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Pollinators differ in their contribution to the male fitness of a self-incompatible composite

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Abstract

Premise: Reproductive fitness in plants is often determined by the quantity and quality of pollen transferred by pollinators. However, many fitness studies measure only female fitness or rely on proxies for male fitness. Here we assessed how five bee taxon groups affect male fitness in a prairie plant by quantifying pollen removal, visitation, and siring success using paternity assignments and a unique pollinator visitation experiment.

Methods: In *Echinacea angustifolia*, we measured per-visit pollen removal for each pollinator taxon and estimated the number of pollen grains needed for successful ovule fertilization. Additionally, we directly measured pollinator influence on siring by allowing only one bee taxon to visit each pollen-donor plant, while open-pollinated plants acted as unrestricted pollen recipients. We genotyped the resulting offspring, assigned paternity, and used aster statistical models to quantify siring success.

Results: Siring success of pollen-donor plants differed among the five pollinator groups. Nongrooming male bees were associated with increased siring success. Bees from all taxa removed most of the flowering head's pollen in one visit. However, coneflower-specialist bee *Andrena helianthiformis* removed the most pollen per visit. Female fitness and proxy measures of male fitness, such as pollinator visitation and pollen removal, did not align with our direct quantifications of male fitness.

Conclusions: Our results illustrate the need for more studies to directly quantify male fitness, and we caution against using male fitness proxy measures. In addition, conservation efforts that preserve a diverse pollinator community can benefit plants in fragmented landscapes.

KEYWORDS

Asteraceae, *Echinacea angustifolia*, male fitness, native bees, plant–pollinator interactions, plant reproduction, pollen movement, pollen removal, siring success, tallgrass prairie

Many flowering plants are visited and pollinated by a diverse array of bees, flies, and other insects. These insect visitors often differ in how they move pollen within and between conspecific plants, thus impacting ecological and evolutionary processes in a plant population (e.g., Aizen et al., 2002; Wilcock and Neiland, 2002; Brosi and Briggs, 2013; Devaux et al., 2014). For example, insects that primarily forage within a floral display increase geitonogamous pollen transfer, thus increasing inbreeding rates in self-compatible species (Karron et al., 2004; Brunet and Sweet, 2006) and potentially interfering with pollination in self-incompatible plants (reviewed by Mitchell et al., 2009). In contrast, insect visitors with either large foraging ranges or those that do not actively groom pollen may increase

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gene flow between plant populations (e.g., Herrera, 1987; Rader et al., 2011). Understanding how various pollinator behaviors like these influence a plant's reproductive fitness helps us to better assess and mitigate the risk for plant populations facing increasingly fragmented habitats.

Since most flowering plants are bisexual, their reproductive fitness is determined by how many seeds they produce (female fitness) and by how many offspring they sire (male fitness), both of which can be directly influenced by floral visitors. For example, the interpopulation variation in the separation of anthers and stigmas can determine whether floral visitors contact both organs during a visit (Armbruster et al., 1989; Solís-Montero and Vallejo-Marín, 2017). If a visitor only contacts the stigma and carries pollen from other plants, it could increase the female fitness of a plant but not the male fitness since it did not pick up pollen from the anthers. Additionally, in dichogamous plants, floral visitors can preferentially visit flowers in one sexual phase over the other phase (e.g., Zych, 2007; Koski et al., 2018).

Variations such as these in female and male fitness can lead to conflicting sexual selection on floral traits (Briscoe Runquist et al., 2017). For many organisms, there is greater opportunity for selection via male fitness versus female fitness because male fitness should be limited by mating opportunities, while female fitness is limited by resource availability (Bateman, 1948). Since most plants are bisexual, male fitness is predicted to drive the evolution of traits associated with increased pollinator service (e.g., floral morphology; Delph and Ashman, 2006). Yet, in mate-limited plant populations, there is likely high variation in both female and male fitness, potentially leading to a population experiencing strong selection through both functions and thus a greater potential for sexual conflict (e.g., Wilson et al., 1994; Ashman and Morgan, 2004; Briscoe Runquist et al., 2017). How variations in pollinator preferences impact the presence and degree of conflicting sexual selection is largely unknown (but see Sahli and Conner, 2011).

Despite this potential for conflicting sexual selection, many studies use female fitness alone as a proxy for the plant's overall reproductive fitness (Rico-Gray and Thien, 1989; Olsen, 1996; Tooker and Hanks, 2006; Ne'eman et al., 2010). In fact, there is a significant bias in the literature toward studies investigating female fitness compared to those investigating male fitness (Caruso et al., 2019). This disparity can likely be attributed to the relative difficulty of measuring each—assessing seed set for female fitness versus tracking the fate of pollen grains for male fitness. Previous studies have used fluorescent dye (Waser, 1988; Campbell, 1991; Adler and Irwin, 2006), visual sightings (Schmitt, 1983; Fenster, 1991), and pollen-color polymorphism (Thomson and Thomson, 1989) to estimate pollen export. More recent methods of applying quantum dots to pollen grains (Minnaar and Anderson, 2019; Konzmann et al., 2020; Moir and Anderson, 2023 [in this issue]) allow researchers to track individual pollen grains instead of using powdered dye, which can be more sensitive to floral morphs or transported differently compared to pollen (Thomson et al., 1986, Adler and Irwin, 2006). However, these methods have logistical limitations and are only proxies for successful pollen movement (i.e., a pollen grain carrying the sperm that fertilizes an ovule). One widespread and effective method of directly tracking successful pollen movement is using genetic markers, including microsatellites and single-nucleotide polymorphisms (SNPs), to determine which plant sired each seed (reviewed by Ashley, 2010; Colicchio et al., 2020). Yet, examining the paternity of sired seeds can only determine the total male fitness and cannot parse out the impact on male fitness by different floral visitors. To accomplish this, we need to determine the outcome of pollen grains picked up by specific floral visitors.

To determine whether pollinator taxa differ in their potential to impact male fitness, we can quantify how much pollen each taxon removes during a visit. Yet, pollen removal by a floral visitor does not guarantee that any of the removed pollen grains will fertilize ovules on other plants. In fact, a pollen grain can have many fates other than deposition on conspecific stigmas (reviewed by Minnaar et al., 2019). We also know that different floral visitors vary greatly in their pollen transfer efficacy. For example, some bees may have weak floral constancy, which results in increased pollen loss to heterospecific styles (Minnaar et al., 2019). Other bees can be very efficient at collecting pollen but not at depositing it (Hargreaves et al., 2009). In systems with pollen-foraging bees, there can be wide variation in how much pollen an individual bee collects per visit. Specialized pollen-foraging bees in some systems are highly efficient at collecting pollen but not at depositing it (Wilson and Thomson, 1991; Parker et al., 2016) potentially causing pollen depletion in plant populations. In fact, some specialized bees deposit proportionally less pollen from their primary pollen source compared to other plants they visit (Weinman et al., 2023 [in this issue]).

Furthermore, we cannot assume that the deposition of more pollen grains on a stigma results in higher seed set (thus increasing the pollen-donor's siring success). In resource-limited populations, we would not expect to find a strong relationship between pollen deposition and seed set (Zimmerman and Pyke, 1988). Yet even in pollen-limited populations, this relationship may not be straightforward. In many pollen-limited populations, we see that the relationship between pollen deposition and seed set flattens at high amounts of pollen deposition, likely because that individual flower is no longer limited by compatible pollen, even if the population still is (Zimmerman and Pyke, 1988; Wagenius et al., 2007). In fact, there may be no relationship between pollen deposition and seed set in uniovulate plants since the single ovule can be fertilized if at least one compatible pollen grain is deposited (but see Chamer et al., 2015). To overcome the uncertainties of these proxy measurements, we need a more direct method of measuring male fitness, which incorporates a method for tracking the fate of pollen grains picked up by different floral visitors.

In this study, we investigated the pollination of *Echinacea angustifolia*, a prairie perennial that depends on a generalist insect community of bee and fly species for successful pollination (Wagenius and Lyon, 2010). Previous research on *E. angustifolia* found that visits by *Andrena helianthiformis*, a coneflower-specialist bee, resulted in significantly more style-shriveling per visit, an indication of ovule fertilization, than other bee floral visitors (Page et al., 2019). However, all bees moved pollen similar distances between sire and maternal plants and deposited pollen from multiple sire plants per visit. Therefore, from a female fitness perspective, a visit by *A. helianthiformis* increased a plant's fitness, but there was no difference in the quality of pollen delivered between the floral visitors, as in other systems (e.g., Valverde et al., 2019). In this study, we focused on how visits by different bee taxa impact the male fitness of *E. angustifolia*. Our objectives were to (1) quantify the per-visit pollen removal rates by the major pollinator taxa visiting *E. angustifolia*, (2) determine the relationship between pollen deposition and the likelihood of ovule fertilization in this uniovulate system, and (3) directly quantify how visits by different pollinator taxa impact a plant's siring success on other *E. angustifolia* individuals. Understanding how floral visitors affect different components of male fitness in *E. angustifolia* will provide a more holistic understanding of how floral visitors impact overall plant reproductive fitness, rather than simply a female-fitness view.

MATERIALS AND METHODS

Study site

In summer 2018, we conducted manipulations and observations in a previously established common garden experimental plot, approximately 60 × 80 m, in western Minnesota, near 45°47′N, 95°40′W. The study plot is a degraded prairie within a landscape that includes remnant prairie, old fields, and prairie restoration. Many *E. angustifolia* plants in this plot were genotyped previously (Ison et al., 2014; Page et al., 2019; Reed et al., 2022). Page et al. (2019) and Reed et al. (2022) provide more details about the plot.

Plant study species

Echinacea angustifolia DC (Asteraceae) is a long-lived perennial, native to the tallgrass prairie of North America. Individual plants are nonclonal and have a sporophytic self-incompatibility system, so they depend on floral visitors to move pollen between plants for reproduction. A flowering plant typically has one flowering head but can have over 10. In our study area, *E. angustifolia* typically begins flowering in late June to early July and finishes flowering in mid to late August, with an individual plant typically flowering for 6 to 36 days (Waananen et al., 2018). Flowering heads have 80–250 uniovulate disc florets and a single row of sterile ray florets (Waananen et al., 2018). Each disc floret is bisexual and goes through a male phase for a day when anthers shed pollen. The next day, a style pushes through the anthers and is receptive to compatible pollen. Flowering begins with the outermost row of disc florets and moves inward in concentric circles up the head. If a style receives compatible pollen, it will shrivel 12–24 h after pollen deposition, indicating ovule fertilization (Wagenius, 2004). Stigmatic surfaces can stay receptive for up to 10 days without compatible pollen deposition (J. L. Ison, personal observations).

Bee study species

Over 26 species of bees visit *E. angustifolia* (Wagenius and Lyon, 2010) in our study area. While *E. angustifolia* has additional floral visitors, including lepidopterans, flies, honey bees, and bumble bees, their visitation rates are much lower (Wagenius and Lyon, 2010; Ison et al., 2018). We focused on the bee taxa with the highest visitation at our study site and classified them into five groups that are distinguishable when they visit flowering heads in the field. These groups are *Andrena helianthiformis*, tribe Augochlorini, *Halictus* spp., small-dark bees, and male *Melissodes* spp. Hereafter, we refer to them as pollinator taxa. For more information on the pollinator taxa, including morphology and how we identified them in the field, please refer to Appendix S1. We note that all taxon identifications took place while the insects were moving in the field, so it is possible some individuals were misclassified.

Pollen removal methods

To estimate per-visit pollen removal by each bee taxon, we observed single visits in our study plot from 9 to 30 July 2018. We prevented visitation for at least 1 day before the observation period using tulle pollinator exclusion bags. On observation days, we counted male-phase florets on each flowering head. We used heads with at least 10 but no less than four male-phase florets. Immediately before observations, we removed the pollinator exclusion bag. Next, we randomly removed three male-phase florets (hereafter, "unvisited florets") using forceps and stored them in 0.5 mL of distilled water.

After removing the unvisited florets, we waited for an insect to land on the flowering head and contact either male or female reproductive parts. Once we observed a single visit on a given head, we collected three male-phase florets (hereafter, "visited florets") as we did for the unvisited florets. At the end of the observation period, we collected three male-phase florets from experimental unvisited heads as an indicator of pollen removal caused by environmental factors, such as wind or handling.

To estimate pollen grains in the samples, we loaded the water-pollen solution onto a hemocytometer (adapted from the method of Koski et al., 2018). We first physically broke apart the male florets in each vial using a sharp pair of forceps, and then vortexed each sample for 30 s. We then loaded 40 μL of this pollen solution into a hemocytometer (Paul Marienfeld GmbH & Co., Lauda-Königshofen, Germany) and counted all pollen grains in a 17.78 μL subset of the sample at 100× magnification. Samples were counted in a random order. While there was substantial variation in pollen production between the unvisited florets, nearly all (85%) of the visited florets had a lower pollen count than unvisited florets collected on the same day from the same flowering head. This indicates that there is more variation between florets from different flowering heads at different phenological stages than there is between florets from the same flowering head at the same phenological stage (for more information, see Appendix S2).

We observed 145 pollinator visits. Any taxon with fewer than five visits was removed from our analysis. We observed 18 visits by *A. helianthiformis*, nine visits by Augochlorini, 57 visits by small-dark bees, and 49 visits by male *Melissodes* spp. We also had 21 heads with no visits that we used to estimate environmental pollen loss.

Pollen removal analysis

We evaluated the relationship between pollinator taxon and the amount of pollen removed by estimating the proportion of pollen removed during a visit (one minus the ratio of visited pollen count over unvisited pollen count). We modeled the proportion of pollen removed as a linear response and tested three predictors: taxon (levels: *A. helianthiformis*, Augochlorini, male *Melissodes* spp., small-dark bees, and no visitors), the count of florets shedding pollen at the time of visit, and the unvisited pollen count. Our maximal model included the three predictors and all two-way interactions. We did not test for a three-way interaction because we did not have all factor-level combinations to assess this interaction. Using stepwise model simplification with backward elimination, we first removed each interaction term and compared each interaction term to the maximal model using a likelihood ratio test (Crawley, 2013). We then removed each predictor and compared each simplified model to the additive model with all three predictors. We also used pollen count removed (unvisited pollen count minus visited pollen count) as a model response.

Pollen deposition methods and analysis

Because *E. angustifolia* is uniovulate, only one compatible pollen grain is needed to fertilize the ovule in a floret. We used hand crosses to investigate this relationship between the number of pollen grains deposited and seed set. We excluded pollinators from 37 flowering heads from 33 flowering plants with pollinator exclusion bags for at least 24 h and then performed hand crosses in sets of nine florets. We removed self-pollen from heads before beginning the crossing process. In each crossing set ($N = 41$), we left three styles uncrossed, although they likely received a small amount of pollen through handling. We crossed the other six styles with a mix of pollen from multiple plants; some sets of crosses had low pollen deposition (applied with a tooth flosser), while others had higher deposition (applied with a toothpick). After performing the crosses, we re-bagged the flowering heads. After 24 h, we removed the uncrossed and three of the crossed styles and placed them in tubes of 70% v/v ethanol. The remaining three styles were left in case 24 h was not enough time for the pollen tubes to grow to the ovule, but we found no difference in seed set between uncollected and collected styles, so these uncollected styles were removed from the data set. We kept track of crosses and distinguished between treatments by painting the subtending bract of each floret (Wagenius et al., 2007).

Seed heads were harvested August–October 2018, and we removed individual achenes (fruits) from each cross. Since *E. angustifolia* achenes expand regardless of whether a seed is present, we x-rayed each achene to determine whether a seed was set (i.e., contained an embryo) (see the supplement of Wagenius et al., 2020 for more details). Next, we used a compound light microscope at 100× and 400× magnification to count all pollen grains on the collected styles after they were soaked in 1 M NaOH for 12–24 h, stained with fusion jelly (Kearns and Inouye, 1993), and placed on a slide. We had a total of 230 florets (control = 108, crossed = 122) with pollen deposition counts and seed set status.

We modeled seed set status for each floret as a binomial response using generalized linear models. We tested two predictors, the log number of pollen grains deposited and if the floret was hand-crossed, as well as their interaction. We conducted model simplification using backward elimination, as described in the pollen removal analysis.

Field methods to assess siring success

In 2018, we manipulated pollinator visitation to determine how floral visits from different pollinator taxa impact the siring success of *E. angustifolia* individuals in an approximately 20 × 40 m section of our study plot over 5 days (6, 10, 11, 16, and 17 July). Although *E. angustifolia* is bisexual, we treated each plant in our study as either a pollen donor (sire) or a pollen recipient (maternal individual). Since most bees in our populations forage for pollen, we did not emasculate any plants, even those classified as pollen recipients, so as not to impact bee visitation and behavior. Each pollen-donor plant was only allowed visits by one pollinator taxon. In contrast, pollen-recipient plants were open-pollinated. All flowering *E. angustifolia* plants within 25 m of our experimental plot were bagged with a pollinator exclusion bag to limit pollen flow from outside of the study area.

On five sunny days around peak flowering time of *E. angustifolia*, we monitored 69–130 actively flowering pollen-donor plants. Once the first bee visited a pollen-donor plant, we identified the visiting bee taxon (hereafter, "target pollinator taxon") and allowed only bees of that taxon to visit that plant for the rest of the observation period. If a bee from a nontarget taxon visited, it was quickly shooed away or caught if it landed and foraged. We recorded all target and nontarget pollinator taxa visits to each pollen-donor plant. We continued monitoring visitation until most pollen was removed from the study area and bees were no longer visiting—typically around noon. At the end of each observation day, we recorded the number of florets that had shed pollen for each pollen-donor plant. On 11 July, we kept the target pollinator taxon the same as on 10 July. For example, if a pollen-donor plant was assigned Augochlorini on 10 July, Augochlorini was also the target pollinator taxon for 11 July. Therefore, 10 and 11 July were lumped into one observation period yielding four total observation periods (6, 10–11, 16, and 17 July) for our analyses.

The day before each of our observation periods, we haphazardly selected 14–26 plants to be pollen recipients (maternal plants) and placed a pollinator-exclusion bag over all flowering heads. Plants early or late in their flowering phenology were not used as pollen-recipient plants because they had few receptive styles or florets shedding pollen. The same pollen-recipient plants were used on 10 and 11 July and on 16 and 17 July (note: the target pollinator taxon could change between 16 and 17 July but not between 10 and 11 July). On each observation day, once we were ready to observe visits to the pollen-donor plants, we removed the bags from the pollen-recipient plants. During the observation period, these plants were open-pollinated and unmonitored since we could not monitor them and also keep vigil over the pollen-donor plants. After the observation period ended, we counted the florets shedding pollen on pollen-recipient plants and re-bagged the flowering heads. These heads were kept bagged until at least 24 h after the observation period. We then observed which styles shriveled on each head, which indicated that the style had received compatible pollen during the observation period. For the pollen-recipient plants used on 16 and 17 July, we recorded which styles shriveled on 17 July, immediately after the observation period, and again on 18 July. Since styles take 12–24 h to shrivel, the 17 July shriveled styles were pollinated during the 16 July observation period. These pollen-recipient plants remained bagged outside of observation periods. For all pollen-recipient plants, the subtended bract of each shriveled style was painted to distinguish between observation periods. From August to October, we collected seed heads from pollen-recipient plants as they matured.

We observed 746 visits of target pollinator taxa to pollen-donor plants throughout the four observation periods (mean 2.2 visits per plant per observation period, ±2 SD). Five pollinator taxa were the target pollinator for at least 12 pollen-donor plants by observation period combinations (Augochlorini = 16, small-dark bee = 147, *Halictus* spp. = 48, male *Melissodes* spp. = 12, and *A. helianthiformis* = 75; Appendix S1). We excluded taxa that were the target pollinator for 12 or fewer pollen-donor plants by observation period combinations from analyses.

Siring success lab methods

In the lab, we used forceps to individually remove the achenes from florets with shriveled styles, i.e., potentially containing a seed that was pollinated during an observation period. In May and June 2019, we germinated achenes using the protocol from Feghahati and Reese (1994) as adapted by Wagenius (2004). Seedlings (hereafter "offspring") were grown in plug trays until the first true leaf could be sampled, about 10–14 days after radicle emergence. Leaf samples were quickly dried in silica gel and stored at room temperature.

We used 10 polymorphic microsatellite loci to assign paternity: Ech03, Ech05, Ech11, Ech13, Ech13Z, Ech15, Ech28, Ech36, Ech37, and Ech47 (Ison et al., 2013). For all 401 offspring, we extracted DNA from the leaf samples using a Qiagen DNeasy Plant Kit (Germantown, MD, USA), as described by Ison et al. (2013). We genotyped the offspring using the procedure of Ison et al. (2013) with the adjustments described by Reed et al. (2022). We also genotyped all 196 flowering plants in our experimental area (both pollen donors and pollen recipients) to determine offspring parentage. Genotyping (including DNA extractions) for some of the flowering plants had been done in previous studies by Ison et al. (2014), Page et al. (2019), and Reed et al. (2022).

We developed two data sets of paternity assignment using two assignment methods. Since all paternity assignment methods have a level of uncertainty, we used two common methods and compared model selection results using each assignment method: Cervus 3.03 (Kalinowski et al., 2007) and MasterBayes 2.57 (Hadfield et al., 2006). For Cervus, we used the delta value (the difference between the natural log of the likelihood ratios between the two sires with the highest likelihood ratios) to assign the most likely sire. We determined the assignment confidence level using the software's user-parameterized simulation. We estimated that 92% of potential sires were genotyped, that 93% of the loci were genotyped, and genotyping error was 3%. Only flowering plants that were actively shedding pollen (including pollen-recipient plants) on an observation day were candidate sires, and we restricted self-pollination. For the analysis, we used assignments at the 80% or higher confidence level.

Using the same genotype data, we also assigned paternity using a Bayesian framework to infer posterior distributions of parameters representing these attributes, including the parent–offspring pedigree, that maximize the model's overall posterior probability. In our Bayesian models, we included parameters for the location, and thus proximities, of the maternal plant and candidate sires and a term to exclude self-pollination. We ran separate models for each observation period so that only plants actively shedding pollen during an observation period could be a potential sire. We used assignments where the candidate sire was assigned in more than 50% of the iterations. For a more detailed comparison of these two paternity assignment methods in this system, please refer to Reed et al. (2022), particularly their Appendix S2.

Siring success analysis

We quantified the effects of visitation by a single pollinator taxon on male fitness of individual plants using the aster statistical approach (Shaw et al., 2008). The fitness response in our model, siring success, was quantified as the sum of seeds sired per individual pollen-donor plant for each observation period. The many instances of zero siring success precluded the use of standard parametric models, so we modeled fitness as an aster graph with two nodes: (1) siring success modeled as a zero-truncated Poisson count conditional on (2) non-zero siring success modeled as binomial. Because we employed two different paternity assignment approaches, we conducted two separate analyses, each with a different response, but both with the same predictors. Our main predictor of interest, pollinator taxon, had five levels across the entire experiment. We included three covariates in all aster models: (1) log number of male florets per plant per day, median = 3.29, range: 0–5.65, (2) number of pollinator visits per plant per day, median = 2, range: 1–10, and (3) observation period, categorical with four levels. Pollen-recipient plants that were assigned as sires to offspring were removed from the aster models because we did not have all the covariates for these plants. We believe excluding these plants did not impact our interpretation since pollen-recipient plants were visited by all pollinator taxa and the goal of the aster analysis was to quantify the contribution towards male fitness for each pollinator taxon separately.

For each of the two responses, we used a model that consisted of only covariates to serve as a null model. Covariates were modeled as main effects in all cases and for each of the two responses, we tested whether it would be appropriate ($P < 0.05$) to include a two-way interaction between any covariate pairs. For the MasterBayes paternity assignments, log-likelihood ratio tests indicated that including a visit by observation day term was appropriate ($P = 0.017$), so we included that term in the null model. For each of the two responses, we compared the null model to a nested model that included one more term, the taxon term modeled as a direct effect on the final node of the aster graph: total siring success. We also compared that nested model to a model that included a term that models the effect of taxon on both nodes of the aster model. We used log-likelihood ratio tests to compare these three models in separate analyses of both responses.

Initial analysis found little evidence that the number of pollinator visits predicted siring success on the pollen-recipient plants. However, this initial approach included all pollinator taxa, and we wanted to see if pollinator visits predicted siring success for just one pollinator taxon. Therefore, we examined whether the number of pollinator visits predicted siring success for the three target pollinator taxa with the most pollen-donor plants and highest variation in visit number—*A. helianthiformis*, *Halictus* spp., and small-dark bees. For each siring success response (i.e., the two paternity assignment methods), we tested two predictors—the number of target pollinator taxon visits and the number of florets shedding pollen—and the two-way interaction. For each of the three target pollinator taxa, we conducted model simplification using backward elimination, as described for the pollen removal analysis. We used R 4.2.1 for all statistical analyses in the study (R Core Team, 2022).

RESULTS

Pollen removal and deposition

Pollinators did vary in the proportion of pollen they removed during a visit, quantified as one minus the ratio of pollen count on visited florets over pollen count on unvisited florets (Table 1). There was also a pollinator effect when the response was the total number of pollen grains removed (Table 1). In addition, the number or proportion of grains removed varied based on how many florets were shedding pollen on a flowering head, and the number of pollen grains produced in those anthers. There was no evidence for two-way interactions between the number of florets with anthers shedding pollen and the pollinator taxon, or the interaction between the pollinator category and the before-visit pollen count (Table 1, $P > 0.05$). When the response was the proportion of pollen removed during a visit, there was a significant interaction between the number of florets with anthers shedding pollen and the unvisited pollen count (Table 1, $P < 0.05$). Two main effects (pollinator taxa, and unvisited florets pollen count) significantly improved the fit of both models (Table 1, $P < 0.05$).

Per visit, *Andrena helianthiformis* removed the most pollen grains, both proportionally and by total number of pollen grains (Figure 1). The other pollinator taxa, Augochlorini, male *Melissodes* spp, and small-dark bees, removed more pollen compared to unvisited florets. *Andrena helianthiformis* removed 70% of the pollen estimated for "typical" heads, i.e., those with the median number of male florets shedding pollen (12) and the median number of pollen grains present before a visit (60). In contrast, other pollinator taxa removed 34–52% of the

TABLE 1 Likelihood ratio tests for stepwise model simplifications using backward elimination of the pollen removal experiment. *P*-values are for the *F*-test of the null hypothesis that a model simplified by excluding the focal term is not different from a model including the focal term. The order in which terms were eliminated did not change results of model simplification. The full models included three main effects: p = pollinator taxon (categorical predictor, five levels), a = number of florets with anthers shedding pollen (linear predictor), b = unvisited floret pollen count (linear predictor), and three interaction terms. For the proportion of pollen removed model, the results in line 4 indicate the minimal model should include the a:b interaction term. The results in line 5 compare that model to the model in line 3 and indicate that the minimal model should include the p main effect term. The final proportion of pollen removed model includes all three main effects and the a:b interaction term. For the number of pollen grains removed model, the results in lines 6 and 7 compare that model to the model in line 5 and indicate that the final model should include the p and b main effect terms. *N* = 124 observed pollinator visits. Bolded lines indicate the final selected models for each model response.

Model response: proportion of pollen removed	Test term	Model df	Residual SS	Test df	*F*	*P*
1. p + a + b + a:b + p:b + p:a		113	17.43			
2. p + a + b + a:b + p:b	p:a	117	17.901	4	0.7632	0.55
3. p + a + b + a:b	**p:b**	**121**	19.31	**4**	**2.3024**	**0.06**
4. p + a + b	a:b	122	20.101	1	4.9572	*0.03*
5. a + b + a:b	p	125	22.324	4	4.721	*0.001*

Model response: number of pollen grains removed	Test term	Model df	Residual SS	Test df	*F*	*P*
1. p + a + b + a:b + p:b + p:a		113	37879			
2. p + a + b + a:b + p:a	p:b	117	38108	4	0.1704	0.95
3. p + a + b + a:b	p:a	121	39499	4	1.0676	0.38
4. p + a + b	a:b	122	40171	1	2.0598	0.15
5. p + b	**a**	**123**	**40838**	**1**	**2.0253**	**0.16**
6. b	p	127	45443	4	3.4678	*0.01*
7. p	b	124	71579	1	92.59	*<0.0001*

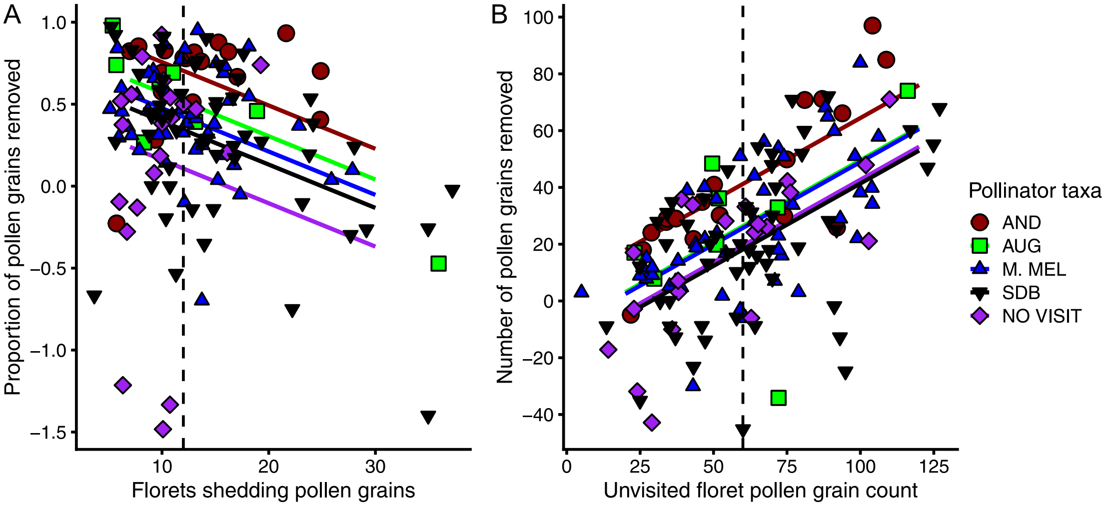

FIGURE 1 Pollen removal per visit by pollinator taxon. (A) Estimated proportions of pollen removed (1 minus the visited floret pollen count divided by the unvisited floret pollen count) decreased with more male florets shedding pollen. The dashed vertical line at 12 indicates median male floret count. (B) Number of pollen grains removed (unvisited floret pollen grain count minus the visited floret pollen grain count) increases with the unvisited floret pollen grain count. The dashed vertical line at 60 indicates the median unvisited pollen count. Best fit lines for each taxon are based on minimal adequate linear models (in bold in Table 1). Pollinator abbreviations: AND = *Andrena helianthiformis*; AUG = Augochlorini; M. MEL = male *Melissodes* spp.; SDB = small-dark bee; NO VISIT = pollen counts are from unvisited florets collected at the end of the observation period. Note: data are jittered horizontally.

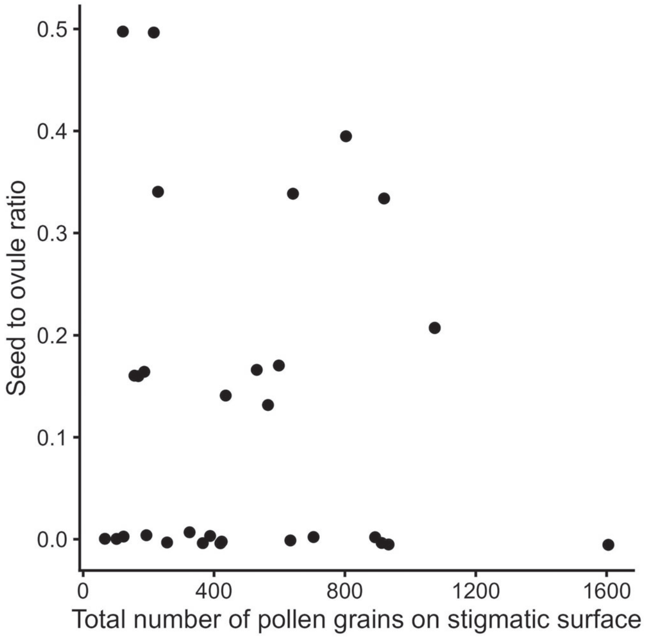

FIGURE 2 The ratio of seeds to ovules versus the number of pollen grains deposited on the stigmatic surface. There was no evidence for a relationship between seed set and pollen grains on the stigmatic surface (generalized linear model, $N = 202$ heads, deviance = 0.026, $P = 0.87$; Appendix S3). Each dot represents the mean of a set of hand crosses, each set originally composed of six florets, typically three styles that were crossed and three that were left uncrossed (mean, with an average of 5.5 florets per cross). Data are jittered vertically.

pollen, and an unvisited head lost 11% of its pollen (Figure 1A), as estimated for "typical" heads. When more florets were shedding pollen on a flowering head, all pollinator categories removed proportionally less pollen, but the rank order of the categories stayed the same (Figure 1A).

We found no relationship between the likelihood of a seed being set and the number of pollen grains deposited on a style or if the style was hand-crossed ($N = 202$, deviance = 0.026, $P = 0.87$; Figure 2; Appendix S3). The interaction term between the predictors also did not improve model fit ($N = 198$, deviance = 2.88, $P = 0.41$). Of 230 individual florets, 30 had a visible seed on the x-ray image (12% of uncrossed florets and 18% of crossed florets).

Paternity assignment

Of the plants that sired offspring, the mean number of offspring sired per observation period was 2.0 (±1.5 SD, range 1–9). Over half (55%) of the sire plants did not sire any offspring during any given observation period. Of the 401 genotyped offspring, 191 were not assigned a sire in Cervus either because there were too many allele mismatches with potential sires or because the delta value had a confidence of <80%. 210 offspring were assigned a sire, with 79 assigned at the 95% confidence interval, and 131 assigned at the 80% confidence interval. Open-pollinated pollen-recipient plants were assigned as sire to 35 of the offspring. MasterBayes assigned the same sire to 272 offspring in at least 50% of the iterations. The programs did not always assign the same sire to the offspring, but the level of assignment differences was similar to what was found in a larger data set of *E. angustifolia* from two experimental plots (Reed et al., 2022).

TABLE 2 Model comparisons to test for effects of pollinator taxon on siring success using two techniques for assigning paternity to offspring. The formula for each null model is shown above the analysis of deviance table; deviance is double the log-likelihood ratio. Varb refers to the aster graph, which comprises two nodes, or fitness components, non-zero siring success (binomial), and count of offspring (zero-truncated Poisson). To account for variation in field conditions among individual plants and replicated observation days, all models include three fixed-effect covariates: counts of male florets shedding pollen (log-transformed lmmf), counts of pollinator visits to the plant (tv), and categorical observation period with four levels (be). We modeled only covariate main effects except in the case of paternity assignments using MasterBayes (see Methods). The predictor of interest is pollinator taxon (pol), which is categorical with five levels. Comparisons were first made between the null model and a model with the pollinator taxon by offspring count interaction (pol:offCt) to test for an effect of pollinator taxon on total plant fitness. Comparisons of that model with a model including a main effect pol term test for an effect of taxon on total plant fitness after accounting for an effect of taxon on both fitness components combined. Parameter estimates of bolded models are shown in Figure 3. $N = 302$ observations of pollen-donor plants by observation period. Bolded lines indicate the final selected model term for each model response.

Model response: Cervus paternity assignment delta values > 80% confidence interval

Null model: resp ~ varb + lmmf + pv + op

Model term	Model df	Model dev	df	Deviance	*P*
	7	−442.5			
pol:offCt	**11**	**−436.1**	**4**	**6.43**	**0.17**
pol	15	−432.8	4	3.28	0.51

Model response: MasterBayes paternity assignment in at least 50% of the iterations

Null model: resp ~ varb + lmmf + pv + op + pv:op

Model term	Model df	Model dev	df	Deviance	*P*
	7	−446.8			
pol:offCt	**11**	**−434.8**	**4**	**11.97**	**0.02**
pol	15	−433.9	4	0.86	0.93

Pollinator taxa and siring success

Fitness of individual plants through male function, or siring success, consistently differed among the five pollinator taxa when employing the >50% MasterBayes approach for assigning paternity ($P < 0.05$; Table 2). The Cervus at 80% confidence interval method showed reduced differences among groups ($P = 0.17$; Table 2). Plants visited by male *Melissodes* spp. bees had the greatest estimated fitness, followed by those visited by *A. helianthiformis*. Plants

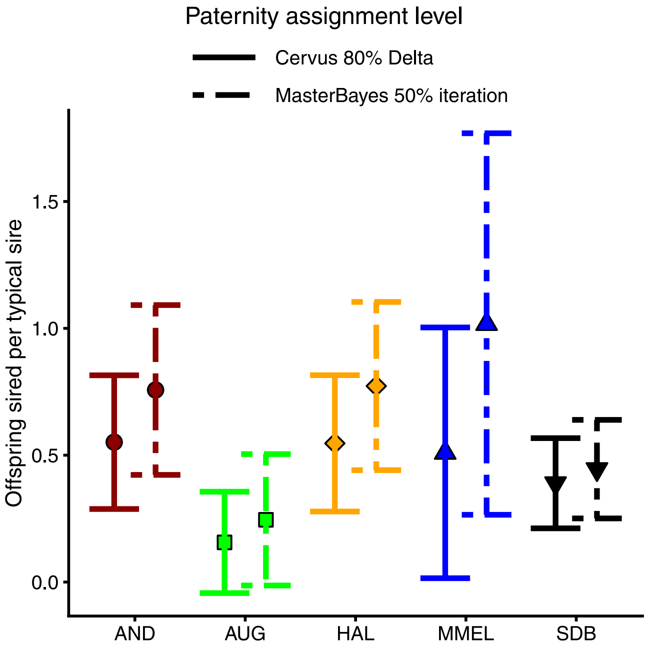

FIGURE 3 Estimated number of offspring sired (mean ± 95% confidence interval) per pollinator taxon based on paternity assignments using Cervus or MasterBayes. Estimates are from the minimal adequate aster model of male fitness for a "typical" pollen-donor plant (in bold in Table 1). A typical plant had the median number of pollinator visits (2), the median number of male florets shedding pollen (27) and flowered during observation period July 10-11. Pollinator abbreviations: AND = *Andrena helianthiformis*; AUG = Augochlorini; HAL = *Halictus* spp.; MMEL = male *Melissodes* spp.; SDB = small-dark bee.

visited by the broader groups of bees (*Halictus* spp. and small-dark bees) had intermediate fitness. The siring success of plants visited by male *Melissodes* spp. was estimated to be 3.3–4.1 times greater than for plants visited by Augochlorini (Figure 3).

All fitness estimates account for three covariates that were included in all aster models: number of male florets, number of pollinator visits, and observation period. Most covariate effect sizes were comparable to those of pollinator taxon. In contrast, the effects of the number of visits were almost always negligible, whether quantified as counts of visits by all visitors or just target taxon visits. We conducted an additional analysis to further investigate this covariate, as described in the next section. Covariates were modeled as main effects in all circumstances with the addition of a pollinator visits by observation period interaction term for the model using MasterBayes assignments (Table 2).

The two responses, reflecting different paternity assignment methods, yielded consistent patterns of pollinator taxon effects. The differences in absolute fitness result from the number of offspring assigned in each method (Figure 3). Model selection for each of the responses revealed pollinator effects on total siring success, as evidenced in comparisons of null models with models that include a pollinator taxon effect on the offspring count (offCt) node, but details differed. The model with the response using 80% confidence interval in Cervus provided very weak evidence of any pollinator taxon effect ($P = 0.17$, Table 2), while the model using MasterBayes >50% iterations showed strong evidence ($P = 0.02$, Table 2). A significant pollinator taxon by offspring count (pol:offCt) term indicates a pollinator taxon effect on total siring success, the final node in the aster graph. We also compared each of those models with a model that includes a pollinator taxon main effect. A model with this main effect quantifies an effect of pollinator taxon on total siring success after accounting for the effect of pollinator taxon on both fitness components together. Both responses showed little to no evidence to include this additional main effect of taxon ($P \geq 0.10$).

Pollinator visitation and siring success

For the single-pollinator taxon analysis, we found mixed evidence that the number of pollinator visits predicted siring success. For plants visited by *Halictus* spp., pollinator visits significantly predicted siring success when the response, siring success, used MasterBayes paternity assignment. However, the effect for *Halitctus* spp. was not significant for the responses based on the Cervus assignment method (Figure 4; Appendix S4). There was no evidence that the number of pollinator visits predicted siring success for small-dark bees and *A. helianthiformis* ($P > 0.05$; Figure 4; Appendix S4). For

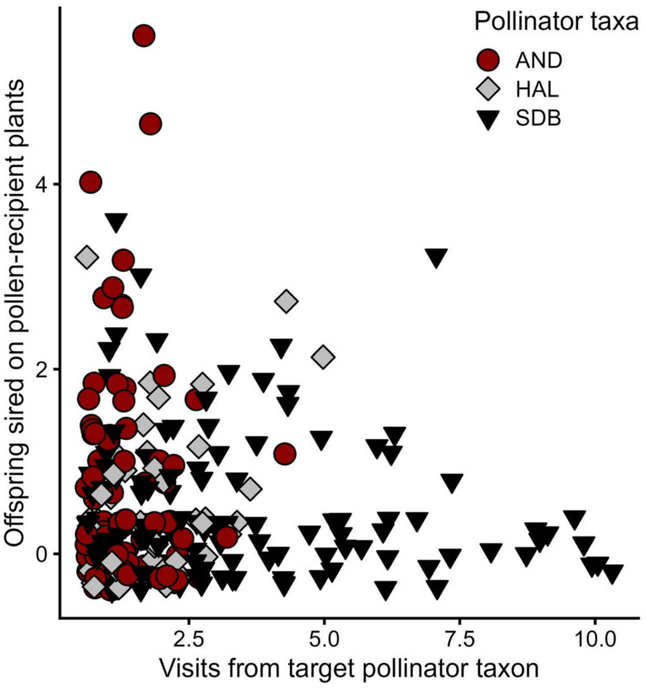

FIGURE 4 Number of offspring sired on pollen-recipient plants by the number of pollinator taxa visits for the three most observed pollinator taxa: *Andrena helianthiformis* (AND); *Halictus* spp. (HAL); small-dark bees (SDB). Shown are paternity assignments using Cervus. The number of pollinator visits was not predictive of the number of offspring sired for these pollinator taxa ($P > 0.05$). Results from model selection for both paternity assignments can be found in Appendix S4.

small-dark bees, the number of florets shedding pollen has some predictive power for siring success (Appendix S4).

DISCUSSION

Pollinator taxa affect plant siring success

Our experiment revealed that the identity of pollinator visitors affected siring success of *E. angustifolia*. Siring success in plants visited by Augochlorini was the lowest, while plants visited by male *Melissodes* spp. had over triple the siring success, though this was only significant for the MasterBayes paternity assignments (Figure 3). Many other aspects of the environment and characteristics of the plants contributed to substantial variation in siring success among individuals, such as the observation day and number of florets producing pollen on that day. Nonetheless, our experimental design and statistical approach enabled us to quantify substantial differences among pollinator taxa, even with relatively small sample sizes for some taxa.

Generalist and specialist pollinator taxa had similar impacts on siring success. Plants visited by oligolectic *A. helianthiformis* had similar siring success to those plants visited by three generalist bee taxa in our study: *Halictus* spp., male *Melissodes* spp., and small-dark bees. As generalists, these bees are expected to visit more heterospecific coflowering species, which may reduce siring success. However, in natural populations near this study site, Richardson et al. (2021) found that most generalist bees visiting *E. angustifolia* carried primarily conspecific pollen. Interestingly, generalist bees in the Augochlorini tribe carried more heterospecific pollen than any other major *E. angustifolia* pollinator (Ison et al., 2018; Richardson et al., 2021), which could explain their reduced contribution to siring success in this experiment. Augochlorini bees dominate the pollinator community in small remnant populations and late in the flowering season (Ison et al., 2018). Their low-quality but high-quantity visitation could contribute to the commonly observed pollen-limited reproduction in small remnant *E. angustifolia* populations.

Most of our pollinator taxa comprised females actively collecting and grooming pollen. Interestingly, plants visited by the nongrooming taxon, male *Melissodes* spp., had siring success equal to or greater than those visited by the grooming taxa (Figure 3). Previously, we found no differences in the distance grooming and nongrooming bee taxa move *E. angustifolia* pollen (Page et al., 2019). However, other studies have found or discussed that nongrooming insects (e.g., flies, male bees) or limited-grooming birds move pollen farther or more effectively than grooming female bees (Rader et al., 2011; Krauss et al., 2017; Tang et al., 2019). Our findings are the first indication that nongrooming behavior could increase siring success. Yet other research suggests increased grooming behavior increases pollen carryover because it "stirs" the pollen on the bee's body so that not just pollen from the most recent flower is "on top" and able to be deposited (Marcelo et al., 2022 [preprint]). Thus, it is still unclear how nongrooming behavior impacts pollen carryover, though the increased siring success from male bee visits in this study could indicate increased pollen carryover.

Pollinator taxa have high per-visit pollen removal rates

Pollinator taxa varied in their per-visit pollen removal, with oligolectic *A. helianthiformis* removing the most, and no significant difference in per-visit removal among the three generalist bee taxa (Augochlorini, male *Melissodes* spp., small-dark bees; Figure 1). We note that pollinator taxa visited during the same time of day, suggesting that all visitors have access to pollen at least during the beginning of their foraging bouts in our system. All pollinators removed a large portion (30–68%) of a flowering head's pollen during a single visit. Consequently, it could only take two or three visits for an individual from any pollinator taxa to remove almost all pollen from a flowering head at these rates. Though this calculation likely overestimates pollen removal in subsequent visits, it emphasizes that only a few bees remove an individual plant's entire daily pollen load.

If some floral visitors are effective at collecting pollen but ineffective at depositing it, it may lead to pollen depletion or even pollen robbing (Hargreaves et al., 2009; Solís-Montero et al., 2015; Parker et al., 2016; Koski et al., 2018). Pollen depletion has been hypothesized as an underappreciated mechanism for pollen limitation and reproductive failure, particularly in small-isolated plant populations (Wilcock and Neiland, 2002). The high rates of pollen removal we observed, especially by *A. helianthiformis*, could suggest pollen depletion occurs in this system. However, the lack of correlation between seed set and the quantity of conspecific pollen deposited in our hand-crossing experiment indicates that variation among pollinators in pollen deposition and potential pollen depletion abilities may have little impact on male fitness of plants. Our findings support previous work by Page et al. (2019) that only one pollen grain is needed to fertilize an ovule, indicating that pollen depletion likely is not a major cause of pollen limitation in *E. angustifolia* and potentially in other uniovulate systems.

Pollinator visitation rates and pollen removal do not predict plant siring success

Due to the logistical challenges of directly measuring a plant's siring success, measurements such as pollinator visitation rates and pollen removal have commonly been used as proxies for male fitness (Queller, 1983; Young and Stanton, 1990; Klinkhamer et al., 1994; Irwin and Brody, 2011; Sun et al., 2018). Few studies have directly compared a proxy measure to actual fitness (but see the review by Schaeffer et al., 2013). When we compared pollen removal to siring success across pollinator taxa, however, no clear pattern emerged. *Andrena helianthiformis* removed the most pollen per visit, but plants visited by *A. helianthiformis* had a similar siring success rate compared to plants visited by other taxa, with the exception of Augochlorini. Therefore, pollen removal does not appear to be an appropriate proxy for male fitness.

Unlike what others have found for female fitness (Vázquez et al., 2005; Sahli and Conner, 2006), we found that for male fitness, the number of pollinator visits per plant per observation period was not a consistent predictor of siring success (Figure 4; Appendix S4). Therefore, we caution against using pollinator visitation as a proxy for male fitness. Similar to pollen removal, this lack of relationship between pollinator visitation rates and male fitness impact could be attributed to *E. angustifolia*'s uniovulate nature since increased pollen deposition on a stigmatic surface does not increase the likelihood of ovule fertilization (Figure 2). In fact, several studies of pollination in *E. angustifolia* found little relationship between pollinator visitation and female fitness (Wagenius and Lyon, 2010; Ison et al., 2018; Richardson et al., 2021).

Impact on male and female reproductive fitness among pollinator taxa

In this study, we found that pollinator taxa had differing degrees of impact on male reproductive success compared to their impact on female reproductive success, as quantified by Page et al. (2019) in the same study area. In other systems, it makes sense that pollinators might differentially influence male and female fitness. Male and female function can be spatially or temporally separated, meaning pollinators may preferentially visit one of the floral sex phases or will not contact both male and female reproductive organs (Zych, 2007; Deschepper et al., 2018). In *E. angustifolia*, individual florets are protandrous, but male- and female-phase florets are right next to each other, and many pollinators contact both during one visit (see videos of Page et al., 2019). Therefore, the disparity we observed between male and female fitness impacts within pollinator taxa is surprising. More research on pollen carryover in this system could help to elucidate the reason. For example, V. G. Marcelo et al. (2022 [preprint]) found that grooming bees have increased pollen carryover and thus greater diversity in their deposited pollen. Therefore, in our system, a grooming bee like *A. helianthiformis* might deposit diverse, high-quality pollen that sets a lot of seeds (increasing female fitness), but the high diversity of the pollen load means that few seeds are set by any one sire's pollen per visit (average impact on male fitness).

The observed differences in male and female fitness impacts have implications for estimating pollinator-mediated selection. For example, if we had used female fitness as a proxy for overall reproductive fitness, we likely would have overestimated the potential for selection on plant traits that increase *A. helianthiformis* visits. More broadly, the variation in fitness for both male and female function in *E. angustifolia* means that pollinator selection on a plant trait could occur through either function. Other studies have attempted to quantify the strength of selection via pollinators through male versus female fitness with mixed results (reviewed by Ashman and Morgan, 2004; Sahli and Conner, 2011). Our work and previous work by Page et al. (2019) demonstrate that pollinator taxa have a strong impact on both male and female fitness in this system, and therefore, there is strong potential for pollinator-mediated selection.

Implications for conservation

Like many prairie plants, *E. angustifolia* populations are often found in fragmented remnants (Samson et al., 2004; Gage et al., 2016). These small remnants are at increased risk of reproductive failure due to mate limitation, which is influenced by the local pollinator community (Aguilar et al., 2006). A better understanding of how the pollinator community impacts the reproductive fitness of *E. angustifolia* will help us to implement more effective conservation measures for this plant and other common prairie species. Previous research focused on female fitness suggested that we focus our conservation efforts on *A. helianthiformis* because it sets the most seeds per visit (Page et al., 2019). However, here we found that *A. helianthiformis* does not sire more seeds per visit compared to most taxa. We also found that visitation by Augochlorini resulted in low siring success. Since Augochlorini is the most common pollinator taxon in the small remnants, as previously established (Ison et al., 2018), their low siring success may put these remnants at increased risk of reproductive failure. To address this, we recommend broad conservation of the other pollinator taxa—*A. helianthiformis*, small-dark bees, *Halictus* spp., and male *Melissodes* spp.—to help promote male fitness and pollinator diversity in these fragmented landscapes. For example, a promising recent study found that prescribed burns can increase the number of active ground-nesting bee nest sites in prairie remnants (Brokaw et al., 2023).

Our work also illustrates the value of directly quantifying male fitness in plants. Though direct quantification is logistically difficult, we found that two commonly used proxies for male fitness, pollen removal and visitation rates, are ineffective at predicting male fitness. Additionally, there was a disconnect between the most effective pollinators from a female fitness perspective (Page et al., 2019) versus a male fitness perspective. A comprehensive understanding of reproductive fitness is particularly important in fragmented populations, which are at higher risk of reproductive failure (Aguilar et al., 2006; Wagenius and Lyon, 2010). We found that most pollen-donor plants sired no offspring in pollen-recipient plants, leaving a small pool of individuals to sire all the offspring. This high variance in male fitness could reduce effective population sizes, with consequences for population dynamics (Kulbaba and Shaw, 2021) and perhaps increasing the likelihood that these already vulnerable populations go extinct (Caballero, 1994). More studies directly estimating male fitness in fragmented habitats would improve our understanding of effective population sizes, informing more comprehensive and better tailored conservation plans.

AUTHOR CONTRIBUTIONS

J.L.I., S.W., Z.Z., E.X.J., and M.A.S. designed the field manipulations. Z.Z., E.X.J., M.A.S., collected microscopy data. A.E.P., L.A.H., M.A.S., M.M.N.I., and R.M.J. collected genotype data. A.E.P., J.L.I, S.W., and M.A.S. analyzed the data. A.E.P. and J.L.I wrote the initial draft of the manuscript with contributions from S.W., M.A.S., L.A.H., and Z.Z. All authors contributed to manuscript revisions.

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DATA AVAILABILITY STATEMENT

Data and analysis scripts are available for this study and can be accessed at https://openworks.wooster.edu/facpub/418/ (Pearson et al., 2023).

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REFERENCES

Adler, L. S., and R. E. Irwin. 2006. Comparison of pollen transfer dynamics by multiple floral visitors: experiments with pollen and fluorescent dye. *Annals of Botany* 97: 141–150.

Aguilar, R., L. Ashworth, L. Galetto, and M. A. Aizen. 2006. Plant reproductive susceptibility to habitat fragmentation: review and synthesis through a meta-analysis. *Ecology Letters* 9: 968–980.

Aizen, M. A., L. Ashworth, and L. Galetto. 2002. Reproductive success in fragmented habitats: Do compatibility systems and pollination specialization matter? *Journal of Vegetation Science* 13: 885–892.

Armbruster, W. S., S. Keller, M. Matsuki, and T. P. Clausen. 1989. Pollination of *Dalechampia magnoliifolia* (Euphorbiaceae) by male euglossine bees. *American Journal of Botany* 76: 1279–1285.

Ashley, M. V. 2010. Plant parentage, pollination, and dispersal: how DNA microsatellites have altered the landscape. *Critical Reviews in Plant Sciences* 29: 148–161.

Ashman, T. L., and M. T. Morgan. 2004. Explaining phenotypic selection on plant attractive characters: male function, gender balance or ecological context? *Proceedings of the Royal Society of London, B, Biological Sciences* 271: 553–559.

Bateman, A. J. 1948. Intra-sexual selection in *Drosophila*. *Heredity* 2: 349–368.

Briscoe Runquist, R. D., M. A. Geber, M. Pickett-Leonard, and D. A. Moeller. 2017. Mating system evolution under strong pollen limitation: evidence of disruptive selection through male and female fitness in *Clarkia xantiana*. *American Naturalist* 189: 549–563.

Brokaw, J., Z. M. Portman, B. Bruninga-Socolar, and D. P. Cariveau. 2023. Prescribed fire increases the number of ground-nesting bee nests in tallgrass prairie remnants. *Insect Conservation and Diversity* 16: 355–367.

Brosi, B. J., and H. M. Briggs. 2013. Single pollinator species losses reduce floral fidelity and plant reproductive function. *Proceedings of the National Academy of Sciences, USA* 110: 13044–13048.

Brunet, J., and H. R. Sweet. 2006. The maintenance of selfing in a population of the Rocky Mountain columbine. *International Journal of Plant Sciences* 167: 213–219.

Caballero, A. 1994. Developments in the prediction of effective population size. *Heredity* 73: 657–679.

Campbell, D. R. 1991. Comparing pollen dispersal and gene flow in a natural population. *Evolution* 45: 1965–1968.

Caruso, C. M., K. E. Eisen, R. A. Martin, and N. Sletvold. 2019. A meta-analysis of the agents of selection on floral traits. *Evolution* 73: 4–14.

Chamer, A. M., D. Medan, A. I. Mantese, and N. J. Bartoloni. 2015. Impact of pollination on sunflower yield: Is pollen amount or pollen quality what matters? *Field Crops Research* 176: 61–70.

Colicchio, J., P. J. Monnahan, C. A. Wessinger, K. Brown, J. R. Kern, and J. K. Kelly. 2020. Individualized mating system estimation using genomic data. *Molecular Ecology Resources* 20: 333–347.

Crawley, M. J. 2013. The R book. Wiley, Chichester, UK.

Delph, L. F., and T.-L. Ashman. 2006. Trait selection in flowering plants: How does sexual selection contribute? *Integrative and Comparative Biology* 46: 465–472.

Deschepper, P., R. Brys, and H. Jacquemyn. 2018. The impact of flower morphology and pollinator community composition on pollen transfer in the distylous *Primula veris*. *Botanical Journal of the Linnean Society* 186: 414–424.

Devaux, C., C. Lepers, and E. Porcher. 2014. Constraints imposed by pollinator behaviour on the ecology and evolution of plant mating systems. *Journal of Evolutionary Biology* 27: 1413–1430.

Feghahati, S. M. J., and R. N. Reese. 1994. Ethylene-, light-, and prechill-enhanced germination of *Echinacea angustifolia* seeds. *Journal of the American Society for Horticultural Science* 119: 853–858.

Fenster, C. B. 1991. Gene flow in *Chamaecrista fasciculata* (Leguminosae) I. Gene dispersal. *Evolution* 45: 398–409.

Gage, A. M., S. K. Olimb, and J. Nelson. 2016. Plowprint: tracking cumulative cropland expansion to target grassland conservation. *Great Plains Research* 26: 107–116.

Hadfield, J. D., D. S. Richardson, and T. Burke. 2006. Towards unbiased parentage assignment: combining genetic, behavioural and spatial data in a Bayesian framework. *Molecular Ecology* 15: 3715–3730.

Hargreaves, A. L., L. D. Harder, and S. D. Johnson. 2009. Consumptive emasculation: the ecological and evolutionary consequences of pollen theft. *Biological Reviews* 84: 259–276.

Herrera, C. M. 1987. Components of pollinator 'quality': comparative analysis of a diverse insect assemblage. *Oikos* 50: 79–90.

Irwin, R. E., and A. K. Brody. 2011. Additive effects of herbivory, nectar robbing and seed predation on male and female fitness estimates of the host plant *Ipomopsis aggregata*. *Oecologia* 166: 681–692.

Ison, J. L., L. J. Prescott, S. W. Nordstrom, A. Waananen, and S. Wagenius. 2018. Pollinator-mediated mechanisms for increased reproductive success in early flowering plants. *Oikos* 127: 1657–1669.

Ison, J. L., S. Wagenius, D. Reitz, and M. V. Ashley. 2013. Development and evaluation of microsatellite markers for a native prairie perennial, *Echinacea angustifolia* (Asteraceae). *Applications in Plant Sciences* 1: 1300049.

Ison, J. L., S. Wagenius, D. Reitz, and M. V. Ashley. 2014. Mating between *Echinacea angustifolia* (Asteraceae) individuals increases with their flowering synchrony and spatial proximity. *American Journal of Botany* 101: 180–189.

Kalinowski, S. T., M. L. Taper, and T. C. Marshall. 2007. Revising how the computer program Cervus accommodates genotyping error increases success in paternity assignment. *Molecular Ecology* 16: 1099–1106.

Karron, J. D., R. J. Mitchell, K. G. Holmquist, J. M. Bell, and B. Funk. 2004. The influence of floral display size on selfing rates in *Mimulus ringens*. *Heredity* 92: 242–248.

Kearns, C. A., and D. W. Inouye. 1993. Techniques for pollination biologists. University of Colorado Press, Denver, CO, USA.

Klinkhamer, P. G. L., T. J. 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.

Konzmann, S., M. Kluth, D. Karadana, and K. Lunau. 2020. Pollinator effectiveness of a specialist bee exploiting a generalist plant—tracking pollen transfer by *Heriades truncorum* with quantum dots. *Apidologie* 51: 201–211.

Koski, M. H., J. L. Ison, A. Padilla, A. Q. Pham, and L. F. Galloway. 2018. Linking pollinator efficiency to patterns of pollen limitation: small bees exploit the plant–pollinator mutualism. *Proceedings of the Royal Society, B, Biological Sciences* 285: 20180635.

Krauss, S. L., R. D. Phillips, J. D. Karron, S. D. Johnson, D. G. Roberts, and S. D. Hopper. 2017. Novel consequences of bird pollination for plant mating. *Trends in Plant Science* 22: 395–410.

Kulbaba, M. W., and R. G. Shaw. 2021. Lifetime fitness through female and male function: influences of genetically effective population size and density. *American Naturalist* 197: 434–447.

Marcelo, V. G., F. M. Darcie Marquitti, M. Vallejo-Marin, and V. L. G. D. Brito. 2022. Pollinator grooming behavior alters pollen landscapes on bees bodies and increases pollen carryover to other flowers. preprint. bioRxiv: 2022-07. https://www.biorxiv.org/content/10.1101/2022.07.14.499901v1.abstract

Minnaar, C., and B. Anderson. 2019. Using quantum dots as pollen labels to track the fates of individual pollen grains. *Methods in Ecology and Evolution* 10: 604–614.

Minnaar, C., B. Anderson, M. L. de Jager, and J. D. Karron. 2019. Plant–pollinator interactions along the pathway to paternity. *Annals of Botany* 123: 225–245.

Mitchell, R. J., R. E. Irwin, R. J. Flanagan, and J. D. Karron. 2009. Ecology and evolution of plant–pollinator interactions. *Annals of Botany* 103: 1355–1363.

Moir, M., and B. Anderson. 2023. Pollen layering and male–male competition: quantum dots demonstrate that pollen grains compete for space on pollinators. *American Journal of Botany* 110; e16184 [in press].

Ne'eman, G., A. Jürgens, L. Newstrom-Lloyd, S. G. Potts, and A. Dafni. 2010. A framework for comparing pollinator performance: effectiveness and efficiency. *Biological Reviews* 85: 435–451.

Olsen, K. M. 1996. Pollination effectiveness and pollinator importance in a population of *Heterotheca subaxillaris* (Asteraceae). *Oecologia* 109: 114–121.

Page, M. L., J. L. Ison, A. L. Bewley, K. M. Holsinger, A. D. Kaul, K. E. Koch, K. M. Kolis, et al. 2019. Pollinator effectiveness in a composite: a specialist bee pollinates more florets but does not move pollen farther than other visitors. *American Journal of Botany* 106: 1487–1498.

Parker, A. J., N. M. Williams, and J. D. Thomson. 2016. Specialist pollinators deplete pollen in the spring ephemeral wildflower *Claytonia virginica*. *Ecology and Evolution* 6: 5169–5177.

Pearson, E., Z. Zelman, L. A. Hill, M. A. Stevens, E. X. Jackson, M. M. N. Incarnato, R. M. Johnson, et al. 2023. Pollinators differ in their contribution to the male fitness of a self-incompatible composite [Data Analysis Files]. Website: https://openworks.wooster.edu/facpub/418

Queller, D. C. 1983. Sexual selection in a hermaphroditic plant. *Nature* 305: 706–707.

R Core Team. 2022. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Website: https://www.R-project.org/

Rader, R., W. Edwards, D. A. Westcott, S. A. Cunningham, and B. G. Howlett. 2011. Pollen transport differs among bees and flies in a human-modified landscape. *Diversity and Distributions* 17: 519–529.

Reed, W. J., J. L. Ison, A. Waananen, Frank. H. Shaw, S. Wagenius, and R. G. Shaw. 2022. Genetic variation in reproductive timing in a long-lived herbaceous perennial. *American Journal of Botany* 109: 1861–1874.

Richardson, L. K., M. K. Gallagher, T. E. Hayes, A. S. Gallinat, G. Kiefer, K. Manion, M. Jenkins, et al. 2021. Competition for pollination and isolation from mates differentially impact four stages of pollination in a model grassland perennial. *Journal of Ecology* 109: 1356–1369.

Rico-Gray, V., and L. B. Thien. 1989. Effect of different ant species on reproductive fitness of *Schomburgkia tibicinis* (Orchidaceae). *Oecologia* 81: 487–489.

Sahli, H. F., and J. K. Conner. 2006. Characterizing ecological generalization in plant-pollination systems. *Oecologia* 148: 365–372.

Sahli, H. F., and J. K. Conner. 2011. Testing for conflicting and nonadditive selection: floral adaptation to multiple pollinators through male and female fitness. *Evolution* 65: 1457–1473.

Samson, F. B., F. L. Knopf, and W. R. Ostlie. 2004. Great Plains ecosystems: past, present, and future. *Wildlife Society Bulletin* 32: 6–15.

Schaeffer, R. N., J. S. Manson, and R. E. Irwin. 2013. Effects of abiotic factors and species interactions on estimates of male plant function: a meta-analysis. *Ecology Letters* 16: 399–408.

Schmitt, J. 1983. Density-dependent pollinator foraging, flowering phenology, and temporal pollen dispersal patterns in *Linanthus bicolor*. *Evolution* 37: 1247–1257.

Shaw, R. G., C. J. Geyer, S. Wagenius, H. H. Hangelbroek, and J. R. Etterson. 2008. Unifying life-history analyses for inference of fitness and population growth. *American Naturalist* 172: E35–E47.

Solís-Montero, L., and M. Vallejo-Marín. 2017. Does the morphological fit between flowers and pollinators affect pollen deposition? An experimental test in a buzz-pollinated species with anther dimorphism. *Ecology and Evolution* 7: 2706–2715.

Solís-Montero, L., C. H. Vergara, and M. Vallejo-Marín. 2015. High incidence of pollen theft in natural populations of a buzz-pollinated plant. *Arthropod-Plant Interactions* 9: 599–611.

Sun, H.-Q., B.-Q. Huang, X.-H. Yu, C.-B. Tian, Q.-X. Peng, and D.-J. An. 2018. Pollen limitation, reproductive success and flowering frequency in single-flowered plants. *Journal of Ecology* 106: 19–30.

Tang, J., Q.-M. Quan, J.-Z. Chen, T. Wu, and S.-Q. Huang. 2019. Pollinator effectiveness and importance between female and male mining bee (*Andrena*). *Biology Letters* 15: 20190479.

Thomson, J. D., and B. A. Thomson. 1989. Dispersal of *Erythronium grandiflorum* pollen by bumblebees: implications for gene flow and reproductive success. *Evolution* 43: 657–661.

Thomson, J. D., M. V. Price, N. M. Waser, and D. A. Stratton. 1986. Comparative studies of pollen and fluorescent dye transport by bumble bees visiting *Erythronium grandiflorum*. *Oecologia* 69: 561–566.

Tooker, J. F., and L. M. Hanks. 2006. Tritrophic interactions and reproductive fitness of the prairie perennial *Silphium laciniatum* Gillette (Asteraceae). *Environmental Entomology* 35: 537–545.

Valverde, J., F. Perfectti, and J. M. Gómez. 2019. Pollination effectiveness in a generalist plant: adding the genetic component. *New Phytologist* 223: 354–365.

Vázquez, D. P., W. F. Morris, and P. Jordano. 2005. Interaction frequency as a surrogate for the total effect of animal mutualists on plants. *Ecology Letters* 8: 1088–1094.

Waananen, A., G. Kiefer, J. L. Ison, and S. Wagenius. 2018. Mating opportunity increases with synchrony of flowering among years more than synchrony within years in a nonmasting perennial. *American Naturalist* 192: 379–388.

Wagenius, S. 2004. Style persistence, pollen limitation, and seed set in the common prairie plant *Echinacea angustifolia* (Asteraceae). *International Journal of Plant Sciences* 165: 595–603.

Wagenius, S., J. Beck, and G. Kiefer. 2020. Fire synchronizes flowering and boosts reproduction in a widespread but declining prairie species. *Proceedings of the National Academy of Sciences, USA* 117: 3000–3005.

Wagenius, S., E. Lonsdorf, and C. Neuhauser. 2007. Patch aging and the *S*-Allee effect: breeding system effects on the demographic response of plants to habitat fragmentation. *American Naturalist* 169: 383–397.

Wagenius, S., and S. P. Lyon. 2010. Reproduction of *Echinacea angustifolia* in fragmented prairie is pollen-limited but not pollinator-limited. *Ecology* 91: 733–742.

Waser, N. M. 1988. Comparative pollen and dye transfer by pollinators of *Delphinium nelsonii*. *Functional Ecology* 2: 41–48.

Weinman, L. R., T. Ress, and R. Winfree. 2023. Individual bee forageess are less efficient transporters of pollen for the plants from which they collect the most pollen into their scopae. *American Journal of Botany* 110: e16178 [in press].

Wilcock, C., and R. Neiland. 2002. Pollination failure in plants: Why it happens and when it matters. *Trends in Plant Science* 7: 270–277.

Wilson, P., and J. D. Thomson. 1991. Heterogeneity among floral visitors leads to discordance between removal and deposition of pollen. *Ecology* 72: 1503–1507.

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.

Young, H. J., and M. L. Stanton. 1990. Influences of floral variation on pollen removal and seed production in wild radish. *Ecology* 71: 536–547.

Zimmerman, M., and G. H. Pyke. 1988. Reproduction in *Polemonium*: assessing the factors limiting seed set. *American Naturalist* 131: 723–738.

Zych, M. 2007. On flower visitors and true pollinators: The case of protandrous *Heracleum sphondylium* L. (Apiaceae). *Plant Systematics and Evolution* 263: 159–179.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

APPENDIX S1. Supplemental information about the pollinator bee taxa in the study.

APPENDIX S2. Variation in pollen count per sample between unvisited and visited florets in the pollen removal experiment.

APPENDIX S3. Analysis of deviance table comparing generalized linear models for the pollen deposition experiment.

APPENDIX S4. Analysis of deviance table comparing generalized linear models of siring success for the three most common pollinator taxa.

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