in which pollen is the only reward available to

Table 2. Effect of intra-individual variation of pollen availability in the flowers of *Solanum rostratum* on the buzz pollination behaviour of *Bombus terrestris*. (A) Visit-level response to differences in pollen available per flower. (B) Flower-level response to differences in pollen available per flower. (C) Plant-level responses to intra-individual variation in pollen availability among flowers. For definitions of response variables, see Table 1. The *P*-value column indicates the significance of the explanatory variable as assessed with a likelihood ratio test.

Level	Response variable	Explanatory variable		Estimate	s.e.	*P*-value
(1) Visit-level response	Buzz duration (VBD)	Flower treatment	Intercept (Treatment A)	−0.598	0.053	<0.001
			Treatment B	−0.026	0.040	
			Treatment C	−0.246	0.043	
			Treatment D	−0.007	0.038	
(2) Flower-level response	Visit number (FVN)	Flower treatment	Intercept (Treatment A)	1.515	0.073	<0.001
			Treatment B	−0.038	0.073	
			Treatment C	−0.352	0.080	
			Treatment D	−0.165	0.067	
	Visit length (FVL)	Flower treatment	Intercept (Treatment A)	3.801	0.103	<0.001
			Treatment B	−0.345	0.117	
			Treatment C	−1.165	0.121	
			Treatment D	−0.587	0.121	
	Cumulative buzzing time (FTB)	Flower treatment	Intercept (Treatment A)	2.347	0.095	<0.001
			Treatment B	−0.240	0.096	
			Treatment C	−0.998	0.107	
			Treatment D	−0.349	0.091	
	Proportion of exogamous visits (FEV)	Flower treatment	Intercept (Treatment A)	−0.043	0.117	<0.001
			Treatment B	0.555	0.146	
			Treatment C	0.807	0.162	
			Treatment D	0.538	0.133	
(3) Plant-level response	Number of visits (PNV)	Plant treatment	Intercept (natural intra-individual variation)	2.362	0.072	0.513
			Increased intra-individual variation	−0.036	0.055	
	Visit length (PVL)	Plant treatment	Intercept (Natural intra-individual variation)	4.520	0.072	0.058
			Increased intra-individual variation	−0.194	0.103	
	Total time spent buzzing (PTB)	Plant treatment	Intercept (Natural intra-individual variation)	3.007	0.079	0.645
			Increased intra-individual variation	−0.036	0.079	
	Total number of buzzes (PNB)	Plant treatment	Intercept (Natural intra-individual variation)	3.721	0.070	0.317
			Increased intra-individual variation	−0.070	0.070	
	Fraction of exogamous visits (PEV)	Plant treatment	Intercept (Natural intra-individual variation)	0.378	0.074	0.232
			Increased intra-individual variation	0.120	0.101	

attract pollinators. In this type of system, the dual role of pollen as both the vehicle for male gametes and the 'food' for pollinators makes intra-individual variation of potential importance in shaping both patterns of visitation and, eventually, plant fitness. We have shown via experimentation that bee pollinators can assess pollen rewards dynamically, as shown by their behavioural responses to encountering morphologically identical, but rewardless, flowers mixed with average and higher-than-average flowers in the same inflorescence. The responses by the pollinators to this local heterogeneity in resource availability range from fine behavioural tunning when encountering a lower-than-average floral reward (changes in the length of individual buzzes measured in hundreds of milliseconds) to the plant-level response revealed when a bee becomes more likely to re-visit a plant after encountering a higher-than-average reward

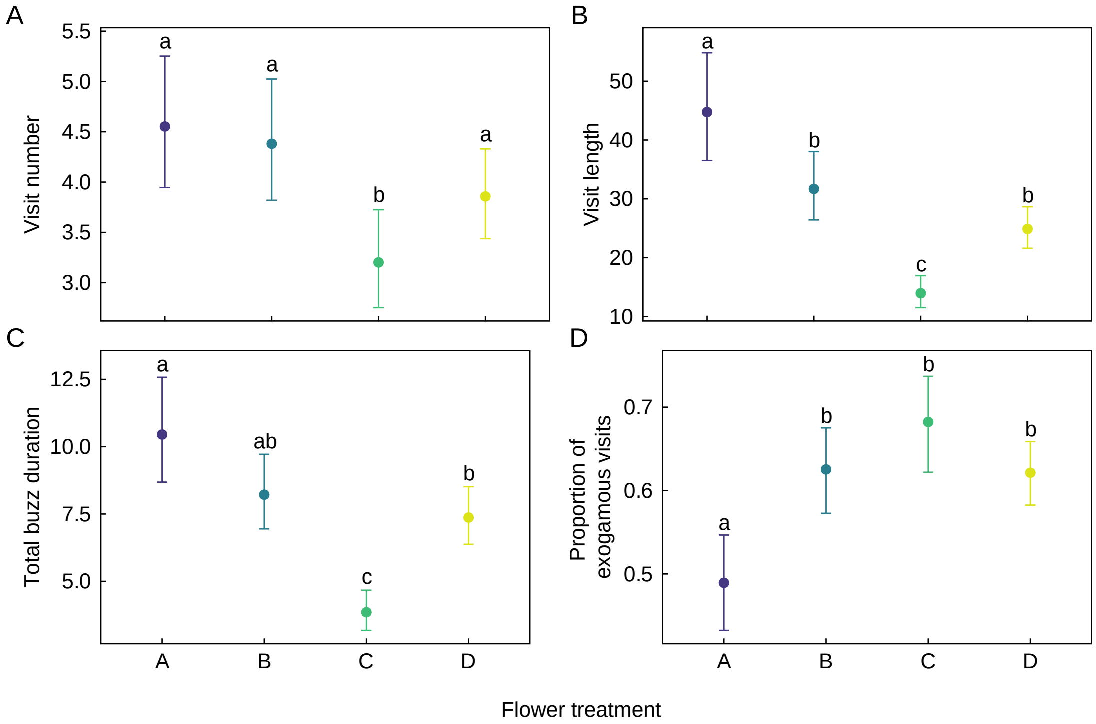

Fig. 4. Flower-level behavioural response of *Bombus terrestris* to different levels of pollen availability. The symbols represent estimated marginal means derived from the fitted mixed-effects models, and error bars indicate 95 % confidence intervals, fitted using a negative binomial and log link (panel A; visit number), a gamma distribution and log-link (panels B and C; visit length and total buzz duration) or a beta-binomial with logit link (panel D; proportion of exogamous visits), and back transformed to the original units. Flower treatments are as follows: A = no blocked feeding anthers; B and D = two blocked feeding anthers; and C = all four feeding anthers blocked. All flowers had the pollinating anther also blocked. Flower treatments A–C occurred in natural intra-individual variation plants, and treatment D in increased intra-individual variation plants. Different small-cap letters denote statistically significant differences assessed with pairwise Tukey's tests. $n = 615$ flowers across 234 plants, visited by 30 bees.

flower. Yet, bees do not use information gathered from multiple flowers of the same plant to inform their foraging decisions and are equally likely to visit with natural or increased levels of intra-individual variation in pollen rewards. We hypothesize that the rules of foraging behaviour described here might help plants to buffer against the fitness effects (negative or positive) of intra-individual variation. We thus predict that, in ecological scenarios like those tested here, natural selection on intra-individual variation in pollen rewards should be weak, even when selection on mean pollen production per flower could be strong. Whether this pattern extends to natural populations, larger floral displays and other pollen-foraging pollinators remains to be determined.

Intra-individual variation in pollen production

Our results suggest that intra-individual variation contributes significantly to the variation in pollen presentation (pollen packing) of individual plants (Harder and Thomson, 1989), and that plants might not regulate primary pollen production tightly. Within-plant variation in pollen production could be a by-product of developmental instability caused by either intrinsic or environmental factors affecting normal development in floral buds (Polack, 2003; Parraguirre *et al.*, 2025). The variation could also be predictable, for example if the number of pollen grains is associated with flower size and if flower size changes with position in the inflorescence (Diggle and Miller, 2004; Wang *et al.*, 2022). In *Solanum carolinense*, however, the number of pollen grains does not vary predictably with position along the inflorescence (Vallejo-Marín and Rausher, 2007). In systems similar to those studied here, inferences about pollen production in an individual obtained from a single flower should be taken with great caution. Nevertheless, the high intra-individual variation observed here implies that pollinators sampling multiple flowers per plant should find significant uncertainty in reward level, potentially influencing their foraging decisions even when they are able to assess the pollen reward of individual flowers.

Bees assess cryptic pollen rewards even in high intra-individual variation contexts

Evaluation of pollen rewards in buzz-pollinated flowers poses a challenge to visiting pollinators because pollen removal does not necessarily affect the visual appearance of the anther, which will usually retain its appearance even when completely emptied of pollen (Vogel, 1978). However, previous work has shown that bees change their visitation and buzzing behaviour in response to pollen availability (Buchmann and Cane, 1989; Harder, 1990; Nunes-Silva *et al.*, 2013), and we extend this by showing that bees respond to flower-level variation in concealed pollen rewards even in the context of high intra-individual variation. We observed that bees made shorter, fewer buzzes and shorter, fewer visits when encountering pollen-less flowers. Bees on buzz-pollinated *Primula conjugens* (Harder, 1990) shorten their buzzes when visiting depleted flowers. In flowers of *Solanum lycopersicum*, where the first visit removes most pollen grains, bumblebees shorten their visit length but not the mechanical properties of their buzzes in subsequent visits (Nunes-Silva *et al.*, 2013). Blocking pollen access or artificially removing pollen from flowers of *Solanum elaeagnifolium* also reduce the handling time, number of buzzes and number of grooming events (Buchmann and Cane, 1989). Likewise, blocking the feeding anthers of heterantherous *S. rostratum* results in shorter visits (Vallejo-Marín *et al.*, 2009). Furthermore, in non-buzz-pollinated plants, such as *Mimulus*, bumblebees can even respond to pollen quality and show differential visitations to plants with different proportions of inviable (empty) pollen (Robertson *et al.*, 1999). But bees do not only respond to reduced pollen rewards. We found that above-average-rewarding flowers received longer visits and were buzzed for longer than other flowers with average-level rewards. Thus, bees also respond, in part, to increased reward levels. However, our experiment suggests that there is perhaps a fitness cost of producing highly rewarding flowers, because bees were more likely to re-visit a plant after encountering these flowers, and this could result in increasing rates of geitonogamous (self) pollen import and reduced pollen export to other individuals. Altogether, our study shows that flower-level assessment of pollen rewards occurs even in the presence of high intra-individual pollen variation (>80 % higher than the natural CV).

The ability of bees to distinguish flowers rapidly and accurately, based on their available pollen rewards, even in cryptic systems, such as in buzz-pollinated flowers, should have ecological and evolutionary advantages for the bee and/or the bee colony in social species. Assessment of pollen rewards requires the energy-demanding and complex behaviour of floral buzzing (Vallejo-Marín, 2019; Rossi *et al.*, 2026). Thus, limiting the time and energy expenditure of buzzing empty flowers can potentially result in considerable energy-saving benefits. However, we observe that even pollen-less flowers receive visits and are buzzed by bumblebees, and this has also been shown in previous studies (Buchmann and Cane, 1989; Russell *et al.*, 2017). This suggests that visually concealing pollen inside brightly coloured anthers strongly limits the ability of bees to determine the reward level before the onset of buzzing. This partial concealment might be particularly important for nectarless poricidal flowers that rely on attracting pollen foragers. Poricidal flowers are known to act as strong dispensing mechanisms (Harder and Barclay, 1994; King and Buchmann, 1996; Vallejo-Marín and Lundgren, 2026), which continue to release increasingly smaller numbers of pollen grains as they are visited. Extending this pollen-dispensing curve as much as possible by attracting pollinators even when reward levels are low should have potential benefits for pollen export and male fitness.

Intra-individual variation effects on foraging patterns

Bumblebees can make patch-level evaluation of pollen rewards and develop preferences for different sites depending on patch-level pollen/protein availability (Rasheed and Harder, 1997*a*). Nevertheless, owing to the potential complexity of fine quantification of pollen removal from cryptic anthers, bees might have limited options to guide their foraging behaviour when visiting different buzz-pollinated plants within a site. Our results do not support the risk-averse behaviour observed by Biernaskie *et al.* (2002) when encountering variable nectar rewards, because bees in our experiment were not more likely to leave a plant after visiting a pollen-less flower neither conducted fewer visits in highly variable plants. The behaviour of switching to a different flower when encountering rewardless flowers has been shown in another experiment that assessed foraging behaviour of bumblebees visiting arrays of plants with ten artificial flowers (Smithson and Gigord, 2003). They showed that when foraging for nectar, a reward that can be assessed in real time as the bee visits the flower (Cresswell, 1999), bees seem to be more likely to leave and avoid plants with a high proportion of empty flowers. However, this is not what we observed here in our experiment of pollen rewards using smaller (three-flowered) inflorescences. Our findings also do not support those of Maldonado *et al.* (2023), who found higher rates of visitation in individuals with more variable nectar rewards, because we found no differences in visitation or buzzing among treatments at the plant level. Previous work with artificial flowers and nectar rewards has thus found contrasting consequences of intra-individual variation on visitation patterns that differ from the lack of plant-level effect of variability in pollen rewards that we observed here.

Possible explanations for the differing results between nectar-reward studies and our pollen-reward experiment include: (1) pollen-foraging bees rely on simple flower-level rules rather than integrating information at the plant level (Harder, 1990); (2) the average reward level of the plant outweighs reward variance in foraging decisions; (3) plant-level effects are more likely to be detected in larger floral displays or at longer plant-separation distances than those used here (Biernaskie *et al.*, 2002; Maldonado *et al.*, 2023); and (4) bees might perceive intra-individual variation in pollen rewards differently from variation in nectar rewards. Future studies designed to tease apart these non-mutually exclusive explanations would provide insights into whether and how the foraging patterns observed here are context dependent.

Selection on intra-individual floral variation

Intra-individual variation in floral traits has been studied mostly in the context of plants that produce multiple types of flowers in the same plant (e.g. different sexual morphs, enantiomorphs, cleistogamy) (Barrett, 2002). However, although rarely reported formally, quantitative variation in homologous floral traits, including floral rewards, is likely to be widespread across taxa and should be common (Herrera *et al.*, 2006; Herrera, 2009, 2017; Żywiec *et al.*, 2012; Austen *et al.*, 2015; Dai *et al.*, 2016; Trevizan *et al.*, 2024). The modular nature of floral structures exposes them to varying environmental conditions in both space (different positions along an inflorescence, different inflorescences within a plant) and time (throughout the flowering period within a year or, in iteroparous perennials, over multiple years). A remarkable step forwards in the study of intra-individual variation is the recent study by Parraguirre *et al.* (2025) on the quantitative genetics of intra-individual variation in flower morphology in *S. rostratum*. They show that the intra-individual variation component of some floral traits, including corolla and anther size, has significant additive genetic variation and thus can respond evolutionarily to natural selection.

If quantitative intra-individual variation in floral traits is common, what is the current evidence that it is under selection in natural populations? Arceo-Gómez *et al.* (2017) analysed selection on anther–stigma distance in *Ipomoea wolcottiana* (Convolvulaceae). They found negative directional selection on the CV of this trait, but no evidence of selection on mean values (see also Herrera, 2009). In a study on bee-pollinated *Passiflora incarnata*, Dai *et al.* (2016) found that intra-individual variability in style deflexion reduced pollen deposition but, intriguingly, increased seed weight, leading the authors to speculate that variability had contrasting effects on the number of seeds versus seed quality. More recently, a study on the association between intra-individual variation in multiple floral traits of the hummingbird-pollinated plant *Justicia sebastianopolitanae* (Acanthaceae) failed to detect selection on intra-individual variation as assessed by measuring male fitness using pollen removal estimates (Paglia *et al.*, 2023). Although our study did not measure selection on intra-individual variation in pollen rewards, our findings of no differences in any measurement of foraging behaviour between the natural and increased intra-individual variation treatments suggest little opportunity for selection on this trait. Future studies linking intra-individual variation and fitness across study systems would help to determine whether quantitative intra-individual variation (e.g. in floral reward levels) is a trait that helps or hinders adaptation to different pollination environments.

Conclusion

Our study provides a first approximation of how intra-individual variation might influence plant–pollinator interactions in a visually cryptic, pollen-rewarding, buzz-pollinated system. The experimental approach taken here allowed us to achieve a high-level of detail in describing foraging patterns across >2300 visits and 9700 buzzing events. We showed that bees assess pollen rewards at the flower level but do not integrate information at the plant level, at least in the experimental conditions assessed here. Taken at face value, our results suggest that the observed foraging behaviour should buffer plants from the negative (and positive) effects of intra-individual variation in pollen rewards. Given that primary pollen production appears to be highly variable among the flowers of the same plant and is likely to be increased further as pollinators visit flowers haphazardly and remove pollen differentially, plants might benefit from pollinators making decisions based on local resource levels (flowers) rather than on individual plant variability *per se*.

SUPPLEMENTARY DATA

Supplementary data are available at *Annals of Botany* online and consist of the following. Figure S2: total pollen production in individual flowers of *Solanum rostratum* and *S. sisymbriifolium*. Each row represents a flower, and colours indicate different individuals. For *S. rostratum*, 71 flowers from 12 individuals were sampled. For *S. sisymbriifolium*, 210 flowers from 21 individuals were sampled. Figure S3: relative allocation of pollen production to pollinating anthers (PA) in heterantherous *Solanum rostratum* expressed as the proportion of pollen produced in the pollinating anther (PA) relative to the total pollan in the flower. Each row corresponds to a flower, and individuals are shown with different colours. $n = 71$ flowers from 12 individuals. Figure S1: distribution of harmonic ratios for buzzes produced by 30 *Bombus terrestris* workers during experimental trials. Each ridge represents a single bee and shows the density of buzzes based on the natural logarithm of the harmonic ratio. Vertical red dashed lines indicate the manually selected threshold used to classify buzzes as either floral or flight.

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AUTHOR CONTRIBUTIONS

Conceptualization, Data curation, Formal analysis, Visualization: F.G.-A.L. and M.V.-M. Methodology: F.G.-A.L., M.V.-M. and L.R.F.M. Investigation: F.G.-A.L. Writing—original draft: F.G.-A.L. and M.V.-M. Writing—review and editing: F.G.-A.L., M.V.-M. and L.R.F.M. Resources and supervision: MVM. Resource acquisition: F.G.-A.L., L.R.F.M. and M.V.-M.

AI USE

ChatGPT (GPT-5.1) was used to assist during *R* coding, including for data manipulation, statistical analysis and plotting, and for minor text editing.

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