Nectar addition changes pollinator behavior but not plant reproduction in pollen-rewarding *Lupinus argenteus*

Jacob M. Heiling[1,2,3,4,6] (iD) , Judith L. Bronstein[2,5], and Rebecca E. Irwin[1,2]

PREMISE: In addition to its role as the male gamete, pollen is often used as a food reward for pollinators. Roughly 20,000 species of angiosperms are strictly pollen-rewarding, providing no other rewards to their pollinators. However, the influence of this strategy on pollinator behavior and plant reproduction is poorly understood, especially relative to the nectar-reward strategy. We performed a field experiment using the strictly pollen-rewarding *Lupinus argenteus* to explore how the absence of nectar influences pollinator behavior and plant reproduction.

METHODS: We added artificial nectar to *Lupinus argenteus* individuals to simulate a phenotype that would reward pollinators with both nectar and pollen. We compared bee pollinator behavior, via direct observation, and female reproduction between nectar-added and nectarless control plants.

RESULTS: Bees exhibited behavioral responses to the novel reward, collecting nectar as well as pollen and spending 27% longer per flower. Pollen transfer increased with flower visit duration. However, plants in the study population were not pollen-limited; consequently, the observed changes in pollinator behavior did not result in changes in female components of plant reproduction.

CONCLUSIONS: The addition of nectar to pollen-rewarding plants resulted in modest increases in per-flower pollinator visit duration and pollen transfer, but had no effect on reproduction because, at the place and time the experiment was conducted, plants were not pollen-limited. These results suggest that a pollen-only reward strategy may allow plants that are visited by pollen foragers to minimize some costs of reproduction by eliminating investment in other rewards, such as nectar, without compromising female plant fitness.

KEY WORDS   *Bombus*; floral reward strategy; foraging; nectar addition; novel phenotype; pollen limitation; pollinator reward; single-visit deposition.

Nectar is likely the most widespread reward that flowering plants provide to pollinators, but it is not the ancestral condition in angiosperms (Armbruster, 2012). Plant gametophytes (pollen and ovules) are thought to have been among the first floral resources for which insects visited flowers and were the first floral rewards (Simpson and Neff, 1983; Luo et al., 2018). Many angiosperm species offer both pollen and nectar rewards, other species are strictly nectar-rewarding, while others have maintained or secondarily derived a strictly pollen-reward strategy. In some cases, all three of these strategies can be found within a plant family (e.g., Papaveraceae, Solanaceae, Orobanchaceae, and Fabaceae; Simpson and Neff, 1983; Macior et al., 2001; Etcheverry et al., 2012).

The use of pollen as the sole reward represents a paradox, i.e., pollen-rewarding plants pay for the movement of gametes with gametes. Every grain of pollen consumed by a pollinator is a lost reproductive opportunity (Armbruster, 2012). Strictly pollen-rewarding plants often make more pollen per flower than do close relatives that offer nectar and pollen (Etcheverry et al., 2012, but see Cruden, 2000 for a comparison across broad plant families), presumably to offset lost reproductive opportunities due to pollen consumption (Darwin, 1859). Why should a plant use its gametes as a reward? Inroads to addressing this question may be made by first asking more proximate questions about pollen rewards. In particular, comparing the pollen-reward strategy to other strategies may

inform our understanding of its ecological consequences for plant reproduction. Here we ask, how might offering nectar affect pollinator behavior and plant fitness in pollen-rewarding plant species? A better understanding of how the different reward strategies mediate pollinator behavior, and how reward-mediated variation in pollinator behavior influences plant reproduction, are first steps in understanding why some lineages have maintained or re-derived this character state while others (often close relatives) have not.

Here we explore whether benefits to plants would differ between the pollen-only and the pollen + nectar reward strategies, and whether such differences could be explained by pollinator foraging behavior. We did so by comparing the behavior of pollinators and components of reproduction in the strictly pollen rewarding species silvery lupine (*Lupinus argenteus* Pursh, Fabaceae) to individual plants whose flowers were augmented with artificial nectar. While bees engaged in pollen foraging are primarily seeking pollen rewards, pollen foragers are known to also collect nectar during pollen foraging trips (Heinrich, 1976). Thus, *L. argenteus* individuals bearing both pollen and novel nectar rewards effectively represent richer resource patches for foraging bees than natural, nectarless, plants. As a result, we predicted an increase in pollinator visitation (i.e., more frequent or longer foraging at more rewarding flowers or plants, from the perspective of the bee), either via increased numbers of visits, increased time spent per flower, or both, in nectar-added plants. This prediction follows from the Marginal Value Theorem (Charnov, 1976), which predicts, in part, that foragers will spend longer in patches with higher-than-average resource availability, a prediction that has been supported in several experimental and observational pollinator foraging studies (e.g., Pyke, 1978; Thomson, 1986). If such increased visitation results in increased pollen deposition, and if seed set is pollen-limited, we predicted an increase in female reproductive success in nectar-added plants relative to controls. Importantly, while increased visit frequency and duration often result in increased pollen deposition, this relationship cannot be assumed for all systems (King et al., 2013), and must be assessed empirically. Specifically, we asked four questions: (1) Will pollinators discover and collect nectar if it is added to the flowers of a species in which they have not previously encountered nectar? Finding that bee pollinators do feed on added nectar, we asked: (2) How does nectar addition affect pollinator foraging behavior and plant reproductive success? Finding a significant positive effect of nectar addition on flower visit duration but not on female reproductive success, we then asked: (3) How does time spent per flower affect single-visit pollen deposition in *L. argenteus*; and (4) Does *L. argenteus* exhibit pollen limitation?

We note that while the mating cost imposed by pollen consumption is a cost to reproductive success through male function, benefits associated with the pollen-reward strategy may come through male function, female function, or both (Stanton et al., 1986; Wilson et al., 1994). Due to the difficulty of reliably assessing reproductive success through male function in the field (Snow and Lewis, 1993), and the unique difficulty of accessing or manipulating the androecium of *Lupinus* species without destroying other floral structures, in this study we focused our direct measurements of plant reproduction through female components alone. However, because movement patterns of pollinators can predict patterns of male mating success (Klinkhamer et al., 1994; Harder and Barrett, 1995; Stanton et al., 1986), our assessments of pollinator behaviors on nectar-added and control plants can serve as indirect proxies for variation in male mating success.

MATERIALS AND METHODS

Study system

Lupinus argenteus is a long-lived herbaceous perennial, occurring in dry meadows and rocky slopes from the montane to alpine zones across the western United States and into northern Mexico. We studied *L. argenteus* in the Gunnison National Forest in the Elk Mountains of Colorado, USA near the Rocky Mountain Biological Laboratory (RMBL). The papilionaceous flowers are blue to purple with white banner spots and are borne in whorls on indeterminate stalks. Plants produce 1 to 40+ flowering stalks at a time (median 4 stalks per plant) with 1 to 20 flowers per stalk (median 6 flowers per stalk). Flowers are receptive and pollen is viable for approx. 5 d, remaining on the stalk (following color change of the banner petal from white to purple) for an additional 5 to 7 d (Gori, 1989). A single plant produces a few to nearly 1000 flowers in a season (mean = 300 flowers/season at our site). As with other lupines (Dunn, 1956; Müller, 1873), *L. argenteus* is strictly pollen-rewarding (Gori, 1989); flowers are invariably dry when dissected and staining flowers with neutral red reveals no floral nectaries (J. Heiling, personal observations). *Lupinus argenteus* is self-incompatible and pollinated by pollen-foraging bees, primarily in the genera *Bombus*, *Andrena*, and *Megachile* (Dunn, 1956; Gori, 1989; Harder, 1990). Pollen is dispensed from a pore in the distal end of semi-fused keel petals (Müller, 1873) which must be depressed away from the axis formed by the banner and petiole to eject pollen. A fixed volume of pollen is dispensed at each visit (Harder, 1990), with the keel refilling with pollen as anthers dehisce following removal. Bees activate the piston-like mechanism by bracing their heads on the banner petal (Figs. 1A, 2B), clutching the wing petals (Fig. 1A.2) that enclose the keel (Fig. 1A) with their mid- or forelegs (Fig. 2B), and then extending their legs (Fig. 2C), resulting in deposition of pollen on their abdomen or thorax (Dunn, 1956). Bees typically approach lupine flowers with their glossas extended (Fig. 2A) and probe the inner corolla space with their glossas while positioning themselves (Fig. 2B). Despite the absence of nectar rewards in natural lupine corollas, the corolla probing behavior observed in lupines has been interpreted as attempted nectar seeking (Heinrich, 1976); nectar accumulation occurs in this inner corolla space in many nectar-producing papilionaceous flowers (e.g., *Vicia* spp. [Fabaceae]; Stpiczyńska, 1995). Alternatively, the use of the glossa in this way may facilitate landing and positioning (e.g., Fig. 2A). Regardless of the cause of this behavior, it presented the opportunity to place artificial nectar in a location that pollen foraging bees would be likely to discover it. Finally, while collecting pollen from lupine flowers, bees' bodies do not come in contact with the area between the wing and keel petals (Fig. 1B.5), with their abdomen or thorax (depending on body size) only contacting the tip of the keel (Fig. 1B.4) while operating the piston mechanism (Fig. 2C). As a result, the inner corolla area (between the wing and keel petals) is an ideal location in which to deposit artificial nectar (Fig. 1B.6).

Do pollinators respond to nectar addition by removing nectar?

To assess whether bees discover and collect artificial nectar in lupine flowers, we added 2 μL of artificial nectar (hereafter, referred to simply as nectar; 40% v/v sucrose, concentration chosen to mimic the co-flowering community, Luo et al., 2014) to all open flowers on three *Lupinus argenteus* individuals (one individual per d on
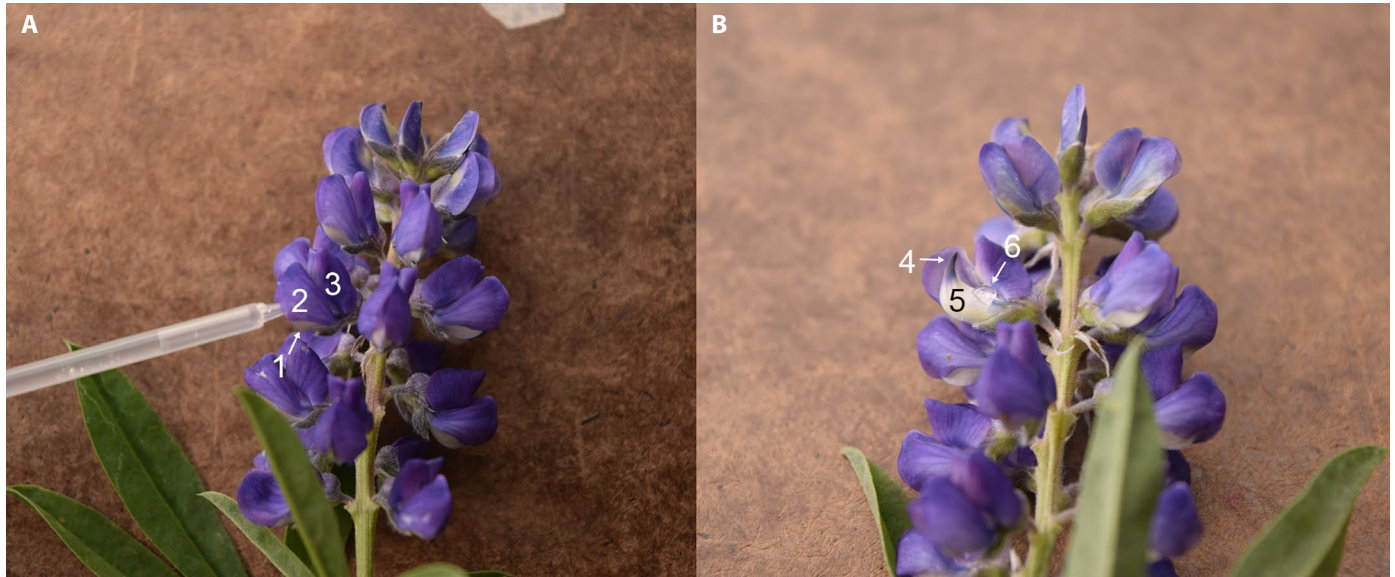
FIGURE 1. *Lupinus argenteus* corolla structure and the artificial nectar placement method and location. (A) Inflorescence with micro-pipette tip placed between keel (1) and wing (2) petals to deposit artificial nectar. Banner petal (3) is also labeled. (B) One wing petal removed to reveal keel tip (4), body of fused keel petals (5), and 2 μL droplet of artificial nectar (6).

25–27 July 2017) at our study site (Bellview Bench, N: 39.00490°, W: –107.03182°, 3139.74 m a.s.l. plot area approx. 11 × 11 m). We deposited nectar between the keel and wing petals (Fig. 1A, B) using a micropipette (Fisherbrand, HandyStep, Thermo Fisher Scientific, Waltham, Massachusetts, USA). We then directly observed the stalks. When a bee initiated a visit to a stalk, we allowed her to visit several flowers, but shooed her away before she revisited any flowers or visited all flowers on the stalk. We then used 5 μL microcapillary tubes to measure the residual nectar in each visited flower and in a neighboring unvisited flower on the same stalk. Once a stalk was visited, we removed it from the plant.

We used a paired t-test to compare the within-stalk mean nectar volumes of visited and unvisited flowers. We conducted the analysis on nectar volume means per stalk for visited vs. unvisited flowers to prevent pseudoreplication. R version 3.5.1 (R Core Team, 2018) was used for all statistical analyses.

How does nectar addition affect pollinator foraging behavior and plant reproductive success?

We arbitrarily selected 98 *Lupinus argenteus* individuals at Bellview Bench (the majority of *L. argenteus* at the site) and randomly assigned them to one of two treatments: (1) nectar addition (pollen + nectar rewarding plants, N = 49); or (2) control (pollen-rewarding only, N = 49). Daily nectar addition to all open flowers on nectar-added plants (with order randomized daily) began soon after flowering (on 14 July 2016) and was discontinued when plants passed peak bloom (13 August 2016). Floral display size (open flowers per plant) was recorded on all plants each day of nectar addition. We probed flowers on control plants with a dry micropipette tip to control for flower handling. On both the nectar-added and control plants, we cleaned the micropipette tip with ethanol between plants to prevent pollen transfer. To avoid diluting the added nectar with morning dew, we added it once plants were dry, approx. 10:00–11:00 AM.

We directly observed bee activity for 14 d between 19 July and 4 August 2016. Observations were conducted for 1 h per d immediately following nectar addition; pilot trials using unvisited flowers (protected with organza bags to prevent bee visitation) showed that nectar volume decreased measurably in field conditions after that period of time, likely due to evaporation. All observations were documented using a digital voice recorder. When we spotted a bee visiting an experimental plant, we followed her and recorded her behavior until she left the study area or flew out of sight. We recorded (1) the approaches to a plant that resulted in flower visitation, summed across the flowering season, to each individual plant (hereafter "total visits"), (2) the number of flowers probed per plant per plant visit, and (3) the time spent per flower (in seconds). We also recorded the species and caste of each visitor; only females (workers and queens) were observed visiting *Lupinus argenteus*. Each time a bout was observed, the bee received a unique ID in our dataset. Because we did not mark bees, it was not possible to determine whether a bee had been observed previously and so we were unable to analyze behavior at the level of the individual bee. Accordingly, we treated visitor ID as a random effect in all analyses.

We did not bag experimental stalks following observations. While it is possible that subsequent visits to experimental plants following the removal or evaporation of nectar may have reduced the overall signal of the nectar treatment on female reproductive success, we chose to leave stalks unbagged in order to facilitate a more biologically realistic design, in which plants remained available to visitors regardless of their reward status.

In order to assess the effect of nectar addition on female reproductive success, we collected all fruit-bearing stalks from nectar addition and control plants between 29 August and 4 September 2016, allowing fruits to dry on the stalk before dissecting them to count the seeds in each fruit. We weighed the seeds per plant to the nearest 0.1 mg.

We used the following packages in R to statistically analyze the data. We used *stats* to fit linear models (LM), *MASS* to fit generalized

linear models (GLM) (Venables and Ripley, 2002), *lme4* to fit linear and generalized mixed effects models (LMM and GLMM) (Bates et al., 2014), and *'lmerTest'* (Kuznetsova et al., 2015) and *'piecewiseSEM'* (Lefcheck, 2016) to perform inference tests and to estimate pseudo r-squares for GLMMs, respectively.

To understand how nectar addition affected pollinator behavior, we analyzed (1) total visits per plant, (2) number of flowers probed per plant visit, and (3) time spent per flower. We assessed the effect of nectar treatment on total visits using a generalized linear model with a negative binomial error distribution (data were overdispersed, dispersion parameter = 2.04; θ = 1.602, maximum likelihood) with nectar treatment as the main predictor and mean floral display (mean open flowers per individual per d across the season) as a covariate. To assess the effect of nectar treatment on the number of flowers probed per visit, we fit a Poisson GLMM with nectar treatment and floral display size (on the day a visit was observed) as fixed effects. Random effects included visitor ID (to account for multiple observations of a single individual bee), plant ID (to account for resampling of individual plants within d and across the season) and date nested within plant. We scaled display size using the base R function *scale()* to reduce Eigenvalue size and improve model fit. We compared the time visitors spent per flower on nectar addition and control plants using natural log transformed mean flower visit duration (transformed to meet assumptions of Gaussian distribution) as the response with nectar treatment (addition vs. control) and display size (scaled as above) as fixed effects in a LMM, with visitor ID and date nested within individual plant as random effects.

We measured three components of female reproductive success: (1) total seed production (all mature seeds per plant); (2) mean seed set per fruit per plant; and (3) mean weight per seed per plant. Because we did not mark individual flowers, and because *Lupinus argenteus* fruits do not leave scars on the stalk, we could not calculate proportion fruit set. To assess the effect of nectar treatment (addition vs. control) on total seed production per plant, we fit a negative binomial GLM (seed count data were overdispersed, θ = 0.765, maximum likelihood) with total seed production and mean floral display size as fixed effects. Seeds per fruit (Shapiro–Wilk: W = 0.920, $P < 0.0001$) and mean seed mass (W = 0.946, $P = 0.003$) were not normally distributed. Accordingly, we assessed the effects of nectar treatment on the mean number of seeds per fruit per plant and mean mass of mature seeds using two-sample Wilcoxon tests (R function *wilcox.test()* in the *'stats'* package).

How does time spent per flower affect single-visit pollen deposition?

To determine whether pollinator effectiveness (measured as single-visit pollen deposition on a stigma) changed as a function of the length of a pollinator visit, we used the interview-stick method (Thomson, 1981). We affixed unvisited *Lupinus argenteus* inflorescences to the end of a 1 m rod, and treated the flowers with either 0, 2, or 4 µL of nectar. We used a range of nectar volumes to promote variation in visit duration, not to test for nectar volume preference among visitors. Inflorescences on interview sticks (Thomson, 1981) were offered to bees observed foraging on *L. argenteus* in our site. When a bee accepted a flower, we recorded the duration of the visit using digital voice recorders, shooing the bee away when she exited the first flower that she accepted. We then removed the stigma from that flower with forceps and stored it in a vial on ice. In the lab on the same day, we mounted stigmas on glass microscope slides with

basic fuchsin gel (Kearns and Inouye, 1993) and counted the number of conspecific and heterospecific pollen grains at 400× under a compound microscope (N = 24 stigmas). We ran interview-stick trials on three d between 28 July and 12 August 2017. Heterospecific pollen deposition was numerically low (< three grains found on three stigmas) and was not include in our analyses.

To assess the effect of visit duration on single-visit pollen deposition, we fit a negative binomial GLMM (pollen count data were overdispersed, dispersion parameter = 2.62; θ = 3.098, maximum likelihood) with visit duration, nectar treatment (2 and 4 µL nectar addition and control) and their interaction as fixed effects, and plant ID included as a random effect to account for sampling multiple stigmas within interview sticks. To quantify the effect of nectar volume on visit duration, we also fit a LMM with visit duration as the response and nectar volume (0, 2, or 4 µL) as a fixed effect and interview-stick ID as a random effect.

Is *Lupinus argenteus* reproduction pollen-limited?

To evaluate whether seed set in the *L. argenteus* population at Bellview Bench was pollen-limited, we randomly assigned 25 individual plants to a pollen supplementation treatment and another 25 to a control group on 7 July 2017. For plants in the pollen supplementation treatment, beginning at the onset of flowering and continuing until senescence (11 July–17 August 2017), we hand-pollinated all open flowers every third day during the approx. 96-h period of stigma receptivity (Gori, 1989). We used pollen mixed from five individuals (10-20 flowers each) which were situated 10 to 20 m away from experimental plants to reduce the likelihood of both outbreeding depression and bi-parental inbreeding (Waser and Williams, 2001). To ensure pollen viability, we limited pollen collection to flowers with white banner spots from the terminal or sub-terminal whorl in anthesis; pollen viability declines in *L. argenteus* after approx. 48 h post-anthesis (Gori, 1989). To apply pollen to stigmas, we used two pairs of forceps to gently pull back the banner and wing petals, exposing the stigma, and applied pollen to the stigma with a camel hair paintbrush (Kearns and Inouye, 1993). This pollen addition left a visible dab of pollen on the stigma. Hand-pollination using similar methods in other plant species results in increased pollen addition to flowers relative to open-pollinated control flowers (Kearns and Inouye, 1993). We counted each open flower on each plant and, using an oil-based paint pen (Sharpie, Oak Brook, Illinois, USA), applied a dot of blue paint to the adaxial surface of the calyx to prevent re-treating or re-counting flowers on subsequent days. To control for the effects of handling, we manipulated the flowers of control plants with forceps during counting.

At the end of the flowering period, we enclosed all stalks in organza bags to prevent dehiscent seed loss and frugivory by deer and marmots during fruit ripening. We harvested plants when all of their fruit were ripe (dry and brittle), and counted all seeds per fruit per plant, weighing seeds to the nearest 0.1 mg. We recorded: (1) proportion fruit set (number of seed-bearing fruits divided by the total flowers per plant); (2) total seeds per plant; (3) mean number of seeds per seed-bearing fruit; and (4) mean seed mass.

To assess the effect of hand pollination on female reproductive success, we fit linear models with treatment (hand pollination vs. control) as our predictor and with total number of flowers (to test for effects of plant size or resources) as a covariate. The covariate was not significant in any model and was dropped. This model passed Durbin-Watson (Durbin and Watson, 1971) and studentized
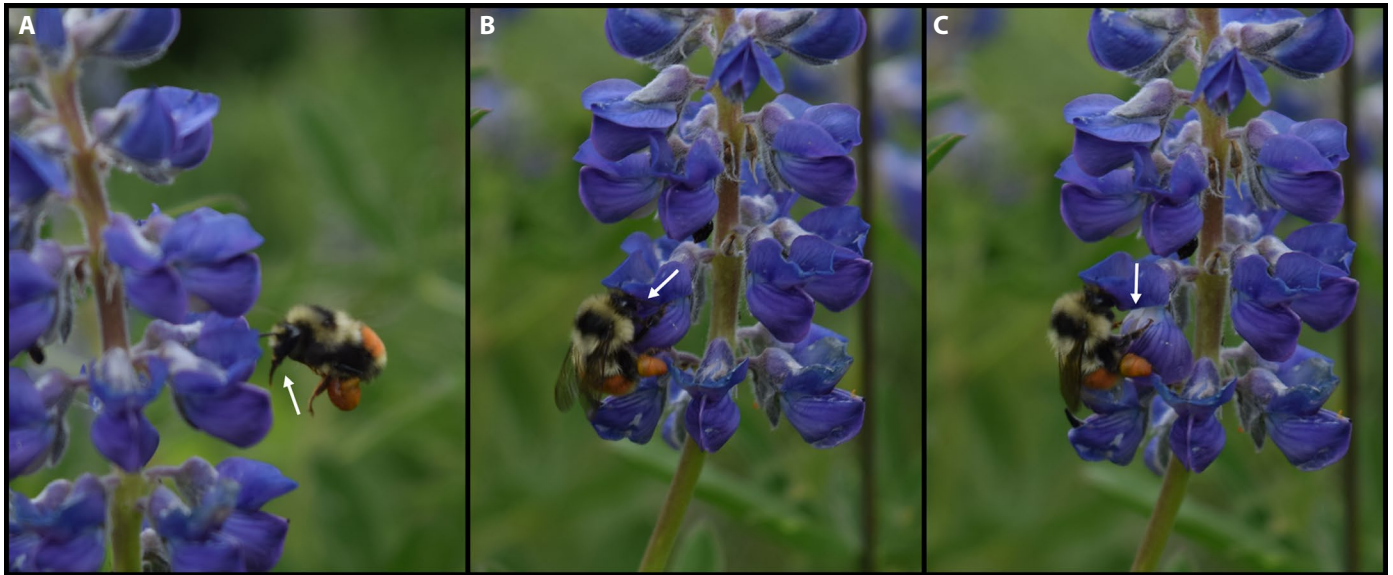
FIGURE 2. A typical pollen collecting visit by a bumble bee (*Bombus bifarius* worker pictured) to an unmanipulated (not treated with artificial nectar) *Lupinus argenteus* inflorescence. (A) *Bombus bifarius* bearing corbicular pollen loads approaching a *Lupinus argenteus* flower with glossa extended (indicated by arrow); (B) a bee braces her head against banner petal and grips wing petals with her forelegs. As head is pressed into banner, glossa extends into gap between wing and keel petals (indicated by arrow); (C) Wing petals are depressed away from keel with fore and midlegs, causing expulsion of a pollen droplet from keel pore onto anterior abdomen segments. Glossa is no longer in the keel-wing gap. The keel surface within the keel-wing gap (indicated by arrow) is not in contact with the body of the bee at any point.

Breusch-Pagan (Breusch and Pagan, 1979) tests for residual autocorrelation and heteroskedasticity, respectively. Seeds per fruit were not normally distributed (Shapiro-Wilk: $W = 0.907$, $P = 0.004$). Accordingly, we assessed the effects of supplemental hand-pollination on the mean numbers of seeds per fruit per plant using two-sample Wilcoxon rank sum tests. We used a t-test to assess the effects of hand-pollination on mean seed mass.

RESULTS

Do pollinators respond to nectar addition by removing nectar?

We observed 21 total visits by 9 individual bees (five *Bombus bifarius*, two *Megachile* sp., one *B. californicus*, and one *B. flavifrons*) to nectar-added stalks in the nectar-removal experiment. Bees collected both nectar and pollen during these visits. Bees were directly observed inserting their glossas into the space between the wing and keel petals where we had deposited the nectar droplet, then pulsating their abdomens, consistent with nectar consumption. Visited flowers had 50% less added nectar compared to unvisited flowers (paired t-test: $t_8 = -4.89$, $P = 0.001$; Fig. 3). Moreover, we recovered none of the added nectar from 11 of the 21 visited flowers.

How does nectar addition affect pollinator foraging behavior and plant reproductive success?

Over the 2016 season, we manipulated a total of 86,900 flowers (nectar added: 45,746 flowers; control: 41,154 flowers) on the 94 focal plants that flowered (nectar added: N = 45 plants; control: N = 49 plants). We observed 170 bee approaches that resulted in visits to focal plants, totaling 2947 individual flower visits, across

>14 h of observation. We observed eight bee taxa foraging on the focal plants, five identified to species (*Bombus appositus*, *B. bifarius*, *B. californicus*, *B. flavifrons*, and *B. mixtus*) and three identified to genus (*Andrena* sp., *Megachile* sp., and *Osmia* sp.). The most common visitors were *B. bifarius*, *B. flavifrons* (both ~ 2.89 mm intertegular distance; Pardee et al., University of Texas at Austin, unpublished manuscript), and a similarly sized *Megachile* sp. Together, these three taxa accounted for 93% of all observed visits (51%, 35%, and 7% of visits, respectively). *Bombus* visitors were all females, predominantly workers, with one queen *B. bifarius* and one queen *B. flavifrons* (out of 57 and 42 bees, respectively). All bee species responded similarly to nectar-added and control plants, with no significant differences in behavioral responses between species (analyses not shown). As a result, we present results of models on pollinator foraging behavior in which all of the bee species were pooled.

Bees spent 27% longer per flower on nectar-added than on control plants ($t_{40.27} = 3.832$, $P < 0.001$; Fig. 4). However, neither total visits (control: 1.510 ± 0.302 (mean ± SE) visits per plant, nectar-added: 1.979 ± 0.313 visits per plant; $t_{95} = 1.152$, $P = 0.252$) nor number of flowers probed per visit (control: 18.73 ± 2.69 flowers per visit, nectar-added: 16.12 ± 1.65 flowers per visit; $Z = 1.100$, $P = 0.271$) differed between treatments. There was a significant positive effect of the covariate mean floral display size on the total number of times plants were visited (estimate = 0.024 ± 0.003 SE, $t_{95} = 8.078$, $P < 0.0001$) and on the number of flowers probed per plant visit (estimate = 0.301 ± 0.085 SE, $Z = 3.569$, $P = 0.0004$). However, there was no effect of mean floral display size on time spent per flower ($t_{54.11} = 1.824$, $P = 0.074$).

Although time spent per flower increased with nectar addition, we found no effect of nectar addition on any metric of plant reproduction, including the number of seeds per plant ($Z = -0.854$,
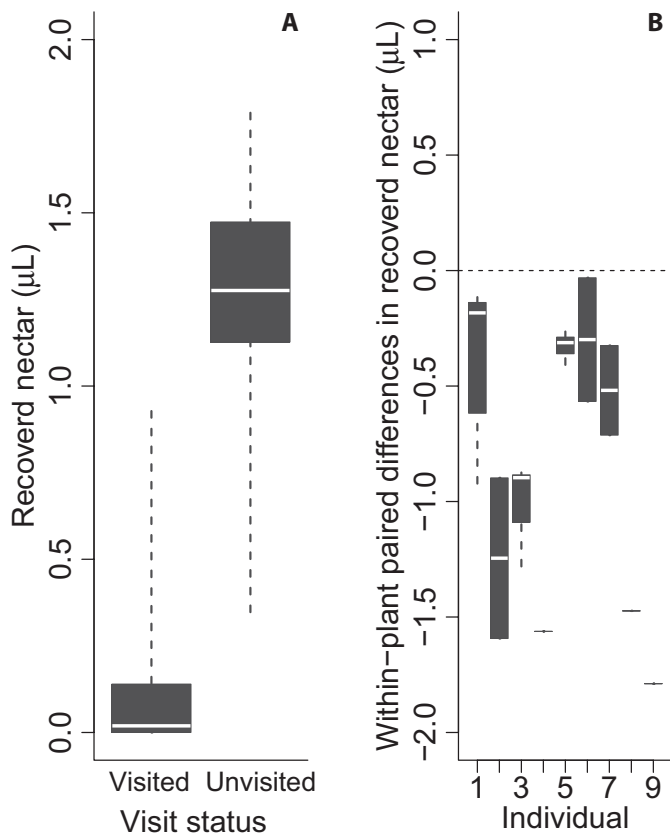
FIGURE 3. Absolute volumes (A) and differences in volume (B) of artificial nectar recovered from paired visited and virgin flowers within single stalks of *Lupinus argenteus* following nectar removal by bees. Visited flowers contained less artificial nectar than unvisited flowers on average. Bars are boxplots, with lower and upper ends of boxes depicting the lower and upper quartiles, respectively. Solid bands indicate medians. Whiskers extend across the data range. N = 9 plants.

$P = 0.393$), number of seeds per fruit ($Z = -1.3729$, $P = 0.085$), or mean seed mass ($Z = -0.515$, $P = 0.303$; Appendix S1). Mean floral display size had a significant positive effect on total seed production (estimate $= 0.028 \pm 0.004$, $Z = 7.465$, $P < 0.0001$; Appendix S2).

How does time spent per flower affect single-visit pollen deposition?

Flower visit duration had a significant positive effect on single-visit pollen deposition (Estimate $= 0.178 \pm 0.051$ SE; conditional $R^2 = 0.616$; $Z = 3.493$, i < 0.0005; Fig. 5). However, nectar volume (0, 2, or 4 µL per-flower) did not have a significant effect on visit duration in these trials ($Z = 0.238$, $P = 0.812$).

Is *Lupinus argenteus* reproduction pollen-limited?

We found no evidence that reproduction in the study population was pollen-limited. There was no effect of supplemental hand pollination relative to open-pollinated controls on proportion fruit set ($F_{1,37} = 1.240$, $P = 0.273$), total seed production ($F_{1,37} = 0.102$, $P = 0.752$), mean seed production per fruit ($Z = 0.107$, $P = 0.543$), or mean seed mass ($t_{30.4} = -0.671$, $P = 0.507$; Appendix S3).

DISCUSSION

Rewards are the foundation of resource-service mutualisms, and floral rewards are the primary ecological link between pollinator behavior and plant fitness (Parachnowitsch et al., 2018). The existence of resource-service mutualisms depends on the ability of the partner that provides resources to balance its production costs against the benefit it derives from interacting with the consumer (Bronstein, 2001). However, we know little about the selection pressures responsible for driving which floral reward strategies plant species employ, the fitness trade-offs associated with alternative reward strategies, or the feedbacks between floral reward strategies and pollinator behaviors. Here, we have shown that providing pollen as a sole floral reward allows *Lupinus argenteus* to secure sufficient pollination service from pollen-collecting bees, and that an alternative pollen + nectar reward strategy may not change the frequency or outcome of the interactions between this species and its pollinators.

Visitors responded positively to nectar addition by lengthening their visits to flowers, and single-visit pollen deposition to stigmas increased modestly but significantly with flower visit duration. In contrast, nectar addition did not affect the total number of visits plants received or the number of flowers probed per plant visit.

Although nectar addition increased per-flower visit duration and pollen deposition, these effects did not translate into differences in female components of plant reproduction, as would have been expected if plants were pollen limited (Thøstesen and Olesen, 1996). It is therefore likely that, in this population, pollen deposition does not limit reproduction. However, pollen limitation can vary in space and time (Campbell, 1987; Price et al., 2005). In sites or years when pollinator visits are rarer (due to variation in pollinator demography or competition with co-flowering species), increased visit duration, such as what was seen when nectar was added to flowers in this study, could potentially alleviate pollen limitation. While the effect of visit duration on pollen deposition was modest, small differences in pollen deposition could mean the difference between full seed-set and a failure to reproduce for lupines, which bear < 10 ovules per ovary (e.g., Wainwright, 1978). Thus, the absence of nectar production and the use of pollen as a sole pollinator reward in lupines appears to be a reward mode whose effectiveness may depend heavily on temporal and spatial variation in pollinator availability.

The strictly pollen rewarding state may favor the cost savings (i.e., of carbon and water) over reproductive assurance in any given season. One benefit of experimental nectar addition is that it allows assessment of the pollinator-mediated effects of nectar presence on plant reproduction independent of potentially confounding effects of variation in resource investment. For long-lived plants that occur in xeric and/or highly variable environments, such as lupines occurring in the montane to alpine zones of the western United States, year-to-year survival may pose a greater limit to lifetime fitness than the relative success of any single reproductive bout (Bell and Bliss, 1980; Morris and Doak, 1998). Further, water limitation results in limitation of nitrogen fixation in *Lupinus argenteus* (Goergen et al., 2009), potentially compounding the total cost of water relative to nitrogen. Pollen is a relatively nitrogen-rich tissue (Rabie et al., 1983), and nitrogen itself, while typically limiting, may bear a relatively lower cost than water to nitrogen fixers growing in xeric soils. As a result, a water-saving reward strategy (such as providing pollen rather than nectar rewards) could come with substantial lifetime fitness benefits via increased long-term survival and higher nitrogen

fixation rates (Goergen et al., 2009), especially if nectar were costly to produce in lupine, a point which requires further examination.

Another possible, and not mutually exclusive, benefit of the strictly pollen rewarding character state may be the filtering of the pollinator community (fewer groups of pollinators forage for pollen than nectar). Limiting rewards to pollen would reduce visitation by nectar-foraging visitors, potentially reducing competition and heterospecific pollen transfer (e.g., Muchhala, et al., 2010). The very low numbers of heterospecific pollen grains found on the stigmas that we sampled (3 grains total) is consistent with this hypothesis. However, pollinator filtering may be costly if it limits pollinator availability, and may leave plants especially vulnerable to pollinator loss. Thus, whether such filtering would be beneficial or costly is highly context dependent.

The only other experimental field study of which we are aware that compares the effects of nectar only and nectar + pollen reward strategies in a pollen-rewarding species was performed in the Himalayas by Tong et al. (2018). Contrary to the results presented here, Tong et al. (2018) describe negative effects of nectar addition on plant reproduction in the pollen-rewarding *Pedicularis dichotoma* Bonati (Orobanchaceae). They suggest that these effects are the
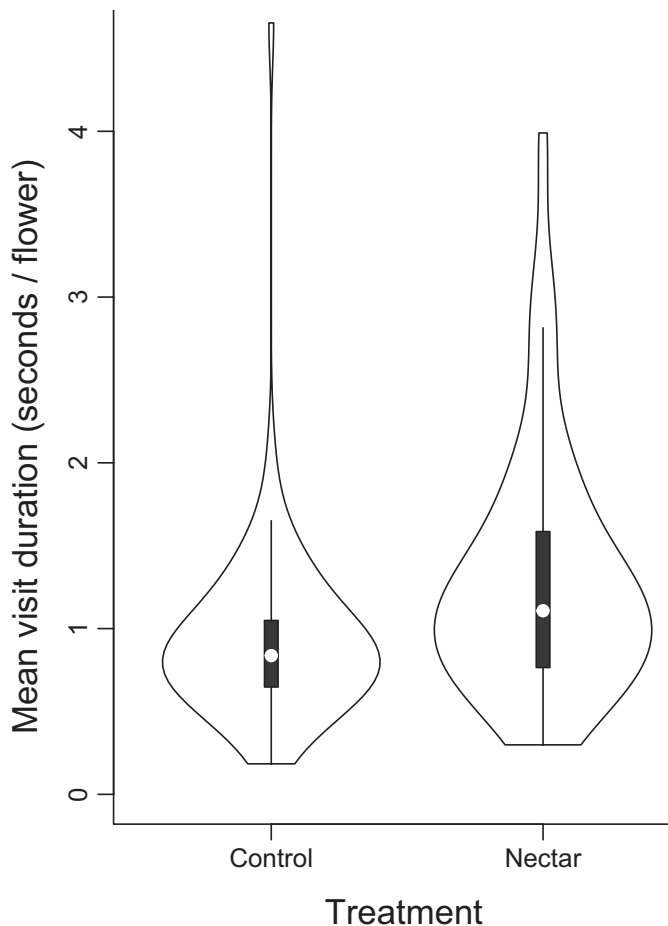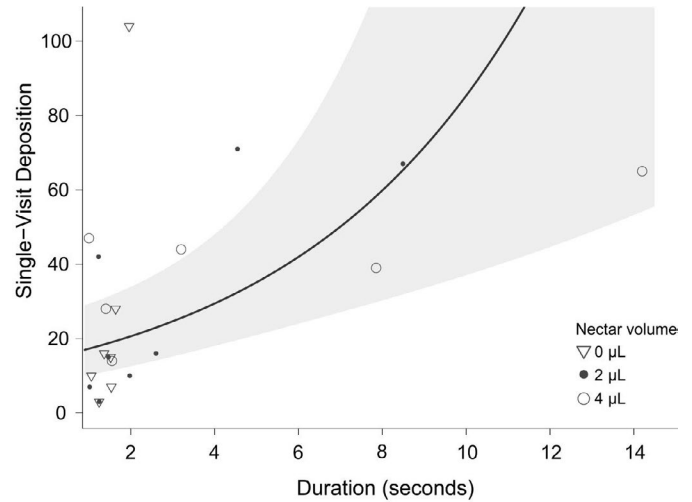
FIGURE 5. Single-visit pollen deposition increased with flower visit duration (sec). Points are raw data, with treatments differentiated by symbol. Band represents 95% CI.

result of altered body positioning and floral handling in bee pollinators foraging on nectar-added and nectarless inflorescences owing to the asymmetrical floral architecture of this species. Conversely, in our study, the handling behavior of pollinators was consistent both within and among bee species (and consistent with observations of others, e.g., Dunn, 1956; Harder, 1990) on the bilaterally symmetrical flowers of *Lupinus argenteus* regardless of the nectar status of flowers. Further, Tong et al. (2018) described an increased visitation frequency to nectar-supplemented flowers, an effect that was absent in *L. argenteus*. We offer two hypotheses to explain this difference. First, community-level floral reward availability varies substantially across communities (Ornelas et al., 2007; Ogilvie and Forrest, 2017) and as a function of soil moisture (Waser and Price, 2016). Subsequently, pollinator behavior varies in response to resource availability (Pleasants, 1981; Irwin and Maloof, 2002). If overall nectar availability were lower in the co-flowering community of *P. dichotoma* compared to that of *L. argenteus*, then artificial nectar may hold a higher relative value to bees in the *P. dichotoma* system. This hypothesis could be evaluated through simple comparative studies of the nectar availability and production rates among the nectar-producing species in each community (e.g., Luo et al., 2014) paired with experimental nectar addition across a range of wetter and drier sites or years. Second, even closely related and co-occurring bee taxa vary widely in their foraging behaviors (e.g., Irwin and Maloof, 2002) and their responses to resource availability (e.g., Ogilvie and Forrest, 2017). Thus, differences in foraging behavior between the pollinators of *P. dichotoma* (*Bombus friseanus*) (Tong et al., 2018) and *L. argenteus* (primarily *B. bifarius*) may drive differences in the outcomes of nectar addition. More studies are needed to understand whether or not there are general patterns associated with the effects of nectar addition on pollinator behavior and plant reproduction in strictly pollen rewarding species.

Although this study focused on measures of plant reproduction via female function, we can use the movement patterns and foraging behaviors that we observed to offer hypotheses regarding patterns of male mating success in response to nectar addition (Klinkhamer et al., 1994; Harder and Barrett 1995; Stanton et al., 1986). Generally, increased flower visit duration could affect male

FIGURE 4. Violin plot of mean visit duration to control (N = 1367 flowers probed, 73 plant visits) and nectar added flowers (N = 1580 flowers probed, 98 flower visits). Boxes are upper and lower quartiles; points are medians; whiskers extend across the data range; curves are rotated probability density functions.

function independently of female function (e.g., Golubov et al., 1999) in two ways. First, pollen removal may increase with visit duration (Harder, 1990; Kudo, 2003), possibly reducing pollen export efficiency due to increased proportional pollen loss (Klinkhamer et al., 1994; Thomson et al., 2000). However, *Lupinus* species characteristically employ dynamic pollen release (i.e., variable quantities of pollen released per visit) as a function of visitation frequency (Harder and Thomson, 1989). Thus, increased visit duration may have little effect on pollen removal even though it did have a significant effect on pollen deposition. Second, when there is a positive relationship between flower visit duration and single-visit deposition, increased visit duration might increase pollen discounting through geitonogamy (de Jong et al., 1993), resulting in decreased male mating success. Generally, individuals with larger floral displays are expected to export more pollen (Queller, 1997; but see Wilson et al., 1994), but they may also suffer higher pollen discounting (Klinkhamer et al., 1994). When such a tradeoff exists, a phenotype that provides larger overall rewards (such as the novel nectar and pollen-rewarding phenotype that we have simulated here) may compound this effect and depress male mating success in larger plants. Conversely, individuals with smaller displays are generally less attractive and may struggle to export their pollen (Klinkhamer and de Jong, 1993). If the presence of nectar could reduce the threshold display size required to compete for pollinators, then it may result in increased pollen export in smaller plants. Thus, the potential influence of a nectar reward in this species on reproductive success through male function might vary with plant size, and may be expected to either magnify (in the case of larger plants) or mitigate (in the case of smaller plants) negative effects of plant size on successful pollen export.

CONCLUSIONS

The evolutionary trajectories of reward strategies should be shaped by the unique combination of limitations and opportunities experienced by a plant lineage and, in turn, determine the species with which they can form and maintain mutualistic interactions. It is likely that pollen-rewarding species evolve in lineages that are able to balance the costs of pollen loss against the benefits of pollen transport better than those that provide other rewards (or no reward at all). If resource and reproductive opportunity costs of consumptive pollen loss are balanced against effective and reliable pollination by pollen foragers, as observed in *Lupinus argenteus*, then there may be nothing to gain from providing alternative rewards such as nectar. Thus, a strictly pollen-rewarding strategy may be the outcome of accepting some opportunity cost (lost pollen) while minimizing other reward allocation costs, without compromising the benefit provided by pollinator visitation.

ACKNOWLEDGMENTS

AUTHOR CONTRIBUTIONS

All authors designed the study and acquired funding. J.H. conducted the investigation, data curation, visualization, and wrote the original draft with input and resources provided by J.B. and R.I. J.H. and R.I. performed the formal analyses. All authors reviewed and edited the manuscript.

DATA AVAILABILITY

All data associated with this manuscript are archived with the Open Science Framework (https://osf.io/4awpt/; https://doi.org/10.17605/OSF.IO/4AWPT).

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

APPENDIX S1. Means and SE of *Lupinus argenteus* reproduction metrics from the nectar- addition experiment.

APPENDIX S2. Means and SE of *Lupinus argenteus* reproduction metrics pollen-limitation experiment.

APPENDIX S3. Total seeds per individual in the nectar addition experiment. Solid line: fitted curve for nectar-added plants (pollen + nectar); dashed line: fitted curve for control plants (pollen only). Circles and triangles are raw data points. Bands are 95% CI.

LITERATURE CITED

Armbruster, W. S. 2012. Evolution and ecological implications of "specialized" pollinator rewards. *In* S. Patiny [ed.], Evolution of plant-pollinator relationships, 44–67. Cambridge University Press, Cambridge, UK.

Bates, D., M. Maechler, B. Bolker, S. Walker, R. H. B. Christensen, H. Singmann, B. Dai, and G. Grothendieck. 2014. Package lme4. R Foundation for Statistical Computing, Vienna, Austria. Website: https://cran.r-project.org/web/packages/lme4/index.html.

Bell, K. L., and L. C. Bliss. 1980. Plant reproduction in a high arctic environment. *Arctic and Alpine Research* 12: 1–10.

Bronstein, J. L. 2001. The costs of mutualism. *American Zoologist* 41: 825–839.

Breusch, T. S., and A. R. Pagan. 1979. A Simple Test for Heteroscedasticity and Random Coefficient Variation. *Econometrica* 47: 1287–1294.

Campbell, D. R. 1987. Interpopulational variation in fruit production: The role of pollination-limitation in the Olympic Mountains. *American Journal of Botany* 74: 269–273.

Charnov, E. L. 1976. Optimal foraging, the marginal value theorem. *Theoretical Population Biology* 9: 129–136.

Cruden, R. W. 2000. Pollen grains: Why so many? *Plant Systematics and Evolution* 222: 143–165.

Darwin, C. 1859. On the origins of species by means of natural selection, 247. John Murray, London, UK.

de Jong, T. J., N. M. Waser, and P. G. Klinkhamer. 1993. Geitonogamy: The neglected side of selfing. *Trends in Ecology and Evolution* 8: 321–325.

Dunn, D. B. 1956. The breeding systems of *Lupinus*, group *Micranthi. American Midland Naturalist* 55: 443–472.

Durbin, J., and G. S. Watson. 1971. Testing for Serial Correlation in Least Squares Regression III. *Biometrika* 58: 1–19.

Etcheverry, A., V. Aleman, M. M. Figueroa-Fleming, T. López-Spahr, D. Gomez, C. A. Yanez, D. M. Figueroa-Castro, and P. Ortega-Baes. 2012. Pollen: Ovule ratio and its relationship with other floral traits in Papilionoideae (Leguminosae): An evaluation with Argentine species. *Plant Biology* 14: 171–178.

Goergen, E., J. C. Chambers, and R. Blank. 2009. Effects of water and nitrogen availability on nitrogen contribution by the legume, *Lupinus argenteus* Pursh. *Applied Soil Ecology* 42: 200–208.

Golubov, J., L. E. Eguiarte, M. A. C. Mandujano, J. López-Portillo, and C. Montaña. 1999. Why be a honeyless honey mesquite? Reproduction and mating system of nectarful and nectarless individuals. *American Journal of Botany* 86: 955–963.

Gori, D. F. 1989. Floral color change in *Lupinus argenteus* (Fabaceae): Why should plants advertise the location of unrewarding flowers to pollinators? *Evolution* 43: 870–881.

Harder, L. D. 1990. Pollen removal by bumble bees and its implications for pollen dispersal. *Ecology* 71: 1110–1125.

Harder, L. D., and S. C. Barrett. 1995. Mating cost of large floral displays in hermaphrodite plants. *Nature* 373: 512–515.

Harder, L. D., and J. D. Thomson. 1989. Evolutionary options for maximizing pollen dispersal of animal-pollinated plants. *American Naturalist* 133: 323–344.

Heinrich, B. 1976. The foraging specializations of individual bumblebees. *Ecological Monographs* 46: 105–128.

Irwin, R. E., and J. E. Maloof. 2002. Variation in nectar robbing over time, space, and species. *Oecologia* 133: 525–533.

Kearns, C. A., and D. W. Inouye. 1993. Techniques for pollination biologists, 155 & 289. University Press of Colorado, Niwot, Colorado, USA.

King, C., G. Ballantyne, and P. G. Willmer. 2013. Why flower visitation is a poor proxy for pollination: Measuring single-visit pollen deposition, with implications for pollination networks and conservation. *Methods in Ecology and Evolution* 4: 811–818.

Klinkhamer, P. G., and T. J. de Jong. 1993. Attractiveness to pollinators: A plant's dilemma. *Oikos* 66: 180–184.

Klinkhamer, P. G. L., T. J. D. de Jong, and J. A. J. Metz. 1994. Why plants can be too attractive – a discussion of measures to estimate male fitness. *Journal of Ecology* 82: 191–194.

Kudo, G. 2003. Anther arrangement influences pollen deposition and removal in hermaphrodite flowers. *Functional Ecology* 17: 349–355.

Kuznetsova, A., P. B. Brockhoff, and R. H. B. Christensen. 2015. Package lmerTest. R package version 2. R Foundation for statistical computing, Vienna, Austria. Website: https://cran.r-project.org/web/packages/lmerTest/index.html.

Lefcheck, J. S. 2016. piecewiseSEM: Piecewise structural equation modelling in r for ecology, evolution, and systematics. *Methods in Ecology and Evolution* 7: 573–579.

Luo, E. Y., J. E. Ogilvie, and J. D. Thomson. 2014. Stimulation of flower nectar replenishment by removal: A survey of eleven animal-pollinated plant species. *Journal of Pollination Ecology* 12: 52–62.

Luo, S. X., L. J. Zhang, S. Yuan, Z. H. Ma, D. X. Zhang, and S. S. Renner. 2018. The largest early-diverging angiosperm family is mostly pollinated by ovipositing insects and so are most surviving lineages of early angiosperms. *Proceedings of the Royal Society B, Biological Sciences* 285: 20172365.

Macior, L. W., T. Ya, and J. Zhang. 2001. Reproductive biology of *Pedicularis* (Scrophulariaceae) in the Sichuan Himalaya. *Plant Species Biology* 16: 83–89.

Morris, W. F., and D. F. Doak. 1998. Life history of the long-lived gynodioecious cushion plant *Silene acaulis* (Caryophyllaceae), inferred from size-based population projection matrices. *American Journal of Botany* 85: 784–793.

Müller, H. 1873. Die Befruchtung der Blumen durch Insekten und die gegenseitigen Anpassungen beider: Ein Beitrag zur Erkenntniss des ursächlichen Zusammenhanges in der organischen Natur, 243. Engelmann, Leipzig, Germany.

Muchhala, N., Z. Brown, W. S. Armbruster, and M. D. Potts. 2010. Competition drives specialization in pollination systems through costs to male fitness. *American Naturalist* 176: 732–743.

Ogilvie, J. E., and J. R. Forrest. 2017. Interactions between bee foraging and floral resource phenology shape bee populations and communities. *Current Opinion in Insect Science* 21: 75–82.

Ornelas, J. F., M. Ordano, A. J. De-Nova, M. E. Quintero, and T. Garland Jr. 2007. Phylogenetic analysis of interspecific variation in nectar of hummingbird-visited plants. *Journal of Evolutionary Biology* 20: 1904–1917.

Parachnowitsch, A. L., J. S. Manson, and N. Sletvold. 2018. Evolutionary ecology of nectar. *Annals of Botany* 123: 247–261.

Pleasants, J. M. 1981. Bumblebee response to variation in nectar availability. *Ecology* 62: 1648–1661.

Price, M. V., N. M. Waser, R. E. Irwin, D. R. Campbell, and A. K. Brody. 2005. Temporal and spatial variation in pollination of a montane herb: A seven-year study. *Ecology* 86: 2106–2116.

Pyke, G. H. 1978. Optimal foraging in hummingbirds: Testing the marginal value theorem. *American Zoologist* 18: 739–752.

Queller, D. 1997. Pollen removal, paternity, and the male function of flowers. *American Naturalist* 149: 585–594.

Team, R. C. 2018. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. Website: https://www.R-project.org.

Rabie, A. L., J. D. Wells, and L. K. Dent. 1983. The nitrogen content of pollen protein. *Journal of Apicultural Research* 22: 119–123.

Simpson, B. B., and J. L. Neff. 1983. Evolution and diversity of floral rewards. *In* C. E. Jones and R. J. Little [eds], Handbook of experimental pollination ecology, 277–293. Van Nostrand Reinhold, NY, USA.

Snow, A. A., and P. O. Lewis. 1993. Reproductive traits and male fertility in plants: empirical approaches. *Annual Review of Ecology and Systematics* 24: 331–351.

Stanton, M. L., A. A. Snow, and S. H. Handel. 1986. Floral evolution: Attractiveness to pollinators increases male fitness. *Science* 232: 1625–1627.

Stpiczyńska, M. 1995. The structure of floral nectaries of some species of *Vicia* L. (Papilionaceae). *Acta Societatis Botanicorum Poloniae* 64: 327–334.

Thomson, J. D. 1981. Field measures of flower constancy in bumblebees. *American Midland Naturalist* 105: 377–380.

Thomson, J. D. 1986. Pollen transport and deposition by bumble bees in *Erythronium*: Influences of floral nectar and bee grooming. *Journal of Ecology* 74: 329–341.

Thomson, J. D., P. Wilson, M. Valenzuela, and M. Malzone. 2000. Pollen presentation and pollination syndromes, with special reference to *Penstemon. Plant Species Biology* 15: 11–29.

Thøstesen, A. M., and J. M. Olesen. 1996. Pollen removal and deposition by specialist and generalist bumblebees in *Aconitum septentrionale. Oikos* 77: 77–84.

Tong, Z. Y., X. P. Wang, L. Y. Wu, and S. Q. Huang. 2018. Nectar supplementation changes pollinator behaviour and pollination mode in *Pedicularis dichotoma*: Implications for evolutionary transitions. *Annals of Botany* 123: 373–380.

Venables, W. N., and B. D. Ripley. 2002. Modern applied statistics with S, 183–206. Springer-Verlag, NY, USA.

Wainwright, C. M. 1978. The floral biology and pollination ecology of two desert lupines. *Bulletin of the Torrey Botanical Club* 105: 24–38.

Waser, N. M., and M. V. Price. 2016. Drought, pollen and nectar availability, and pollination success. *Ecology* 97: 1400–1409.

Waser, N. M., and C. F. Williams. 2001. Inbreeding and outbreeding. *In* C. W. Fox, D. A. Roff, and D. J. Fairbairn [eds.], Evolutionary ecology, concepts and case studies, 84–96, Oxford University Press, Oxford, UK.

Wilson, P., J. D. Thomson, M. L. Stanton, and L. P. Rigney. 1994. Beyond floral Batemania: Gender biases in selection for pollination success. *American Naturalist* 143: 283–296.