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Site fidelity by bees drives pollination facilitation in sequentially blooming plant species

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Abstract. Plant species can influence the pollination and reproductive success of coflowering neighbors that share pollinators. Because some individual pollinators habitually forage in particular areas, it is also possible that plant species could influence the pollination of neighbors that bloom later. When flowers of a preferred forage plant decline in an area, site-fidelity may cause individual flower feeders to stay in an area and switch plant species rather than search for preferred plants in a new location. A newly blooming plant species may quickly inherit a set of visitors from a prior plant species, and therefore experience higher pollination success than it would in an area where the first species never bloomed. To test this, we manipulated the placement and timing of two plant species, *Delphinium barbeyi* and later-blooming *Gentiana parryi*. We recorded the responses of individually marked bumble bee pollinators. About 63% of marked individuals returned repeatedly to the same areas to forage on *Delphinium*. When *Delphinium* was experimentally taken out of bloom, most of those site-faithful individuals (78%) stayed and switched to *Gentiana*. Consequently, *Gentiana* flowers received more visits in areas where *Delphinium* had previously flowered, compared to areas where *Delphinium* was still flowering or never occurred. *Gentiana* stigmas received more pollen in areas where *Delphinium* disappeared than where it never bloomed, indicating that *Delphinium* increases the pollination of *Gentiana* when they are separated in time. Overall, we show that individual bumble bees are often site-faithful, causing one plant species to increase the pollination of another even when separated in time, which is a novel mechanism of pollination facilitation.

Key words: *associational effects; Bombus; facilitation; floral resource; foraging behavior; magnet species effect; phenology; plant-pollinator interaction; pollination; resource depletion; sequential mutualism; site fidelity.*

INTRODUCTION

> *"I have always regretted that I did not mark the bees by attaching bits of cotton wool or eiderdown to them with rubber, because this would have made it much easier to follow their paths."*
>
> — Charles Darwin on his observations of male bumble bee flight paths (Freeman 1968:183).

Neighboring plants can influence the abiotic and biotic environment important for a focal plant's fitness. In particular, plant neighbors can alter the response of animal consumers, like pollinators and herbivores, and thus influence the amount of pollination (Mitchell et al. 2009) or herbivory (Barbosa et al. 2009) experienced by a focal plant. Such associational effects among plant neighbors may affect plant fitness and population processes (Underwood et al. 2014). In communities, plants often flower and share pollinators with other species (e.g., Pleasants 1980, Hegland and Totland 2005). If pollinators change their abundance or foraging behavior in the presence of another plant species, this community context can have negative, positive, or neutral effects on a focal plant's pollination (Rathcke 1983, Moeller 2004, Mitchell et al. 2009). Neighboring plant species are known to influence one another when they flower simultaneously: reproductive success can decline in a plant if pollinators are drawn away (Chittka and Schürkens 2001, Brown et al. 2002) or if too much interspecific pollen transfer occurs (Waser 1978, Morales and Traveset 2008, Flanagan et al. 2009); alternatively, reproductive success can increase if pollinator numbers are jointly enhanced (Johnson et al. 2003, Ghazoul 2006). Few researchers have investigated whether plants may influence another when they flower separately in time, though some studies suggest that earlier plant species may help support the population maintenance or growth of shared pollinators (Waser and Real 1979, Moeller 2004, Moeller and Geber 2005). It is important to understand if associational effects occur through time, because measures of community effects on plant reproduction or selection on traits will be incorrect if considering only contemporary contexts.

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Given what we know about flower-feeding animals, it is possible that a plant could influence another that blooms later through a pollinator behavioral response, rather than through pollinator population size effects hypothesized by Waser and Real (1979). When individuals of many different flower-foraging animals are marked as Darwin regretted not doing—animals such as hummingbirds (Paton and Carpenter 1984), sunbirds (Gill and Wolf 1975), bats (Lemke 1984), butterflies (Gilbert 1980), euglossine bees (Janzen 1971), honey bees (Ribbands 1949), and bumble bees (Manning 1956)—they are seen to confine their feeding to small areas that they return to over days or weeks. Because of this tendency for site fidelity, pollinators may stay faithful to foraging areas rather than plant species when flowers of their preferred plant decline (cf. Bronstein 1995); that is, they may show “site-holdover”. If so, site-faithful pollinators would switch to feed on a different plant species. Such later-blooming plant species or flowers would experience higher visitation because they inherited returning pollinators from a previously preferred plant, compared to areas where the previous plant species never bloomed. Thus, pollinator site-fidelity could mediate sequential pollination facilitation between plants. Thomson (1982) suggests such an effect: visits to other species in a local area seemed to increase when *Aconitum columbianum* was removed. However, we lack rigorous empirical studies to determine such sequential pollination facilitation; doing so requires collecting data on the behavior of individual pollinators and measuring their effects on plant pollination.

Bumble bees (*Bombus* spp.) are abundant and cognitively sophisticated flower visitors in temperate, alpine, and arctic areas (Goulson 2010). Data suggest that they may remain site-faithful when their preferred or “major” (Heinrich 1976) plant declines and visit new plant species, thus mediating sequential pollination facilitation. First, individual adult bumble bee workers live between 2 and 4 weeks (Rodd et al. 1980), so most will experience the rise and fall of different plant species’ flowering phenologies, and thus a decline in their major food plant. Second, bumble bees are flexible in their foraging choices: they can visit less-preferred flower types in addition to their preferred flower (Heinrich 1976, 1979), and are more likely to switch species when flowers are sparse or have low reward (Heinrich 1976, Chittka et al. 1997). Third, within a foraging area, bumble bee individuals persistently return to the locations of previously rewarding inflorescences or plants (Manning 1956, Heinrich 1976, Thomson 1988, 1996, Comba 1999, Cartar 2004). This bee response may operate at broader spatial scales: site-faithful bees may persistently return to areas of the landscape where rewarding plants previously flowered. The tendency for bumble bees to avoid unfamiliar flower types (Forrest and Thomson 2009) may conflict with such site-tenacity, however.

Here we examine how bumble bees respond to the disappearance of their major flower type in a foraging area, and if their response can cause one plant species to affect the pollination of another separated in time. Our manipulative field experiment mimics a natural progression involving two sequentially blooming plant species that share some bumble bee pollinators: the earlier and heavily visited *Delphinium barbeyi* (Ranunculaceae; hereafter *Delphinium*), and the later *Gentiana parryi* (Gentianaceae; hereafter *Gentiana*). We monitored the responses of both individual bees and the visitor assemblage. We ask: (1) are bumble bee individuals faithful to patches of *Delphinium*? (2) When *Delphinium* goes out of bloom in an area, do individual bees move elsewhere to search for their preferred species, or do they stay and switch plant species, i.e., do they display site-holdover?, and (3), are visitation rates and stigma pollen loads in *Gentiana* higher due to visits by former *Delphinium* bees in these areas?

Methods

Study system and site

We carried out the experiment in the alpine meadows of Paradise Basin (38°59′29.49″ N, 107°03′36.84″, 3390 m above sea level), north of the Rocky Mountain Biological Laboratory (RMBL), Colorado, USA. *Delphinium barbeyi* is an abundant long-lived perennial herb in mesic meadows around the RMBL; it flowers mid- to late-summer (Appendix S1: Fig. S1; Inouye et al. 2002). Its flowers are dark purple, zygomorphic, arranged in a raceme, protandrous, and self-compatible (Williams et al. 2001). *Gentiana parryi* is a common long-lived perennial herb in mesic and drier meadows around the RMBL; it flowers in the late summer and early autumn (Appendix S1: Fig. S1). Its flowers are broad, tubular, vertically oriented, and mostly royal blue with an area of white with dark speckles in the lower half. The flowers are protandrous and self-compatible, but require animal pollinators for maximum seed set (Ogilvie 2014). The two species often co-occur in meadows, sometimes within a few meters; *Delphinium* blooms before *Gentiana*, but their phenologies overlap. In the vicinity of our study site, the two species are primarily visited by some of the same medium- and long-tongued bumble bee species (Appendix S1: Fig. S1), including *Bombus kirbiellus*, *B. flavifrons, B. nevadensis, B. appositus,* and *B. fervidus*, while *Gentiana* also receives visits from short-tongued *B. bifarius, B. sylvicola,* and *B. frigidus* (Ogilvie 2014, Ogilvie and Thomson 2015). During this study, bees foraged mainly for nectar. We follow the plant nomenclature in Hartman and Nelson (2001) and bee nomenclature in Williams et al. (2014, 2015).

Experimental design and bee observations

To test whether a plant species can increase the pollination of another separated in time via pollinator site fidelity, we manipulated the placement and timing of the attractive *Delphinium* and the later-blooming *Gentiana* using arrays of cut flower stems. The experiment had two phases involving three plots in a triangle with 200 m sides (Fig. 1). The first phase spanned 2 d and involved *Delphinium* in two plots. The second phase spanned 1 d and involved all three plots: *Gentiana* was added to all areas, while *Delphinium* was removed from one, remained in one throughout, and was always absent from one. We marked individual bumble bees and monitored their individual and collective visits during both phases and on both plant species. Each of the six replicates spanned 3 d.

By removing *Delphinium* from one plot and adding *Gentiana* to all plots, we simulated the local disappearance of a major forage plant and the start of flowering of another species. By marking individual bees, we were able see whether site-faithful individuals respond to the local disappearance of their preferred plant by staying in the same area and switching plant species, or moving elsewhere to remain with their major plant, and ultimately how these responses affect the amount of pollination in *Gentiana*. We included a plot where *Gentiana*

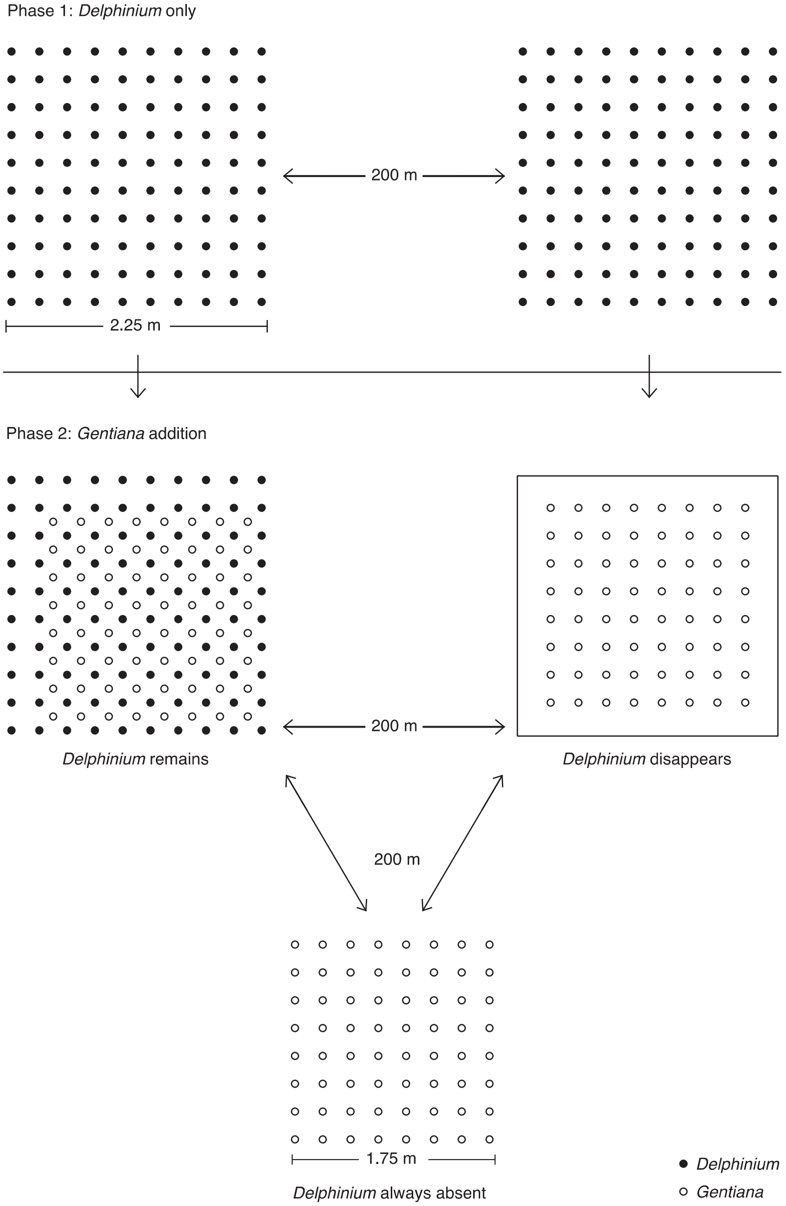

FIG. 1. Experimental design used to investigate how site-faithful bees respond to the local disappearance of their preferred forage plant and the consequences for pollinator-mediated interactions between plant species. The experiment had two phases: the first (top) comprised two patches of *Delphinium barbeyi* separated by 200 m, in which we monitored individual and collective visits over 2 d; in the second phase (bottom), on the third day, we removed *Delphinium* from one patch (indicated by the square), added *Gentiana parryi* to the two patches and to a third area at 200 m distance, and monitored known individual and collective bee visits.

flowered without any *Delphinium* in the area, to know its pollination success independent of *Delphinium.* We carried out six replicates of the experiment in two sites separated by 300 m. We alternated between the two sites so that each was used three times, though we shifted plot treatments and their locations by about 40 m. We ran one replicate at a time and isolated replicates by using alternate sites and leaving a day between the end of one replicate and the start of another. We ran the first two replicates in separate sites in August of 2010, while the remaining four were carried out in August and September of 2011 (each site was used twice in 2011 with an intervening 6 d). When we reused sites in a year, we saw only a couple of individual bees from previous replicates, but we could reliably distinguish them from the current replicate bees. We did not see individual bees move between the two sites.

Phase one: Delphinium only.— In the first phase, we established two small patches of cut *Delphinium* inflorescences, separated by 200 m. We searched within a 200 m radius of each plot and removed any other *Delphinium* inflorescences from the area, of which there were only a few small patches. Each patch comprised a 10 × 10 array of water-filled holders placed at 25 cm intervals (2.25 m on each side); two inflorescences were placed in each holder to total 200 inflorescences per patch (10.73 ± 0.55 flowers per inflorescence, $N = 40$; about 2146 flowers per patch). The flower holders were lengths of plastic irrigation tubing attached to wooden stakes with duct tape and sealed on the inside with wax; they presented *Delphinium* inflorescences at 1 m, a natural height. We intended for bumble bee individuals to consider each patch as a distinct foraging area and to move rarely between them. At a meadow nearby, 52% of closely observed bumble bee individuals foraged in single *Delphinium* patches similar in flower number, and the mean distance they moved between patches was 35 m (Ogilvie 2014). Furthermore, in the same study system, bumble bees were most often seen within 100 m of their marking location (Elliott 2009). The patches were exposed to bees for about 4 h the day before we started observations. During each replicate, we replenished half of the inflorescences before the start of the second and third experimental days. We cut fresh *Delphinium* inflorescences from populations about 2 km away immediately before they were placed in the arrays. Cut *Delphinium* inflorescences continued to open flowers, and produce and replenish nectar, though we did not quantitatively measure nectar production and replenishment rate.

During phase one, two observers simultaneously observed and marked bumble bees visiting the two *Delphinium* patches in warm and clear weather during eight to ten 30 min observation periods on each of the 2 d. We did these observations to establish which individual bees were using a *Delphinium* patch as their main foraging site. During these observation periods, at 5-min intervals (seven per period), we made "scan counts" within 1 min (Martin and Bateson 2007), in which we recorded the number of bees visiting the patch, their species and caste, the number that were marked, and the identity of any mark. We could observe the entire patch at once. The number of visits did not differ between the two plots across the experiment ($P = 0.58$) but did vary among most replicates (negative binomial generalized linear model (GLM) with replicate and plot as fixed effects, $P < 0.005$), so we include replicate as a random effect in our statistical models (see *Data analysis* below). During the observations between these scan counts, we caught unmarked bees from the flowers directly into a restraining device ("bee squeezer", Kearns and Thomson 2001:73), and uniquely marked them between the wing bases with one to three colored paint dots (Sharpie oil-based paint pens; Newell Rubbermaid, Oak Brook, Illinois, USA). The bees that we observed must have initially switched plant species or moved from other locations to forage on our experimental patches of *Delphinium*. To rigorously test our research question, such a situation was unavoidable, but we do not believe that this issue undermines our results.

Phase two: Gentiana added.— On the third day, before bees were active, we started the second phase by removing all *Delphinium* inflorescences from one patch. Within the two areas used during the first phase (in which *Delphinium* disappeared and remained), and a third at 200 m distance to form a triangle with 200 m sides, we established 8 × 8 arrays of cut *Gentiana* flowers in small water-filled florist's aquapicks positioned at 25-cm intervals, alternating with the taller *Delphinium* flower holders in those plots (Fig. 1). We cut *Gentiana* stems the evening before from plants in earlier-flowering populations 6–9 km away, from which flower visitors had been excluded using sandbag-style exclusion bags (Thomson et al. 2011), so that some flowers had receptive and unvisited stigmas. We ensured that flower numbers were approximately equal among arrays; there was a mean of 180 flowers in each array (range 141–230, ±SE 6.4). *Gentiana* was not yet flowering at our field site, so this flower was likely novel to most bumble bees.

Three observers simultaneously observed bumble bee visits to the three plots during eight to ten observation periods similar to the first phase. We only occasionally marked bees (0–11 individuals per plot per replicate), because it seemed that new individual bees were returning to visit *Gentiana*, but we were not exhaustive as we were primarily interested in the response of site-faithful bees to the disappearance of *Delphinium*. We took scan counts at 5-min intervals during 33 min observation periods; in the plot with both plant species, we took interval counts for *Delphinium* two minutes after *Gentiana*. We pooled the number of bees seen during the seven scan counts for each observation period to compare visits to *Gentiana* in the three plots. We could group bees visiting *Gentiana* into those returning individuals with a foraging history on *Delphinium* during phase one ("old repeat bees"), those returning individuals that were new during phase two and without a *Delphinium* history ("new repeat bees"), and those that were unmarked ("unmarked bees"). During phase two, we also measured whether the bumble bees had a preference for *Delphinium* or *Gentiana* flowers by carrying out choice tests outside of observation periods. We presented the two flower types at the end of a stick to free-foraging bees in the arrays, and recorded the first flower type that they chose (the "interview stick" technique, Thomson 1981).

Stigma pollen loads

We compared stigma pollen deposition—and likely pollination success—in *Gentiana* between areas where *Delphinium* disappeared and was always absent. We did not include *Gentiana* stigmas from areas where *Delphinium* remained in this comparison, because pollen grains of the two species are too similar for us to distinguish. At the end of each replicate we collected all *Gentiana* stems and let pollen tubes develop in the lab. We excised the bilobed stigmas, stored them in 70% ethanol, stained the lobes in lactophenol-aniline blue (Kearns and Inouye 1993), mounted them on microscope slides, and photographed them under a high-magnification dissecting microscope. We used a qualitative scale from 0 (no pollen grains) to 5 (maximum coverage of pollen grains) to score each of the two lobes of a stigma from the images. One person scored all samples, blind to the treatment, and periodically compared some scored stigmas to a set representative of each score, to ensure that her qualitative scoring was not drifting during the process.

Data analysis

We completed all analyses in R version 3.2.2 (R Core Team 2015). To determine how returning bees responded to the disappearance of *Delphinium*, we examined the phase two responses of bees that were site-faithful, in phase one, to the plot where *Delphinium* later disappeared. We compared the proportion of site-faithful bees seen again during phase two that (1) stayed in the same plot and visited *Gentiana,* (2) moved areas and visited *Delphinium* where it remained, (3) moved areas and visited *Gentiana* where *Delphinium* remained, and (4) moved areas and visited *Gentiana* where *Delphinium* was always absent. We compared the proportional response of individual bees using a GLM with a binomial error distribution and a logit link function with type of response as a fixed effect. We defined "site-faithful" bees as those that were seen in the plot where *Delphinium* was later removed at least five times during phase one. We also included all other known bees seen during phase two, if they were seen at least once and spent 25% or more of their time in the plot where *Delphinium* later disappeared in phase one.

We determined whether bumble bees had a preference for *Delphinium* or *Gentiana* flowers during phase two by first pooling all choice tests across replicates. For the plots where *Delphinium* was removed or always absent, in which only *Gentiana* was present, we compared the number of choices for *Gentiana* and *Delphinium* flowers with *G*-tests. In the plot where *Delphinium* remained, bees were foraging on either *Gentiana* or *Delphinium* and could choose either species on the interview stick, so we used a two by two contingency table test that compared the flower type choices made by bees foraging on either plant species. For all visitation variables, we pooled the responses of all bumble bee species and castes because we did not have sample sizes large enough to compare them (visits were dominated by one species).

Turning to pollination-related variables, we compared (1) the number of bee visits during pooled scan counts among plots where *Delphinium* remained, disappeared, and was always absent, and (2) stigma pollen load scores between plots where *Delphinium* disappeared and was always absent. We used generalized linear mixed models (GLMMs) because we had non-normal data and non-independent observations (we had repeated observations or measurements on plots within replicates). For both response variables we used models with plot as the fixed effect and replicate as a random effect. By including replicate as a random effect, we accounted for the variation attributable to replicate, which was not our main interest (Zuur et al. 2009). For the visitation response variable, in which the count data were overdispersed, we specified a negative binomial error distribution and log link function with the function *glmmadmb* in the R package *glmmADMB* (Skaug et al. 2014). For the stigma pollen load response variable, in which the scores were bound between 0 and 5, we specified a model with binomial errors and a logit link function using function *glmer* in R package *lme4* (Bates et al. 2015). Unless otherwise noted, we present means and their standard errors in parentheses.

RESULTS

Phase one: bumble bee site-fidelity

We recorded 8697 bumble bees in *Delphinium*-plot scan counts totaling 23 h; counts per minute ranged from 0 and 18 bees in each plot (6.31 ± 0.09). We saw four *Bombus* species and bees of all castes (workers, males, and new queens); *B. kirbiellus* and males were the most common species and caste (Appendix S2: Table S1). We marked a total of 867 bees in the *Delphinium* plots over six replicates of the experiment; between 58 and 86 bees in each plot. We saw between 35.7% and 75.9% of individuals again in each replicate (63.4 ± 6.0%); between 6.5% and 32.2% of marked individuals were resighted frequently (23.4 ± 3.8%) in our observations, in at least 10% of all scan counts. The typical frequency distribution of the number of times individuals were resighted was right-skewed, with a small number of bees returning more frequently to plots. Visits by marked bees accounted for between 48.1% and 89.3% of all bee visits to the *Delphinium* plots (72.5 ± 6.3%), and marked individuals were seen between 1 and 87 times during the first phase of all replicates. Most bees returned only to one *Delphinium* plot, but 4.3–15.4% of returning bees were seen more than 10% of the time in the second plot (7.7 ± 1.6%; i.e., they used both plots as foraging areas).

Phase two: bumble bee response to Delphinium disappearance

In the plots where *Delphinium* disappeared, we saw 62.1–85.1% of site-faithful bees again (76.7 ± 4.3%, $N = 6$; there were 14–39, mean 30, site-faithful bees in each replicate). These returning individual bees responded in four ways to the disappearance of *Delphinium* (Fig. 2). Most individuals responded in one way (85.3 ± 1.6%), though they occasionally showed two (10.5 ± 1.3%) or three responses (4.1 ± 1.5%); we consider each unique response from an individual and not the total sum of responses from each individual. The most common response was for bees to stay in the plot and visit *Gentiana* flowers (78.1 ± 3.0%; Fig. 2). Of the remaining responses, 16.1 ± 2.1% moved areas and visited the *Delphinium* that remained, 5.1 ± 1.5% moved areas and visited *Gentiana*, while one bee (in the entire experiment) moved areas and visited the *Gentiana* where *Delphinium* was always absent (0.06%). The proportions of bees responding in the four ways differed significantly according to the GLM model ($P < 0.025$; Fig. 2).

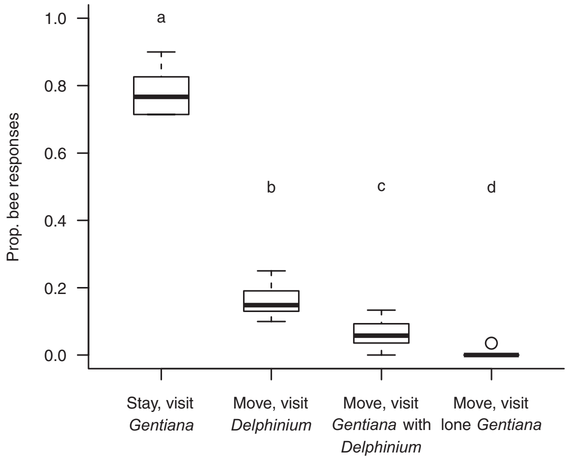

FIG. 2. Tukey boxplots showing the proportional response of site-faithful individual bumble bees to the local disappearance of *Delphinium barbeyi*, their forage plant, and the addition of a new plant species, *Gentiana parryi*. Returning individuals could respond in four different ways: (1) stay in the same area and switch to *Gentiana*, (2) move 200 m and stay foraging on *Delphinium*, (3) move 200 m and switch to *Gentiana* where *Delphinium* remained, and (4) move 200 m and switch to *Gentiana* where *Delphinium* was always absent. We included all unique responses from an individual and the number of individuals varied among replicates ($N = 6$). Different letters indicate significant differences (binomial GLM, $P < 0.025$).

Phase two: bumble bee flower preferences

In the plots where *Delphinium* remained, the flower preference of bees visiting choice bouquets strongly depended on the species they were foraging from (two by two contingency table, $X^2 = 85.6$, $P = <10^{-15}$). Those bees visiting *Delphinium* were more likely to choose *Delphinium* (87.9%) than *Gentiana* (12.1%, $N = 99$), while those visiting *Gentiana* showed no preference (49.1% chose *Delphinium,* 50.9% chose *Gentiana*; $N = 55$). Interestingly, in the plots with only *Gentiana*, flower preference depended on the "ghost" of *Delphinium.* Where *Delphinium* was always absent (where most bees had no recent experience with *Delphinium*), the bees showed no preference for either flower type (47.8% chose *Delphinium,* 52.2% chose *Gentiana*; *G*-test, $G = 0.058$, $P = 0.81$, $N = 46$). However, where *Delphinium* had disappeared, bees preferred *Delphinium*; 71.4% chose *Delphinium*, while 28.6% chose *Gentiana* (*G*-test, $G = 29.64$, $P = 5.2 \times 10^{-08}$, $N = 231$). *Delphinium* was likely preferred over *Gentiana* because of its higher nectar production: though the flowers of *Gentiana* have higher nectar volume, *Delphinium* flowers are higher in nectar sugar concentration (J. E. Ogilvie, *unpublished data*) and were about an order of magnitude more abundant at the patch level in this experiment.

Phase two: Gentiana pollination

During the second phase, we recorded 2205 bee visits to *Gentiana* arrays during 17 h of accumulated scan counts. *Gentiana* flowers received more bumble bee visits in plots where *Delphinium* disappeared (27.08 ± 6.15) compared to plots where *Delphinium* remained (12.95 ± 3.04) or was always absent (5.19 ± 1.61; Fig. 3a). These visit numbers were significantly different between plots (GLMM, $P < 10^{-11}$; Fig. 3a). As array flower number was similar for all plots in a replicate, per-flower visitation rates were highest in the plot where *Delphinium* disappeared, though where *Delphinium* remained, it also increased visitation rates to *Gentiana* flowers relative to areas where *Delphinium* was absent. Given the common response of site-faithful bees that we observed (Fig. 2), pollinator site holdover likely contributed to the greater number of visits when *Delphinium* disappeared. Furthermore, a higher proportion of all visits to *Gentiana* in these areas were by known individuals who had foraged on *Delphinium* during phase one ("old repeat bees"; Fig. 3b), compared to the other areas (three-sample proportions test, $X^2 = 308.93$, $P < 10^{-15}$). In the plot where *Delphinium* was always absent, *Gentiana* flowers received most of their visits from bees that did not have a foraging history with *Delphinium* (38.9% and 44.7% of visits were by unmarked and "new repeat" bees, respectively, totaling 83.6%; Fig. 3b).

We collected between 38 and 56 *Gentiana* stigmas from plots where *Delphinium* disappeared and was always absent (total 554 stigmas). Stigma pollen loads were significantly higher where *Delphinium* disappeared

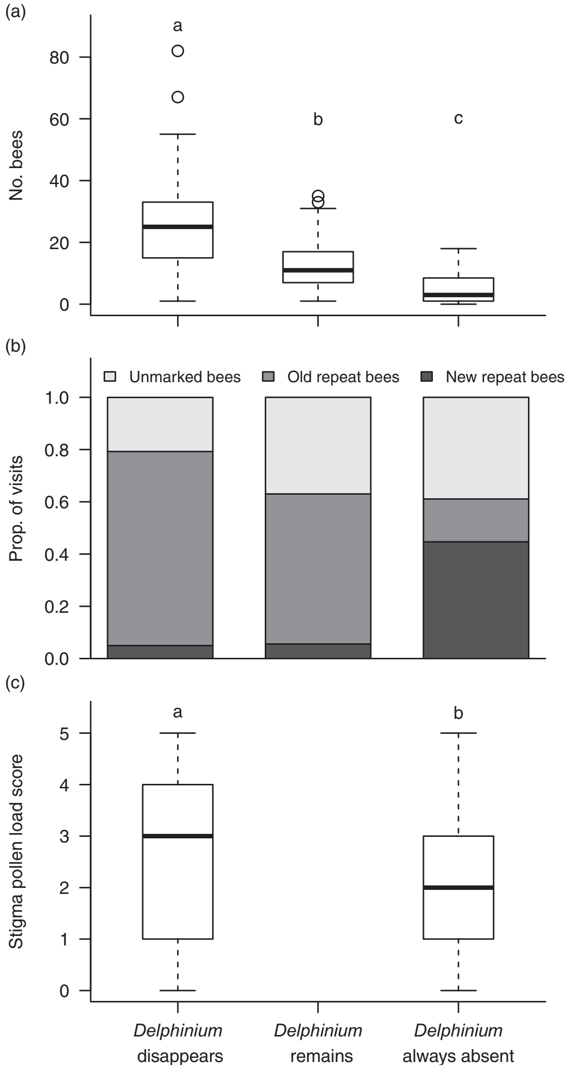

FIG. 3. Pollination levels in *Gentiana parryi* arrays over a day in areas where *Delphinium barbeyi* disappeared, remained, or was always absent. (a) Tukey boxplots showing the number of bee visits during pooled scan counts (7 min). (b) The proportional composition of total visits by individual bees with particular foraging histories, pooled across replicates. Old repeat bees were those marked during phase one of the experiment on *Delphinium*, while new repeat bees were those who were marked on *Gentiana* and returned during phase two. (c) Tukey boxplots showing the amount of pollen on *Gentiana* stigmas on a qualitative scale from 0 to 5 (no pollen grains to maximum coverage of grains). We do not show *Gentiana* stigma pollen load data from the areas where *Delphinium* remained because we could not distinguish pollen grains of the two plant species. Different letters indicate significant differences with GLMMs, $P < 10^{-11}$.

(2.47 ± 0.06) compared to where it was always absent (1.74 ± 0.06; GLMM, $P < 10^{-11}$; Fig. 3c).

DISCUSSION

The foraging habits of individual bumble bees caused one plant species to increase the pollination of another that bloomed later. Many individual bumble bees returned repeatedly to the same small areas to forage on *Delphinium*, an attractive forage plant. When *Delphinium* was experimentally taken out of bloom, most of the known bees that we saw again remained faithful to their foraging area and switched to forage on the newly flowering plant species, *Gentiana*, rather than move to an area within their flight range (Elliott 2009, Ogilvie 2014) to stay faithful to their preferred plant. *Gentiana* pollination was higher in areas where *Delphinium* had been previously flowering compared to areas where it had never occurred, because these area-faithful bees quickly adopted *Gentiana* and became a set of returning visitors. If pollinators commonly have a lag in response to floral resources, or if they show foraging site holdover as we show here, then plant pollination success can be partly determined by the density of floral resources, conspecific or heterospecific, at some time in the recent past. That is, areas may retain a legacy from past floral neighborhoods that can affect flower pollination success; the temporal scale of floral neighborhoods can be broader than researchers have assumed.

Most of our site-faithful bumble bees persisted at a site where their forage plant was depleted, which collectively had measurable ecological consequences for a co-occurring plant. Will pollinators commonly respond this way? We know little about the individual site-fidelity of many flower feeders (Thomson and Chittka 2001), but a taxonomically diverse set of animals are known to visit foraging areas repeatedly. By their very nature, such site-tenacious animals may be likely to stay in a foraging area—if there is a suitable plant species to switch to—so we expect the response to be fairly common. Indeed, animals other than bumble bees will return to previously rewarding nectar locations in the field, including euglossine bees (Janzen 1971), honey bees (Moore et al. 2011), and hummingbirds (Hurly 1996). Furthermore, the species of pollen collected by solitary mason bees seemed to be driven by their site fidelity and not the floral density of available resources (Williams and Tepedino 2003). Thus, pollinator foraging choices governed by site fidelity may affect plant pollination, as we show here, but also pollinator resource collection and fitness. In particular, we expect this site-holdover to be a common response of bumble bees, as individuals are often site-tenacious (e.g., Thomson 1996), and show flexible flower choices at a site (Heinrich 1976, 1979). While bumble bees can be averse to novel flowers (Kawaguchi et al. 2007, Forrest and Thomson 2009), when experiencing depleted rewards in a preferred plant, they may more readily sample different flower types and adopt new specialties (Heinrich 1976, 1979). Furthermore, bees are more likely to visit novel flower types if occupied by conspecific bees (Leadbeater and Chittka 2005, Kawaguchi et al. 2007), which may have accelerated the adoption of *Gentiana* flowers in our study.

Why did site-faithful bees so commonly stay and switch flower types when their preferred flower disappeared?

Persisting at a depleted site could be suboptimal behavior. If foraging animals maximize some sort of foraging currency, a site-faithful animal's response to resource depletion may be based on weighing the benefits and costs of staying or leaving. In this sense, the time and energy costs involved in searching for new foraging areas, and in learning and memorizing new routes to the nest, may vastly exceed the benefits of staying with a familiar flower type. It may be adaptive for bumble bees to persist at a site and opportunistically sample unfamiliar flowers for a few reasons, especially in montane meadows where flowers are continuously available over the growing season. First, a worker's lifespan can span the end and start of different species' flowering phenologies, and colonies must forage from a range of flowers throughout the growing season to grow and reproduce (Heinrich 1976), so visiting new flowers could be a way to keep track of changing resources (Heinrich 1979). Second, foragers may often encounter depleted floral rewards due to exploitation by other foragers (Zimmerman 1988, Renner 2006), but such depletion will be transient, so it would be beneficial for foragers to return. Third, resource-rich patches may tend to be rich through time—if those areas are suitable for a high density of flowering plants—and it would be beneficial for individual foragers to return to resource rich areas. Modeling the benefits and costs of a site-faithful pollinator's response to resource depletion would help inform how general site-holdover behavior might be.

Later-flowering *Gentiana* benefitted from flowering in an area with a legacy of *Delphinium*. *Delphinium* acted as a "magnet plant species," a species with highly rewarding flowers that attracted a disproportionate number of animal visitors relative to other plants in a community (sensu Thomson 1978, Laverty 1992); those persistent visitors then spilled over to *Gentiana*. Importantly, *Delphinium* attracted many returning individuals, as magnet plants might regularly do, though this has not been explicitly measured before. The visitation experienced by *Gentiana* flowers in the plot where *Delphinium* was always absent shows that newly blooming plant species can take some time to recruit flower visitors. However, this lag in the discovery of new resource patches can be reduced if plants adopt visitors already faithful to a site; the action of only a modest number of returning bees increased flower visitation rates and stigma pollen loads in *Gentiana* (9–31 bees per plot in our study). Furthermore, for the temporal pollination facilitation mechanism that we describe, there is little chance for the sequential plant to experience negative reproductive effects due to interspecific pollen transfer, which can occur when pollinator-sharing plants bloom simultaneously (Morales and Traveset 2008, Flanagan et al. 2009).

How likely a site-faithful animal is to stay in an area and switch flower types when a preferred plant declines may be context dependent. A pollinator may be more likely to stay and switch if the flowers of the sequential species are similar in color (cf. Chittka et al. 1997), scent (cf. Waelti et al. 2008), or shape and size (cf. Gegear 2005), to the original plant. Bees may be less likely to stay and switch if the new flower type is complex with concealed rewards, because learning to correctly handle such flowers takes time when they are novel (Laverty 1980). In our experiment, the simple open tubular flowers of *Gentiana* likely made the switch easier for bumble bees. In addition, the tendency of individual bees to return to a depleted foraging site may increase with their experience and the reward received at a site (Townsend-Mehler and Dyer 2012, Al Toufailia et al. 2013). Sequential pollination facilitation through pollinator site fidelity may be most likely in situations involving high-reward plant species that attract an abundance of flower feeders (which are common in most plant communities, e.g., Heinrich (1976)). In our field experiment, we removed *Delphinium* and added *Gentiana* flowers suddenly, whereas in most natural situations flowers of a plant species would decline and begin more gradually. We chose this protocol due to logistical constraints, but expect similar responses to more gradual declines of preferred flowers. In such situations, pollinators may increasingly visit the newly blooming alternative as the preferred flower type fades. The threshold density at which a forager will abandon its preferred flower for another is likely to be extremely variable, dependent on such things as the plant and animal association, and the type, number, and reward level of other flowers available in the plant community. We did not measure the duration of *Delphinium's* positive legacy effect, but it will fade as the memories of individual bees are replaced with more recent rewarding sites or species, or as those bees disappear. We expect that the site fidelity effect would persist at least for a few days, or for about half of the flowering duration of an individual *Gentiana* plant (mean flowering duration 9.6 ± 0.2 d, J. E. Ogilvie, *unpublished data*). Thus, we believe that the site fidelity effect will be ecologically important for *Gentiana* plants.

We show that site fidelity by individual pollinators can cause pollination facilitation between plant species separated in time. Plants may also increase the pollination of later plants in the community if their presence increases pollinator population sizes by offering floral resources through an extended period (Waser and Real 1979, Moeller 2004, Moeller and Geber 2005). We speculate that sequential pollination facilitation, as hypothesized by Waser and Real (1979) and documented here, may be common in plant communities but is difficult to measure because it occurs over broad temporal, and spatial, scales. We also expect that knowing the foraging behavior of pollinator individuals—data that Charles Darwin regretted not collecting—will uncover further interesting and ecologically consequential patterns.

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