Behavioral Ecology, 2024, Vol. 35, No. 6, arae083
https://doi.org/10.1093/beheco/arae083
Advance access publication 4 October 2024
Original Article

Learning to handle flowers increases pollen collection for bees but does not affect pollination success for plants

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Handling Editor: Emilie Snell-Rood

Cooperation and conflict are common in plant–pollinator interactions. Flowering plants often entice pollinators to visit by offering floral food rewards, thereby facilitating pollination. However, pollinators such as bees can learn to improve their collection of floral rewards (such as pollen), changing how they interact with the flower's reproductive organs, which together could reduce pollination success. Consequently, complex flowers that slow pollinator learning might benefit the plant. Yet how pollinator learning and flower complexity interact to affect pollination success is unknown. We therefore asked how differences in complexity of 4 flower types (*Phacelia campanularia, Exacum affine*, *Solanum elaeagnifolium*, and *Erythranthe guttata*) affected learning by pollen-foraging generalist bumble bees (*Bombus impatiens*) and how learning affected pollen collection and pollen deposition on these flowers. We found that bees generally learned how to efficiently handle more complex flower types more slowly. Bees that required more visits to become efficient foragers collected less pollen, with no effect on pollen deposition. Except for the simplest flower type, learning also involved development of motor routines unique to each flower type. Experienced bees overall collected more pollen, but individual differences in motor routines did not affect pollen collection. Conversely, individual differences in motor routines affected pollen deposition, but there was no overall effect of experience. Thus, even though learning overall benefits the bee, it does not alter female (and potentially male) fitness benefits for the plant. We discuss potential reasons for these patterns and consequences for bee behavior and flower evolution.

Key words: bees; cognition; exploitation; flowers; foraging; learning; mutualism; pollen, pollination.

Introduction

Plant–pollinator interactions are a model system for understanding conflicts of interest in mutualisms (Bronstein 2009; van der Kooi et al. 2021). Plant–pollinator interactions typically involve animals visiting flowers to collect food rewards (e.g. nectar and pollen), and in the process, picking up and transferring pollen to conspecific flowers. Because foraging can be dangerous and is expensive in terms of time and energy, pollinators often optimize their foraging efficiency with experience (Lewis 1993; Laverty 1994; Chittka et al. 1999; Raine and Chittka 2008). Although learning is widely accepted to benefit the pollinator, learning could also benefit the plant in terms of pollen export and receipt (Lewis 1993; Barker et al. 2018). Pollinator learning is traditionally thought to increase pollination success, for instance by increasing the likelihood that the pollinator stays true to one flower type ("floral constancy"), thereby potentially reducing pollen loss and transfer to heterospecifics (Waser 1986; Chittka et al. 1999). Yet pollinator learning could also reduce pollination success. For example, experienced pollinators tend to make shorter visits potentially resulting in fewer opportunities to pick up and transfer pollen (e.g. Laverty 1980; Gegear and Laverty 1995; Ramos et al. 2017). Although learning is frequently hypothesized to change how the interests of plants and pollinators are aligned (e.g. Leonard et al. 2011; Evans et al. 2017; Barker et al. 2018; Richman et al. 2021; van der Kooi et al. 2021), evidence of how pollinator learning directly affects pollination success is extremely limited (see Internicola and Harder 2012; Jones and Agrawal 2017; Ramos et al. 2017; Vázquez and Barradas 2017).

Pollinator cognition is particularly well understood in the context of bee–flower interactions (Chittka and Thomson 2005; Giurfa 2007). Although numerous studies have addressed how generalist bees learn to forage for carbohydrate-rich nectar, bees must also collect protein-rich pollen to survive (Simpson and Neff 1981; Kevan and Baker 1983; Kitaoka and Nieh 2009; Nicolson and van Wyk 2011). Yet we lack knowledge of whether pollen foragers learn to optimize their flower handling and whether learning results in increased pollen collection (see Raine and Chittka 2007; Russell et al. 2016). Nectar foragers can require dozens of floral visits to become efficient at handling flowers, particularly when flowers require complex motor routines

Received: 11 May 2024; **Revised:** 14 September 2024; **Editorial decision:** 23 September 2024; **Accepted:** 1 October 2024.

("flower handling skills/techniques") for bees to efficiently access concealed nectar (Laverty and Plowright 1988; Woodward and Laverty 1992; Laverty 1994; Chittka et al. 1999). Because flowers that offer pollen rewards can also possess diverse morphologies and conceal their pollen (Vogel 1978; Buchmann 1983; Table 1), pollen foragers should also have to learn to become proficient at handling flowers (Raine and Chittka 2007; Russell et al. 2016, 2017). Furthermore, although prior studies have focused on changes in time spent handling the flower, how pollen foraging motor routines are modified with experience is poorly understood (see Laverty 1980; Chittka and Thomson 1997; Russell et al. 2016).

Floral morphology often dictates how rewards are partitioned and dispensed and flower morphology frequently differs dramatically among plant species. As a result, efficient collection of rewards is hypothesized to require diverse flower handling techniques (Laverty and Plowright 1988; Lewis 1993; Gegear and Laverty 1995). For instance, collecting nectar from a milkwort flower (*Polygala* spp.) requires the bee to force open the banner from the keel petals to extend its proboscis into the nectary (e.g. Westerkamp and Weber 1999), whereas bees must fully enter into the hood petals of wolfsbane flowers (*Aconitum* spp.) to locate the hidden nectaries (e.g. Laverty 1980; Laverty and Plowright 1988). Furthermore, the amount of learning required to become a proficient nectar forager is hypothesized to depend on flower morphology, with relatively more learning required on complex flowers that require complex handling skills (Laverty 1980, 1994; Muth et al. 2015). Yet how floral morphology affects learning of pollen foraging has been barely explored (Raine and Chittka 2007; Russell et al. 2016).

How a flower's morphology affects bee learning may also affect benefits to both bees and plants (Ramos et al. 2017; Vázquez and Barradas 2017). For example, when less learning is required to become proficient at handling a given flower type, we expect bees to collect rewards sooner at their maximal rate. However, pollinator learning could be beneficial or costly for the plant. For instance, from the plant's perspective, learning might result in a better pollinator, if optimizing flower handling involves the bee contacting the plant's reproductive organs more frequently or reliably. Yet learning could result in a worse pollinator from the plant's perspective if optimizing flower handling involves body positioning that circumvents contact with reproductive organs or making quicker visits that result in less pollen overall transferred among flowers. Furthermore, the more pollen moved into the pollen baskets (largely unavailable for pollination; Parker et al. 2015), the less pollen available for transfer among conspecific flowers (Castellanos et al. 2006; Hargreaves et al. 2009; Wilkins et al. 2022), which could further compound the negative effects of learning on pollination for the plant (assuming that learning results in more pollen collected; see Raine and Chittka 2007).

In this laboratory study, we assessed how floral complexity influenced learning of flower handling by pollen-foraging bumble bees (*Bombus impatiens*), and how acquisition of these skills influenced pollen collection and pollen transfer among flowers. We used fresh flowers of 4 nectarless species (*Phacelia campanularia* (Boraginaceae), *Exacum affine* (Gentianaceae), *Solanum elaeagnifolium* (Solanaceae), and *Erythranthe guttata* (Phrymaceae)) that offer only pollen as a reward and vary in floral complexity and in how pollen is dispensed (Fig. 1; Tables 1 and 2). We hypothesized that (1) more complex flower types would be harder for bees to learn, (2) pollen collection would increase more slowly when learning was more difficult, and (3) pollen transfer to flowers would decline as bees gained experience and decline faster when learning was easier.

Methods

Experimental subjects

To study how pollen foraging experience on different flowers affected flower handling skills, pollen collection, and pollen transfer, we used 81 initially flower-naïve workers from 7 captive commercially obtained colonies (Koppert Biological Systems, Howell, MI, USA) of the common eastern bumble bee, *B. impatiens*. Briefly, following Russell et al. (2017), each colony was maintained on 2 molar solution of sucrose and pulverized honeybee-collected pollen (Koppert Biological Systems) from artificial feeders within enclosed foraging arenas (LWH: 82 cm × 60 cm × 60 cm) set to a 14 h:10 h light:dark cycle.

Following Laverty (1994) and Laverty and Plowright (1988), we reasoned that differences in floral complexity would correspond to differences in learning required to improve extraction of resources. We used fresh flowers from 10 *P. campanularia* (Boraginaceae), 36 *E. affine* (Gentianaceae), 8 *S. elaeagnifolium* (Solanaceae), and 10 *E. guttata* (Phrymaceae) plants (Table 1; Fig. 1), which were grown in a university greenhouse (14:10 h light:dark cycle) and fertilized bi-weekly (PlantTone, NPK 5:3:3, Espoma, Millville, NJ, USA). To prevent desiccation, all freshly cut live flowers were placed into custom water tubes (Russell et al. 2017).

Table 1. Prominent features of the 4 kinds of pollen offering flowers.

Flower	Pistil	Anther	Flower morphology
Phacelia campanularia	Long, slender style with bilobed long and thin stigma	Five small anthers dehiscing completely with exposed pollen	Saucer shaped flower with anthers and style readily accessed
Exacum affine	Small stigma at the end of the single short style	Five short rigid anthers dehiscing by pores (pollen concealed)	Stellate flower with anthers and style readily accessed
Solanum elaeagnifolium	Small stigma at the end of a long style	Five long semi-rigid anthers dehiscing by pores (pollen concealed)	Stellate flower with anthers and style readily accessed
Erythranthe guttata	Long, slender style with bilobed sensitive stigma (closes with pollination)	Four very small anthers dehiscing completely with exposed pollen	Gullet flower with anthers hidden within corolla tube and style sometimes protruding

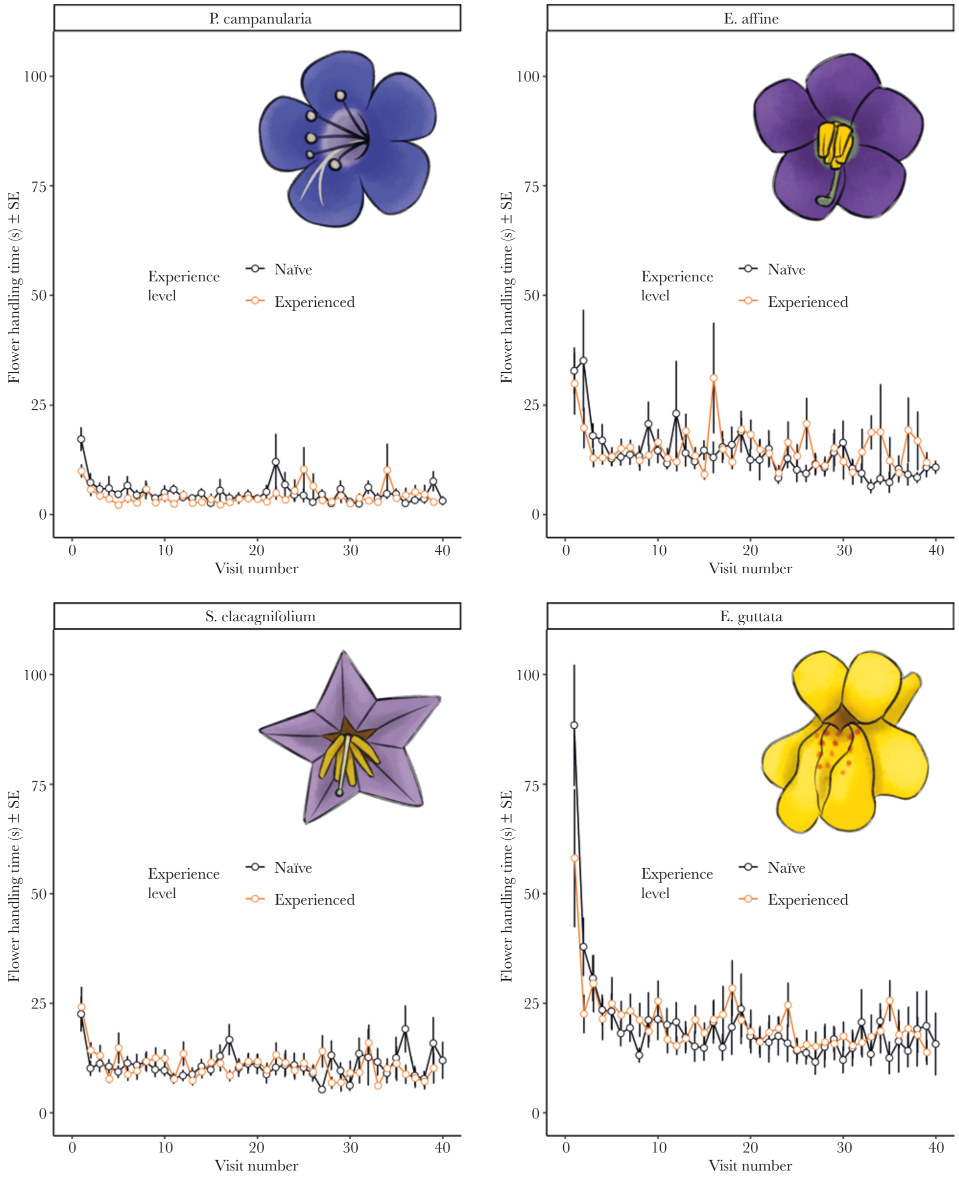

Fig. 1. Handling times shown for the first 40 (out of up to 70) flower visits made by initially naïve (first trial) and experienced (second trial) bees on each plant species. N = 17, 21, 20, 20 initially naïve bees and N = 19, 21, 20, 20 experienced bees foraging on *P. campanularia*, *E. affine*, *S. elaeagnifolium*, and *E. guttata*, respectively.

Table 2. Floral variables of the (mFCI) and their values.

Plant species	Shape/value/ weight = 0.3	Depth/value/ weight = 0.2	Symmetry/value/ weight = 0.2	Corolla segmentation/ value/weight = 0.2	Reward concealment/ value/weight = 0.1	FCI value: sum of weighted score
P. campanularia	Brush/3.5	Low-depth/1	Radial/1.13	Choripetalous/1.5	Unconcealed/1	1.876
E. affine	Brush/3.5	Low-depth/1	Radial/1.13	Choripetalous/1.5	Concealed/3	2.126
S. eleaegnifolium	Brush/3.5	Low-depth/1	Radial/1.13	Sympetalous/2.5	Concealed/3	2.276
E. guttata	Gullet/3.75	Med-depth/2	Bilateral/3	Sympetalous/2.5	concealed/3	2.925

Experimental protocol

To identify appropriate test subjects, we captured bees observed foraging on the artificial nectar feeders, marked uniquely with nontoxic oil markers (Sharpie, CA), and returned them to their colonies. We divided painted flower-naïve bees into 4 treatments, with 2 sub-treatments each. A minimum of 3 colonies were represented per treatment. Treatments differed by plant species used: 20 cut flowers of a given species were spaced 7 cm apart in a 5 × 4 Cartesian grid design on the arena wall. We systematically alternated assignment of bees to each treatment to control for effects of time and day on behavior. Sub-treatments differed in terms of whether a bee was initially flower-naïve ("naïve") or had previously been tested ("experienced"): each bee therefore received 2 consecutive trials with the same plant species, with the naïve and experienced trials separated by approximately 24 h. After its naïve trial, the bee was returned to

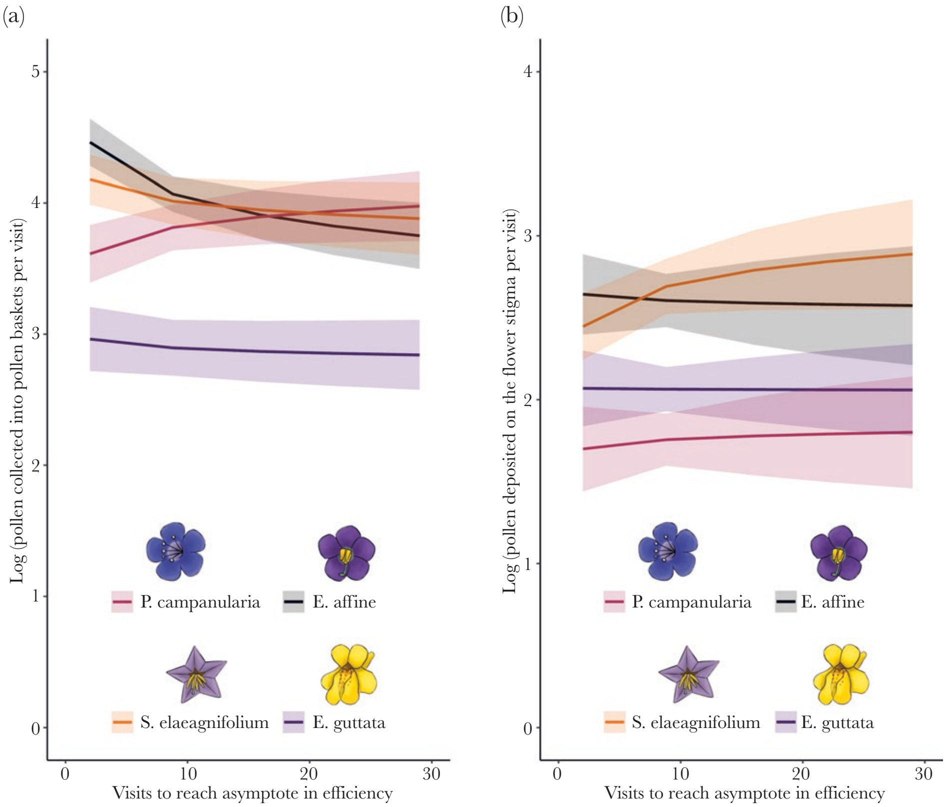

Fig. 2. The quantity of pollen grains (a) in the pollen baskets (corbiculae) or (b) transferred to flower stigmas, as influenced by plant species and number of visits required by initially naïve bees to reach an asymptote in handling efficiency during their first trial. $N = 17, 21, 20, 20$ initially naïve bees foraging on *P. campanularia*, *E. affine*, *S. elaeagnifolium*, and *E. guttata*, respectively. Plotted lines indicate estimated means and shaded regions indicate 95% CIs.

its colony, but after its experienced trial, the bee was permanently removed from the colony, to facilitate a colony to recruit more foragers.

To initiate a behavioral trial, flowers were set up and a single painted flower-naïve worker bee was gently captured from the foraging arena using a 40-dram vial (Bioquip) and immediately released in the center of the test arena following Russell et al. (2017). Prior to release, we visually confirmed the absence of pollen on the bee's body. To estimate how much experience was required to reach an asymptote in flower handling time, we ran several long practice trials (120 visits) and estimated that an asymptote had been reached less than 50% through each practice trial; data from these practice trials was not used in any subsequent analyses. We therefore terminated each naïve behavioral trial after 70 visits (or earlier if the bee stopped visiting flowers for 5 min) to ensure that bees had learned and to avoid bees depleting flowers of pollen rewards or filling their pollen baskets completely. For a subset of flowers and trials, we extracted pollen from the anthers to confirm that pollen had not been depleted after a trial. We terminated each experienced behavioral trial immediately once the bee had made the same number of visits as in its naïve trial. To terminate a trial, we turned off the overhead arena lights and captured the bee in a vial. After terminating a trial, the complete pollen load from 1 pollen basket (i.e. corbicular pollen load) was carefully removed from the bee, and the anthers and styles of all visited flowers were removed. The pollen load, anthers, and styles were stored separately in 70% ethanol for pollen counting (styles and anthers for each trial were separately pooled into microcentrifuge tubes). The arena was cleaned with distilled water and fresh paper towels thoroughly between trials.

Trials were video recorded to enable using an event logging program (BORIS v.8.19.3) to measure the handling time per flower visit (from 3 legs on the flower until physical contact with the flower ended), the latency to begin collecting pollen after landing on the flower (from 3 legs on the flower until the bee began scrabbling or buzzing on the anthers), and the duration of each flower visit during which bees used easily distinguishable categories of pollen collecting handling routines (Fig. 3; see Results for a description). We also recorded whether the bee had collected pollen on a given flower visit. We failed to video-record the naïve trial for 3 bees and these data were therefore unavailable for behavioral analyses.

Assessment of pollen collection and transfer

To examine whether bee foraging experience affected pollen collection, the corbicular pollen load from each trial was stored in separate microcentrifuge tubes with 1 mL of 70% ethanol. We counted pollen in two or three 10 μL aliquots using a hemocytometer (Hausser Scientific, Horsham, PA) at 400× or 100× (Leica DM 500) to arrive at an estimate for the total volume. Estimated pollen counts were rounded to the nearest whole number. To evaluate pollen deposition, the pollen grains on flower stigmas were enumerated. We acetolyzed styles from flowers (following Dafni 1992) pooled by trial, condensed samples by centrifugation to 40 μL, and counted resuspended pollen in two 10 μL aliquots; if we counted zero grains, we counted conspecific grains in all 4 aliquots.

Ranking flower complexity

We first calculated values of the floral complexity index (FCI) following Stefanaki et al. (2015), in which levels of 5 floral variables receive a weighted score that is summed. However, because we used an identical number of individual flowers per plant species during foraging assays and not racemes, we excluded the "functional reproductive unit" variable. In this modified FCI (see Table 2), we substituted this variable for a "reward concealment" variable, which is considered a key characteristic of complex flowers (e.g. Krishna and Keasar 2018; Ornai and Keasar 2020) and accounts for stamen location and form, which are of key relevance for pollen foraging bees and are not considered in the original FCI.

Data analyses

All data were analyzed using R v.4.2.2 (R Development Core Team 2023).

Does foraging experience increase efficiency in handling flowers and affect pollen collection and deposition? We first analyzed how foraging experience affected the relative efficiency of bees in handling flowers during their first trial. Following Laverty (1994) and Laverty and Plowright (1988), we assumed that experienced bees during their second trial had achieved maximal efficiency and that initially naïve bees during their first trial increased their efficiency with foraging experience. We therefore calculated the relative efficiency of a bee during its first trial as a percentage of its maximum efficiency (the mean handling time or latency during its second trial). For all naïve bees, we thus calculated initial foraging efficiency for the first flower visit, as well as the rate of improvement in efficiency (slope from linear regressions) until reaching an asymptote in performance (3 consecutive visits with ≥90% efficiency; following Laverty 1994). For bees that did not reach an asymptote in efficiency for flower handling (3 of 78 bees), all visits were used in regressions following Laverty (1994). We used Kruskal–Wallis Tests (KWT) to assess whether the initial efficiency, slope, or number of visits until reaching the asymptote differed among plant species, and in cases of significance we used a Wilcoxon-Signed Rank Test (WSRT) with corrections for multiple testing to determine pairwise differences. The fit of the regressions was good overall (coefficient of determination [R^2] mean ± SE: handling time: *P. campanularia*: 0.61 ± 0.09; *E. affine*: 0.51 ± 0.09; *S. elaeagnifolium*: 0.51 ± 0.09; *E. guttata*: 0.57 ± 0.07) (see Supplementary Material for representative traces).

Next, we analyzed how individual differences in handling efficiency (above) influenced the quantity of pollen grains collected into bee pollen baskets and deposited on flower stigmas by initially naïve bees. We normalized pollen collected or deposited by the number of flower visits (i.e. for each trial: total pollen divided by total visits made). We specified generalized linear mixed effects models (GLMMs) with a Gaussian distribution (glmmTMB package; Magnusson et al. 2018), specifying type III Wald chi-squared (χ^2)-tests via the Anova() function (car package; Fox 2015). We specified "mean pollen collected" or "mean pollen deposited" as the response variable and explanatory variables as "visits to reach asymptote in efficiency," "rate of improvement in efficiency," "initial efficiency," and 'plant species' (*P. campanularia*, *E. affine*, *S. elaeagnifolium*, or *E. guttata*), with "beeID" as a random effect. To simplify this analysis we applied model selection to the efficiency explanatory variables via backwards elimination using anova() in R. For subsequent GLMMs, of the 3 efficiency variables, only "visits to reach asymptote in efficiency" was thus retained. In cases of significant effects, we ran Tukey's post hoc test using the emmeans() function (emmeans package; Lenth et al. 2024) to determine pairwise differences. We checked for overdispersion, zero inflation, and uniformity for all GLMMs using the DHARMa package (Hartig 2022) and log transformed pollen counts to meet model assumptions.

Do flower handling skills change with experience and affect pollen collection and deposition? To analyze how changes in flower handling skills with experience affected pollen collection and deposition, we used GLMMs in which we specified the response variable as "mean pollen collected" or "mean pollen deposited" (as above) and the explanatory variables as "bee experience level" (naïve or experienced), "plant species" (as above), and "mean percent modified handling routine" (percent of total handling time for a flower that the bee used the given handling routine that had been modified with experience—see Results for details—averaged across all flower visits for the given trial). To meet model assumptions, we added 1% to the latter response variable and square root transformed it. We specified nested random effects for these GLMMs as "bee ID" within "colony ID."

Results

Flower handling efficiency increased with experience and affected pollen collection, but not deposition

Ranking complexity of the 4 flower types via a modified floral complexity index (mFCI) from least to most complex, *P. campanularia* was least complex (mFCI = 1.876), followed by *E. affine* (mFCI = 2.126), *S. elaeagnifolium* (mFCI = 2.276), and *E. guttata* (mFCI = 2.925) (Table 2). Greater complexity roughly corresponded with reduced initial handling efficiency and slower learning for initially naïve bees during their first trial (handling efficiency: handling time on each visit during the first trial as a percentage of the mean flower handling time across the second trial). Bees showed the greatest improvement in handling efficiency across consecutive flower visits on *P. campanularia*, followed by *E. affine*, *S. elaeagnifolium*, and *E. guttata* (least to most complex flower) (70%, 43%, 32%, 30%, respectively), though differences were not statistically significant (Fig. 1; KWT: $\chi^2_3 = 0.34$, $P = 0.953$). Similarly, the bees reached an asymptote in handling efficiency quickest on *E. affine*, followed by *S. elaeagnifolium*, *P. campanularia*, and *E. guttata* (Fig. 1; range: 1 to 26 visits to reach an asymptote in handling efficiency: mean visits ± SE: 5.90 ± 0.96; 5.95 ± 1.44; 7.41 ± 1.6; 8.45 ± 1.42, respectively), although these differences were also not statistically significant (KWT: $\chi^2_3 = 3.99$, $P = 0.263$). Finally, naïve bees visiting *E. guttata* and *P. campanularia* had a significantly lower initial handling efficiency than naïve bees visiting either *E. affine* or *S. elaeagnifolium* (Fig. 1; overall effect: KWT: $\chi^2_3 = 14.54$, $P = 0.0023$; pairwise differences: WSRT: $P = 0.015$; mean % ± SE efficiency relative to experienced state: for *E. guttata*: 41 ± 9; *P. campanularia*: 40 ± 10; for *E. affine*: 74 ± 12; for *S. elaeagnifolium*: 101 ± 22).

Following model selection, we assessed how individual differences in the number of visits to reach an asymptote in handling efficiency affected the quantity of pollen grains initially naïve bees collected into their pollen baskets and deposited on flower stigmas per visit during their first trial. Overall, bees that learned more quickly, collected more pollen, but this effect depended on plant species and did not reflect differences in floral complexity (Fig. 2a; GLMM: effect of visits to asymptote: $\chi^2_1 = 5.65$, $P = 0.018$; effect of plant species: $\chi^2_3 = 186.65$, $P < 0.0001$; effect of plant species × visits to asymptote: $\chi^2_3 = 19.33$, $P = 0.0002$). In contrast,

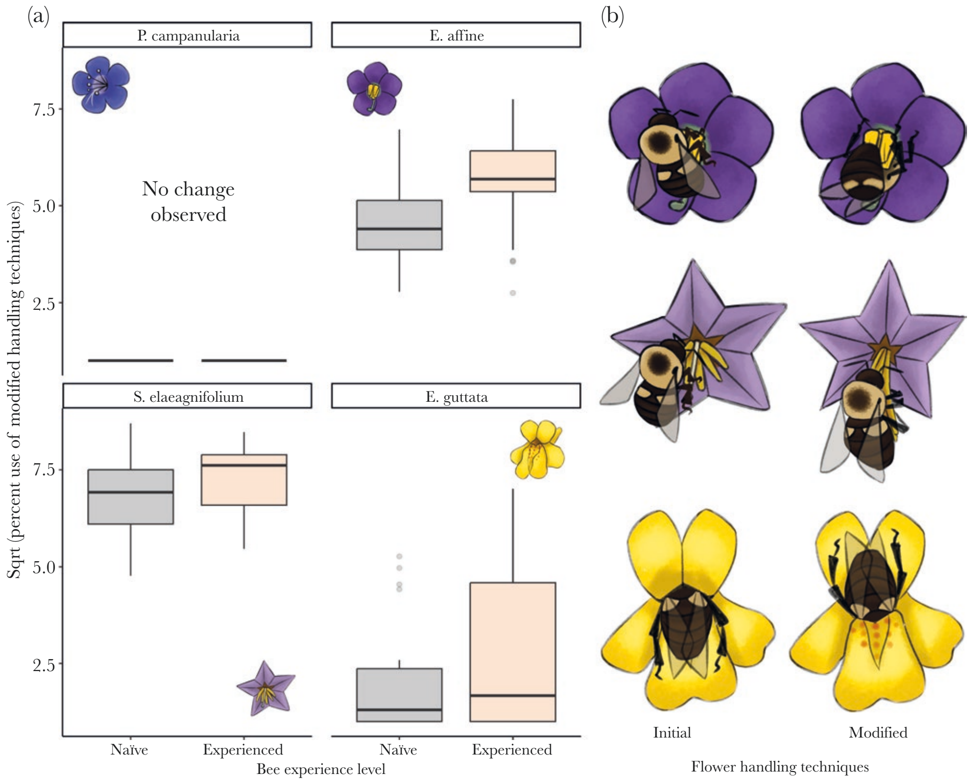

Fig. 3. a) Mean percent of a flower visit during which the modified handling routines were used to extract pollen. Plotted as boxplots. b) Illustrations of the initial and modified handling techniques ("motor routines") used to extract pollen from flowers. No changes in the pollen foraging motor routine were observed for *P. campanularia*. $N = 21, 20, 20$ initially naïve and experienced bees foraging on *E. affine*, *S. elaeagnifolium*, and *E. guttata*, respectively.

individual differences in learning did not affect pollen transfer (Fig. 2b; GLMM: effect of visits to asymptote: $\chi^2_1 = 1.16$, $P = 0.281$). Unsurprisingly, given differences in the amount of pollen offered and stigma size, there were significant differences in pollen receipt among plant species (Figs. 2b and 5b; GLMM: effect of plant species: $\chi^2_3 = 118.74$, $P < 0.0001$; effect of plant species × visits to asymptote: $\chi^2_3 = 2.81$, $P = 0.422$).

Flower handling skills changed with experience and affected pollen deposition, but not collection

Bees modified their flower handling motor routines on 3 of the 4 flower types with experience. We did not find objective changes in handling skills of bees on visits to the comparatively simple flowers of *P. campanularia*. Initially naïve bees primarily bit down and buzzed the dorsal surface of *E. affine* anthers to extract pollen on each visit (Fig. 3; first trial: 79 ± 2.4 of the time), but with experience, bees often inverted themselves on the downward curving anthers, biting them and buzzing while anther pores were appressed to the ventral thorax (Fig. 3; second trial: 32 ± 2.9% of the time). Buzzes, which indicated an attempt at extracting pollen, were identified by their distinctive sound and occurred only after a bee had landed (see Russell et al. 2017). Initially naïve bees on *S. elaeagnifolium* primarily bit and buzzed the individual spread apart anthers (Fig. 3; first trial: per visit, mean % ± SE: 53 ± 3.2%), whereas with experience, bees more commonly clasped 3 or more anthers together while biting and buzzing (Fig. 3; second trial: 53 ± 2.7% of the time). During their first trial, initially naïve bees on *E. guttata* almost entirely scrabbled, using their legs and mandibles to knock pollen from the anthers while upright within the corolla tube (Fig. 3; first trial: 95 ± 2.0% of the time; for a complete description and video of scrabbling behavior, see Russell and Papaj 2016). In contrast, as bees on *E. guttata* gained experience, they began inverting themselves within the corolla tube and buzzed pollen directly from the anthers (Fig. 3; second trial: 12 ± 3.5% of the time). Altogether, on their second trial bees increased their use of the modified handling techniques on *E. affine*, *S. elaeagnifolium*, and *E. guttata* on average by 54%, 14%, and 127%, respectively.

Across plant species, pollen collection significantly increased with bee experience (from first to second trial) (Fig. 4a; GLMM: effect of experience level: $\chi^2_1 = 9.81$, $P = 0.0017$). The degree to which pollen collection improved with experience did not significantly differ among flower types (GLMM: effect of plant species × experience level: $\chi^2_3 = 0.30$, $P = 0.961$), though bees foraging on *P. campanularia*, *E. affine*, *S. elaeagnifolium*, and *E. guttata* (least to most complex) collecting on average 17%, 19%, 26%, and 27% more pollen per visit with experience, respectively. However, individual differences in the use of modified handling techniques did not significantly affect pollen collection (Fig. 5a; GLMM: effect of motor routine: $\chi^2_1 = 2.03$, $P = 0.154$; effect of plant species: $\chi^2_3 = 259.53$, $P < 0.0001$; effect of motor routine × experience level: $\chi^2_1 = 0.56$, $P = 0.455$; effect of motor routine × plant species: $\chi^2_2 = 0.86$, $P = 0.652$; effect of motor routine × experience level × plant species: $\chi^2_2 = 0.27$, $P = 0.873$).

Conversely, pollen transfer was significantly affected by individual differences in handling techniques, but the effect depended on experience level (from the first to second trial) and plant species (Fig. 5b; GLMM: effect of motor routine: $\chi^2_2 = 7.69$, $P = 0.0056$; effect of experience level: $\chi^2_1 = 0.02$, $P = 0.893$; effect of

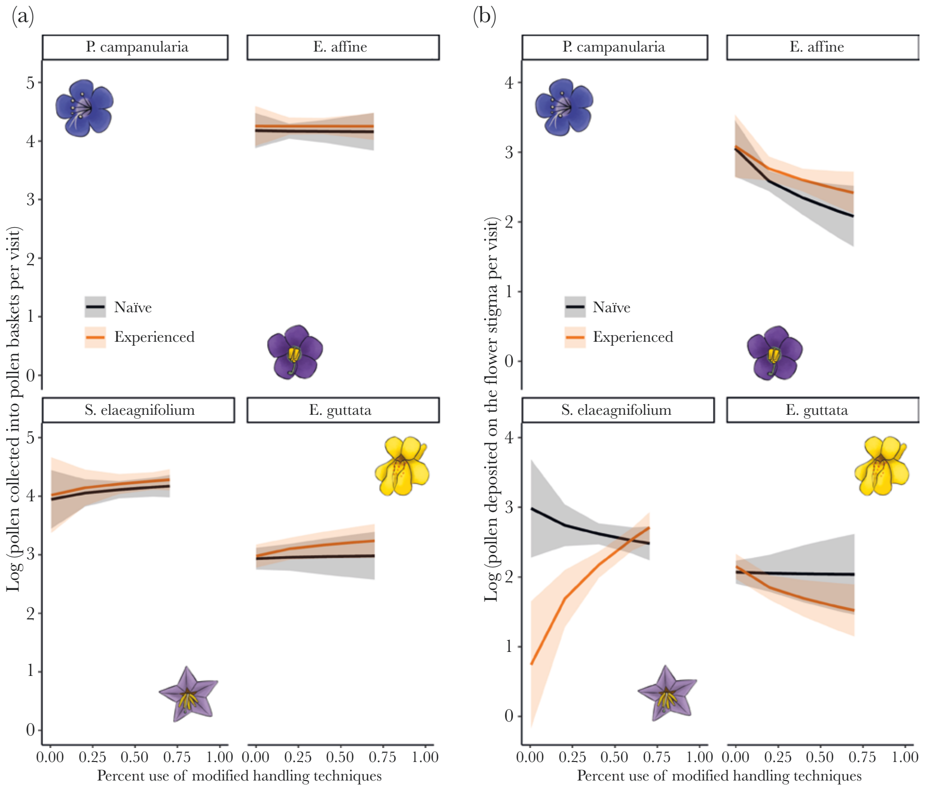

Fig. 4. The quantity of pollen grains a) in the pollen baskets (corbiculae) or b) transferred to flower stigmas, as influenced by mean percent of a flower visit during which the modified handling routines were used to extract pollen, bee experience level (first versus second trial) and plant species. No changes in the pollen foraging motor routine were observed for *P. campanularia*. N = 21, 20, 20 initially naïve bees and N = 21, 20, 20 experienced bees foraging on *E. affine*, *S. elaeagnifolium,* and *E. guttata*, respectively. Plotted lines indicate estimated means and shaded regions indicate 95% CIs.

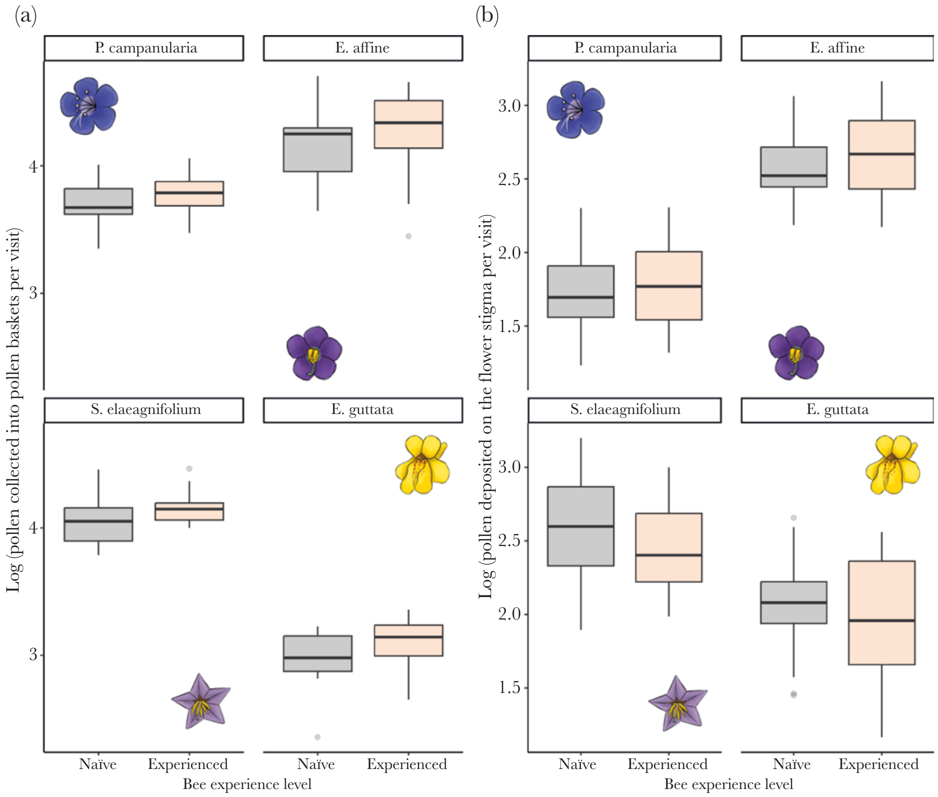

Fig. 5. The quantity of pollen grains a) in the pollen baskets (corbiculae) or b) transferred to flower stigmas, as influenced by bee experience level (first versus second trial) and plant species. Plotted as boxplots. N = 17, 21, 20, 20 initially naïve bees and N = 19, 21, 20, 20 experienced bees foraging on *P. campanularia*, *E. affine*, *S. elaeagnifolium*, and *E. guttata*, respectively.

plant species: $\chi^2_3 = 80.98$, $P < 0.0001$; effect of plant species × experience level: $\chi^2_3 = 13.31$, $P = 0.0040$; effect of motor routine × experience level: $\chi^2_1 = 0.22$, $P = 0.637$; effect of motor routine × plant species: $\chi^2_2 = 7.57$, $P = 0.023$; effect of motor routine × experience level × plant species: $\chi^2_2 = 19.70$, $P = 0.00005$). Although these differences were not statistically significant, experienced bees deposited an average of 18% and 28% more pollen and 49% and 6% less pollen per visit on *P. campanularia*, *E. affine*, *S. elaeagnifolium*, and *E. guttata* (least to most complex), respectively (Fig. 4b).

Discussion

Flower morphology that requires pollinators to learn how to extract rewards is often considered an adaptation that benefits the plant in terms of enhancing pollination (Darwin 1895; Lewis 1993; Laverty 1994; Jones and Agrawal 2017; Barker et al. 2018). We find that learning to manipulate flower morphology by pollen-foraging bumble bees does not significantly affect benefits for the plant in terms of pollen deposition on flowers. Across all 4 plant species tested, both the total amount (Fig. 4) and rate of pollen deposition (Fig. S2) did not differ between experienced and initially flower-naïve bumble bees. In contrast, we found strong benefits of learning for bees in terms of pollen collection. With experience, bees were much more likely to successfully extract pollen (Fig. S1), collected 22% more pollen in their pollen baskets overall (Fig. 4), and did so at a 24% greater rate (Fig. S2). Furthermore, flower complexity significantly affected learning, with complex flowers being generally more difficult to learn. Pollen foraging is often expected to have a greater (and negative) impact on pollination relative to nectar foraging (Wilson and Thomson 1991; Koski et al. 2018; Weinman et al. 2023). Assuming our results are broadly generalizable, more difficult to learn flowers (i.e. generally more complex flowers) do not appear to be more costly to plants (Laverty 1980; Muth et al. 2015), at least in terms of reduced pollen transfer by inexperienced pollinators. Likewise, how experience affected pollen collection by bees differed little among flower types regardless of floral complexity, suggesting that difficult to learn flowers do not serve to protect floral rewards from being overexploited (Laverty 1994).

Given considerable variation in flower complexity, why did not complexity have stronger effects on pollen collection or pollen deposition? We assessed learning of flower handling in multiple ways and not all were strongly affected by flower complexity, potentially precluding finding large effects. Pollen-foraging bees quickly reached an asymptote in their efficiency to handle and locate the anthers of all flower types (Supplementary Material), on par with the fastest improvements observed for bees foraging for nectar on comparatively simple flowers (Laverty 1980, 1994; Laverty and Plowright 1988). Furthermore, bees that learned more quickly collected more pollen, and although this pattern differed among flower types, we found no straightforward relationship with flower complexity. Modest effects of flower complexity on learning of handling efficiency may thus have been a consequence of how quickly bees learned, resulting in other components of floral morphology playing a bigger role in modifying bee pollen collection. Consequently, if handling efficiency drives patterns of reward collection and pollen transfer (as frequently suggested; e.g. Laverty 1980; Lewis 1993; Muth et al. 2015; Keasar et al. 2023), the lack of an effect of flower complexity in this study is not surprising.

Provided rapid learning of efficient handling, more slowly learned components of flower handling may have driven differences in pollen collection between experienced and inexperienced bees. Flower handling motor routines and successful pollen extraction (Fig. S1) took many visits to learn and were strongly affected by flower complexity. Although flower handling motor routines have been described for inexperienced and experienced bees on different flower types (e.g. Laverty 1980; Chittka and Thomson 1997; Russell et al. 2018; Baek et al. 2023), the pace of learning handling techniques of different flower types has been unclear. Our results highlight that not all components of flower handling (e.g. handling efficiency, successful extraction of rewards, and handling motor routines) are learned at the same pace, with motor routines being learned particularly slowly. Additionally, substantial interindividual variation in the use of the modified motor routines suggests that experienced bees were still learning when trials were terminated. Potentially, had we allowed many more flower visits to fully learn motor routines, we might have eventually observed stronger effects of flower complexity on pollen collection and pollen transfer. Consistent with this, pollen transfer was affected by individual differences in the use of motor routines. Likewise, Raine and Chittka (2007) found that bees were still improving their collection of pollen on a single plant species even after more than 300 flower visits. Altogether, determining conclusively how pollinator learning affects pollen fate will require careful assessment of how different learned components of flower handling directly affect pollen collection and/or pollination.

Pollen collection by bees frequently reduces the amount of pollen available for pollination (Hargreaves et al. 2009; Russell et al. 2021; Weinman et al. 2023). Given that bees collected pollen more effectively and efficiently with experience, why did not learning simultaneously reduce pollen transfer? One possibility is that learning also made bees better at depositing pollen on flower stigmas, resulting in no overall change in pollen transfer for experienced versus inexperienced bees. For instance, modification of flower handling techniques with experience often involved the bee altering how it contacted the flower's reproductive organs (Fig. 3b), which could have resulted in more opportunities for pollen transfer to stigmas, even if less pollen overall was available for transfer to stigmas due to improved pollen collection. Surprisingly, we found that modification of handling techniques decreased pollen transfer overall. Another possibility is that learning might have reduced pollen wastage by the bee (i.e. pollen removed from the anthers, but not collected by the bee or deposited on stigmas; Parker et al. 2016; Minnaar et al. 2019). As a consequence, experienced bees would acquire more pollen on their bodies, which could explain increases in pollen collection without necessarily affecting pollen transfer. Quantifying how learning alters pollen wastage and contact with stigmas would thus be useful to more fully characterize effects of pollinator learning on pollination.

Although we only quantified benefits to the plant in terms of pollen transfer, pollinator learning may have affected other components of pollination success. For instance, perhaps experienced bees depleted anthers more thoroughly, which would reduce pollen acquisition and transfer by subsequent visitors (Minnaar et al. 2019). A brief check of visited flowers in our trials revealed that they were not depleted of pollen, but we were unable to quantify whether flowers visited by experienced versus inexperienced bees were relatively more depleted. Additionally, we could not examine whether learning affected the quality of pollen transferred across visits, in terms of xenogamous versus same-flower self-pollen, which is likely affected by changes in flower handling time and the sequence of flower visits (Robertson 1992; Karron et al. 2009; Horsburgh et al. 2011; Minnaar and Anderson

2019; Minnaar et al. 2019). Finally, effects of learning on pollen deposition may not have been observed if bees delivered much more pollen to stigmas than these reproductive structures could physically receive. However, this possibility is unlikely: for each plant species, the maximum quantity of pollen on stigmas after behavioral trials was much greater than the average quantity of pollen transferred to stigmas (greater by 2.1×, 2.7×, 3.4×, and 4.7× for *E. affine*, *P. campanularia*, *E. guttata,* and *S. elaeagnifolium*, respectively), suggesting that on average, stigmas were not saturated with pollen.

In conclusion, more complex flowers are generally more difficult to learn and costs of learning flower handling (Figs. S1 and S2) depend on flower type, just as has been found in a nectar reward context (Laverty 1980, 1994; Gegear and Laverty 1995; Muth et al. 2015; Keasar et al. 2023). Additionally, we present rare quantitative evidence of how pollinator learning influences pollen fate, demonstrating strong positive effects for reward collection, but weak effects on pollen transfer to flowers. Our results thus confirm and extend those of Ramos et al. (2017), who found no effect of flower handling experience by nectar-foraging butterflies on pollinia transfer in milkweeds. Finally, our work has particular relevance for understanding why pollinators such as bees often exhibit floral fidelity (i.e. flower constancy), a pattern of behavior hypothesized to benefit pollinators and to drive flower evolution (Waser 1986; Chittka et al. 1999; Grüter and Ratnieks 2011; Muth et al. 2015; Ramos et al. 2017; Papaj and Russell 2024). Costs associated with learning a given flower type are thought to facilitate floral fidelity, but time penalties incurred while learning to efficiently handle flowers are often small (as we found here) and thus unlikely to facilitate floral fidelity (Woodward and Laverty 1992; Gegear and Laverty 1995; Chittka et al. 1999). In contrast, our results suggest that the costs of learning flower handling motor routines are far larger and thus have the potential to facilitate floral fidelity.

Supplementary material

Supplementary material is available at *Behavioral Ecology* online.

Acknowledgments

We are grateful to Koppert Biologicals for bee colonies, Abilene Mosher for greenhouse care, Russell lab members for discussion, and two reviewers for helpful comments. We acknowledge this work was performed on the unceded traditional territory of the Kiikaapoi, Sioux, and Osage.

Author contributions

Maggie Mayberry (Conceptualization [equal], Data curation [equal], Investigation [equal], Methodology [equal], Project administration [equal], Supervision [equal], Writing—original draft [equal]), Katherine Naumer (Data curation [equal], Investigation [equal], Methodology [equal], Supervision [equal], Writing—review & editing [equal]), Annaliese Novinger (Data curation [equal], Investigation [equal], Methodology [equal], Writing—review & editing [equal]), Dalton McCart (Data curation [equal], Investigation [equal], Methodology [equal], Writing—review & editing [equal]), Rachel Wilkins (Data curation [equal], Investigation [equal], Methodology [equal], Supervision [equal], Writing—review & editing [equal]), Haley Muse (Data curation [equal], Investigation [equal], Methodology [equal], Writing—review & editing [equal]), Tia-Lynn Ashman (Conceptualization [equal], Methodology [equal], Writing—review & editing [equal]), and Avery Russell (Conceptualization [equal], Data curation [equal], Formal analysis [lead], Funding acquisition [lead], Investigation [equal], Methodology [equal], Project administration [equal], Resources [lead], Supervision [equal], Validation [equal], Visualization [equal], Writing—original draft [equal], Writing—review & editing [equal])

Funding

None declared.

Conflicts of interest

None declared.

Ethics approval

All bumble bee experimentation was carried out in accordance with the legal and ethical standards of the United States.

Data availability

Analyses reported in this article can be reproduced using the data provided by Mayberry et al. (2024).

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