

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

Avery E. Pearson^{2,4}, Zeke Zelman², Lauryn A. Hill², Mia A. Stevens^{2,3}, Evan X. Jackson²,
Miyauna M. N. Incarnato², Ren M. Johnson², Stuart Wagenius³, Jennifer L. Ison^{2,5}

²Biology Department, College of Wooster, 1189 Beall Avenue, Wooster, Ohio, 44691, USA.

³Negaunee Institute for Plant Conservation Science and Action at the Chicago Botanic Garden,
1000 Lake Cook Road, Glencoe, Illinois, 60022, USA.

⁴Present address: Department of Biological Sciences, University of North Texas, 1155 Union
Circle #305220, Denton, Texas, 76203, USA.

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Running title: Siring success in *Echinacea* varies by bee visitor

⁵Corresponding author: jison@wooster.edu

ABSTRACT

Premise of the study – 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 assess how five bee taxa affect male fitness in a prairie plant by quantifying pollen removal, visitation, and siring success using paternity assignments and a unique experimental approach.

Methods – In *Echinacea angustifolia*, we measured per-visit pollen removal for each pollinator taxa 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.

Key results – Siring success of pollen donor plants differed among five pollinator taxa. Non-grooming 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. Finally, 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. Additionally, we discuss how conservation efforts focused on preserving a diverse pollinator community can benefit plants in fragmented landscapes.

KEY WORDS: plant-pollinator interactions; male fitness; Asteraceae; *Echinacea angustifolia*, pollen removal; native bees; pollen movement; tallgrass prairie; plant reproduction; siring success

INTRODUCTION

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 in Mitchell et al., 2009). In contrast, insect visitors with either large foraging ranges or those that do not actively groom pollen may increase 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 if 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, it is predicted that male fitness will drive the evolution of traits associated with increased pollinator service, such as 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 towards 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: this issue) allow researchers to track individual 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 SNPs, to determine which plant sired each seed (reviewed in 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 if pollinator taxa differ in their potential to impact male fitness, we can quantify how much pollen each taxon removed during a visit. Yet, pollen removal by a floral visitor does not guarantee that it will successfully set a seed. In fact, there are many routes a pollen grain can take that do not result in deposition on conspecific stigmas (reviewed in Minnaar et al., 2019). We also know that different floral visitors vary greatly in their ability to transfer pollen effectively. 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 (Parker et al., 2016; Wilson and Thomson, 1991)

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: this issue).

Furthermore, we cannot assume that more pollen grains deposited on a stigma result in more seeds 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 at least 26 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 has been found in other systems (e.g., Valverde et al., 2019). In this study, we focus on how visits by different bee taxa impact the male fitness of *E. angustifolia*. Our objectives are: 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 *E. angustifolia*'s male fitness allows us to gain a more holistic understanding of how floral visitors impact overall plant reproductive fitness, rather than a female-fitness-skewed view.

MATERIALS AND METHODS

Study site – In summer 2018, we conducted field manipulations and observations in a common garden experimental plot, approximately 60 m x 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 in previous studies (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, is a long-lived perennial and member of the Asteraceae, native to the tallgrass prairie of North America. Individual plants are non-clonal and have a sporophytic self-incompatibility system, so they depend on floral visitors to move pollen between plants for successful reproduction. A flowering *E. angustifolia* plant typically has one flowering head but can have over ten. 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's flowering period typically ranging from 6-36 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 during one day in which 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 hours 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 observation).

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*, 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 (see Supplementary Data with this article). 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 July 9th to July 30th, 2018. We prevented visitation

for at least one 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 ten 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 removed three male-phase florets (hereafter, ‘visited florets’) using the same methods as unvisited floret removal. At the end of the observation period, we collected three male-phase florets from the bagged 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 (method adapted from 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 seconds. We then loaded 40 μ L of this pollen solution into a hemocytometer (Paul Marienfeld GmbH & Co, in Lauda-Königshofen, Germany) and counted all pollen grains in a 17.78 μ L subset of the sample at 100x 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 lower pollen count than unvisited florets collected on the same day from the same flowering head, indicating that there is more variation between florets from different flowering head 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 which were 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 visitor), 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 backwards 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 hours and then performed hand-crosses in sets of nine florets. We removed self-pollen from heads before beginning the crossing process. In each 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 of from multiple plants; some sets of crosses had low pollen deposition (applied with tooth flosser), while others had higher deposition (applied with a toothpick). After performing the crosses, we re-bagged the flowering heads. After 24 hours, we removed styles from the controls and three of the crossed styles and placed them in tubes of 70% ethanol. The remaining three styles were left in case 24 hours 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 dataset. We kept track of crosses and distinguished treatment 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 if a seed was set (i.e., contained an embryo) because achenes in *E. angustifolia* expand regardless of fertilization status (see supplement information in Wagenius et al., 2020 for more details). Next, we counted the number of pollen grains on the collected styles by first soaking them in 1M NaOH for 12-24 hours and counting pollen grains stained with fusion jelly under a compound light microscope using the 100x and 400x magnification (Kearns and Inouye, 1993). We counted the total number of pollen grains present on the 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 backwards elimination, as described in the pollen removal analysis.

Siring success field methods – In July 2018, we conducted a field manipulation to determine how floral visits from different pollinator taxa impact the siring success of *E. angustifolia* individuals. Our manipulation took place in an approximately 20 m x 40 m section of our study plot over five days (July 6, 10, 11, 16, and 17). While *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 *E. angustifolia*'s peak flowering time, 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. For July 11, we kept the target pollinator taxon the same as on July 10. For example, if a pollen donor plant was assigned Augochlorini on July 10,

Augochlorini was also the target pollinator taxon for July 11. Therefore, July 10 and 11 were lumped into one observation period yielding four total observation periods (July 6, 10-11, 16, & 17) 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 July 10 and 11 and on July 16 and 17 (note: the target pollinator taxon could change between July 16 and 17 but not between July 10 and 11). On each observation day, once researchers 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 hours 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 July 16 and 17, we recorded which styles shriveled on July 17, immediately after the observation period, and again on July 18. Since styles take 12-24 hours to shrivel, the July 17 shriveled styles were pollinated during the July 16 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 of 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, stdev $\pm$ 2). Five pollinator taxa were the target pollinator for at least twelve 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 for eight 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 a 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 ten 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 (Maryland, USA), as described in Ison et al. (2013). We genotyped the offspring following the procedure in Ison et al. (2013) with the adjustments described in 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), Reed et al. (2022).

We developed two datasets 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 there was a genotyping error of 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 on 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 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 to 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 taxon 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 a single pollinator taxon. Therefore, we examined if 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 backwards 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 in unvisited florets (Table 1). There was also a pollinator effect when the response was the total number of 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 category, 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 after a visit, there

was a significant interaction between the number of florets with anthers shedding pollen and the unvisited pollen count (Table 1, $P < 0.05$). All three main effects (number of florets with anthers shedding pollen, pollinator taxa, and unvisited 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 grains (Fig. 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 pollen, and an unvisited head lost 11% of its pollen (Fig. 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 (Fig. 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, $P = 0.87$; Fig. 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 (stdev $\pm$ 1.5, 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 assignments differences was similar to what we found in a larger dataset of *E. angustifolia* from two experimental plots (Reed et al., 2022).

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 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 (Fig. 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 effects 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 for the model using MasterBayes 50% interaction assignments (Table 2).

The two responses reflecting different paternity assignment methods, yielded consistent patterns of pollinator taxon effects, and the differences in absolute fitness result from the number of offspring assigned in each method (Fig. 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 *Halictus* spp. was not significant for the responses based on the Cervus assignment method (Appendix S4; Fig. 4). There was no evidence that the number of pollinator visits predicted siring success for small-dark bees and *A. helianthiformis* ($P > 0.05$; Appendix S4; Fig. 4). For 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 (Fig. 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 co-flowering 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. The generalist bee Augochlorini 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 dominates 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 non-grooming taxon, male *Melissodes* spp., had siring success equal to or greater than those visited by the grooming taxa (Fig. 3). Previously, we found no differences in the distance grooming and non-grooming bee taxa move *E. angustifolia* pollen (Page et al., 2019). However, other studies have found or discussed that non-grooming insects (e.g. flies, male bees) or limited-grooming birds move pollen farther or more effectively than grooming female bees (Rader et al., 2011; Tang et al., 2019; Krauss et al., 2017). Our findings are the first indication that non-grooming behavior could increase siring success. Yet other research suggests increased grooming behavior increases pollen carryover because it ‘stirs’ up 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., 2023; this issue). Thus, it is still unclear how non-grooming 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; Fig. 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.

Interestingly, unvisited florets that were left out for three or more hours in the windy prairie (labeled ‘no visit’ in Fig. 1), had the lowest pollen loss. This suggests that a single visit by any bee removes at least as much pollen as an unvisited flowering head would lose in the entire morning due to wind or other abiotic factors.

High pollen removal rates, like the ones seen here, especially by *A. helianthiformis*, could suggest pollen depletion occurs in our system. 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). However, the lack of correlation between seed set and the quantity of conspecific pollen deposited that we found 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 Schaeffer et al., 2013 review). 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 (Fig. 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 (Fig. 2). In fact, several studies of pollination in *E. angustifolia* found no 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 associated with 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 this. For example, Marcelo et al. (2023: this issue) find 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 *E. angustifolia*'s reproductive fitness will help us to implement more effective conservation measures for this plant and other common prairie species. Previous research from a female fitness perspective 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 on 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.

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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 microscope 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.

DATA AVAILABILITY

Data and analysis scripts are available for this study and can be accessed at:

<https://openworks.wooster.edu/facpub/418/>

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the 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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TABLES

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 = pollen count before removal (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.

[illegible]

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

Table 2. Model comparisons to test for effects of pollinator taxon on siring success using four 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 50% of the MasterBayes iterations (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 Fig. 3. N = 302 observations of paternal 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

FIGURE LEGENDS

Fig. 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 the number of male florets shedding pollen. The dashed vertical line at 12 indicates median male floret count. (B) Number of pollen grains removed (visited floret pollen grain count minus the unvisited 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 taxa 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.

Fig. 2. The ratio of seeds to ovules versus the number of pollen grains per 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 means of a set of hand-crosses, each set originally composed of 6 florets, typically three styles that were crossed and three that were left uncrossed (mean, with an average of 5.5 florets per cross). Note: data are presented by cross and are jittered vertically.

Fig. 3. Estimated number of offspring sired (mean $\pm$ 95% confidence interval) per pollinator taxon based on two paternity assignments - Cervus (hashed-dotted lines) and MasterBayes (long-dashed). 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 taxa abbreviations: AND = *Andrena helianthiformis*; AUG= Augochlorini; HAL = *Halictus* spp.; MMEL = male *Melissodes* spp.; SDB = small-dark bee.

Fig. 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* – red circles (AND), *Halictus* spp. – gray diamonds (HAL), and small-dark bees – black triangles (SDB). Shown are paternity assignments using Cervus. The number of pollinator visits did not predict 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.

Figure 1

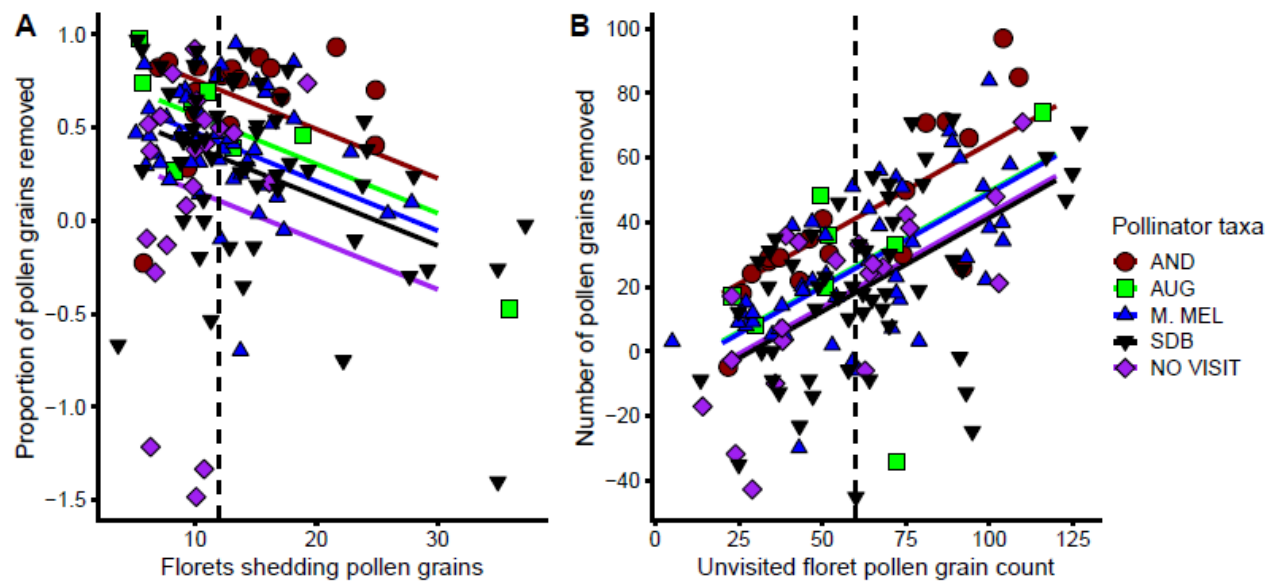


Figure 2

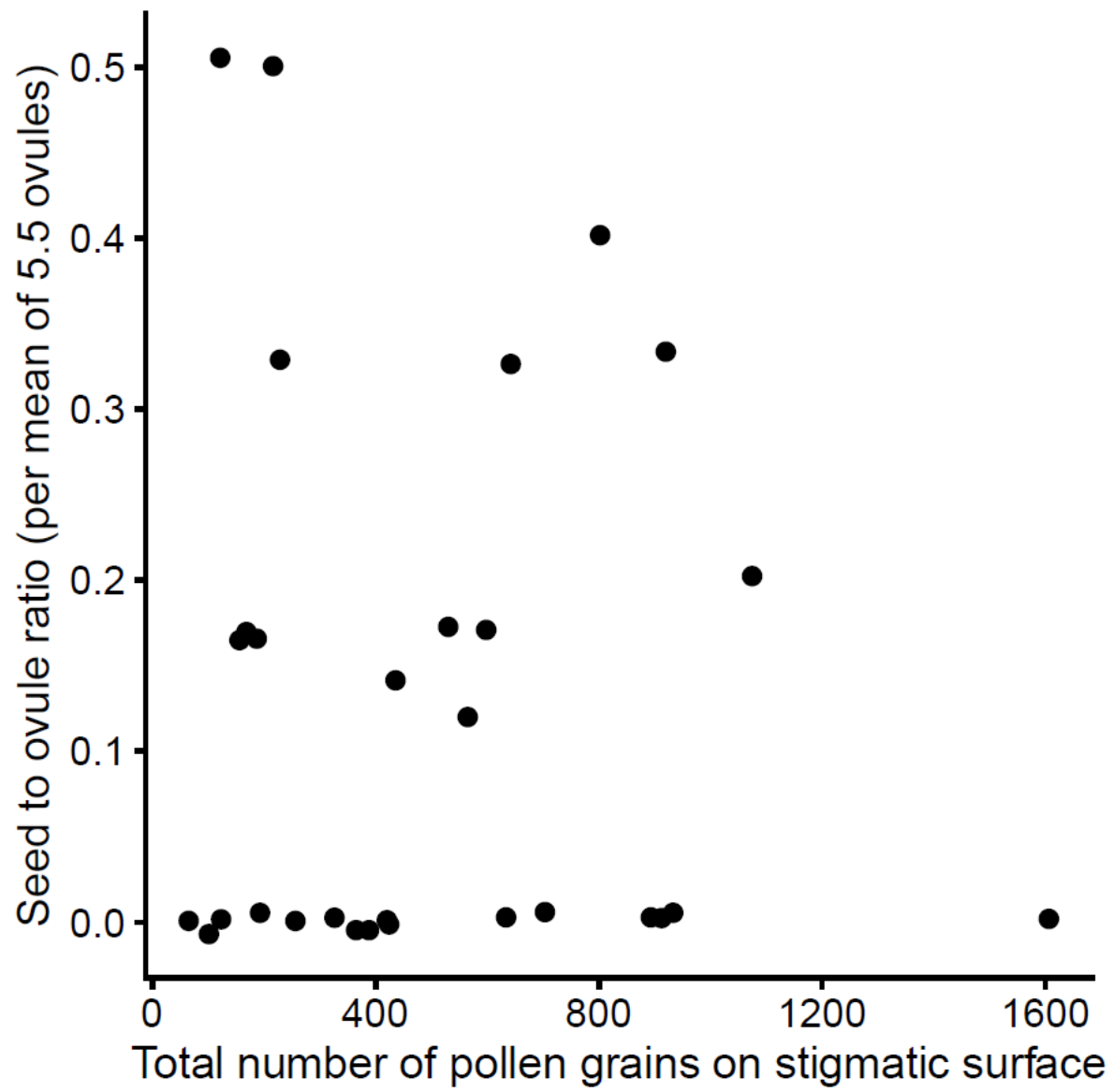


Figure 3

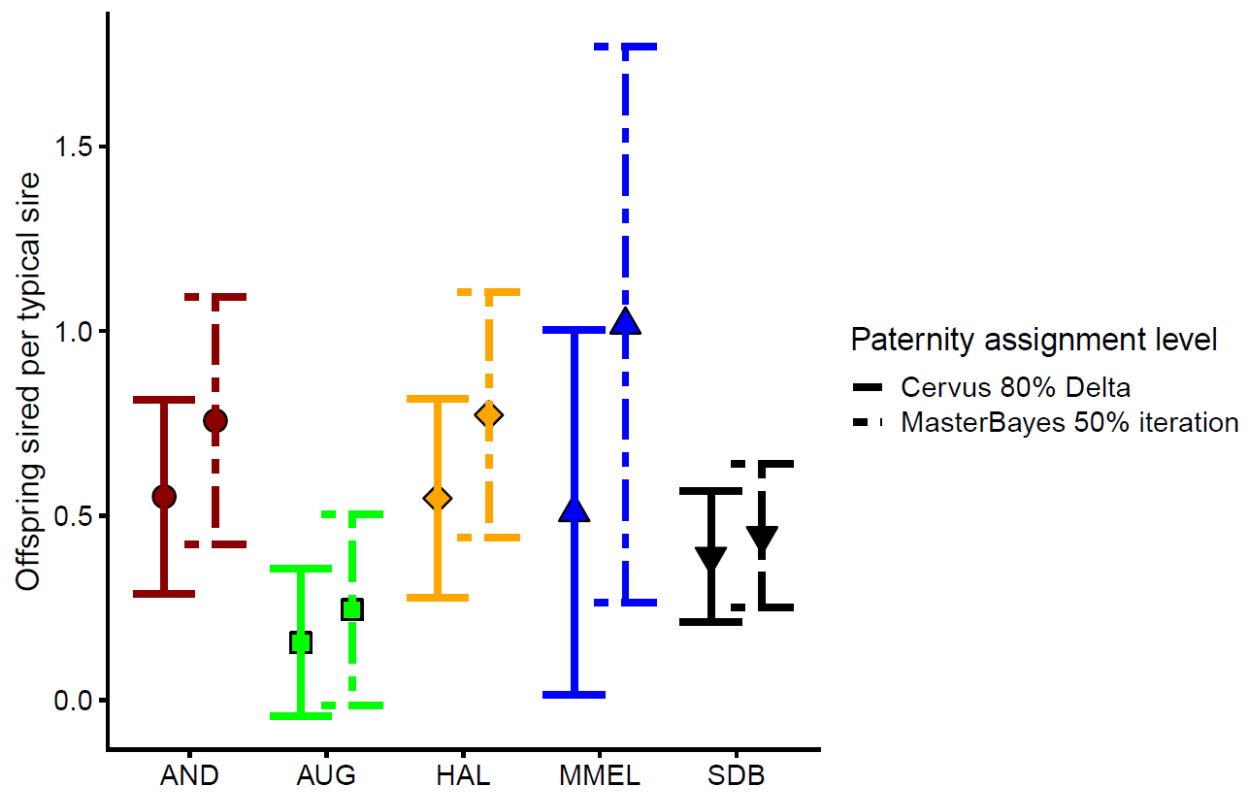
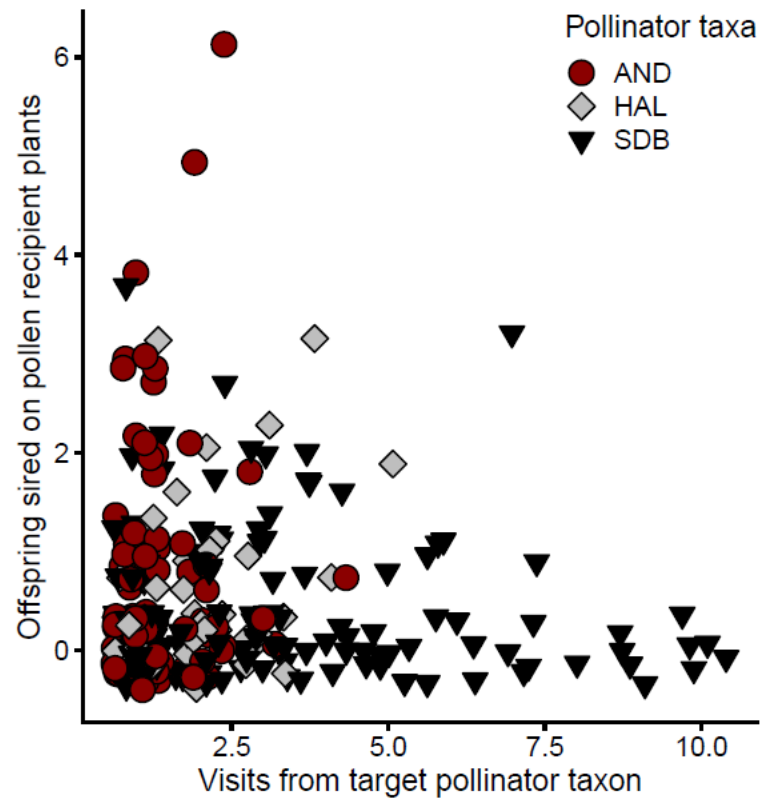


Figure 4



Appendix S1. Supplemental information about the pollinating bee taxa in the study.

We observed five pollinator taxa visiting *Echinacea angustifolia* plants during our study. In the manuscript, we refer to our first pollinator taxon as ‘*Andrena helianthiformis*’. The vast majority of *Andrena* observed visiting *E. angustifolia* in this study and a previous study were *A. helianthiformis* (Page et al., 2019). We refer to our second taxon as ‘Augochlorini’, a tribe in family Halictidae, were easy to distinguish in the field because of their characteristic metallic-green body color. *Augochlorella aurata* (= *Augochlorella striata*) has been the most common visitor of this tribe at our field sites (Wagenius and Lyon, 2010; Ison et al., 2018; Page et al., 2019; Richardson et al., 2021). Our third taxon is referred to as the ‘small-dark bee’ group and this taxon contained floral visitors that we could not identify further in the field because of their small bodies (5-8 mm) and quick movements. This likely included *Ceratina* spp. (Apidae), *Lasioglossum* spp., *Heterosarus* spp., and other Halictids. The fourth taxon in the manuscript is ‘*Halictus* spp.’ (genus *Halictus*). These are medium-sized sweat bees with dark body color and light stripes on their abdomens, and likely included at least three species including *Halictus ligatus*, *H. confusus*, and *H. parallelus* (Wagenius and Lyon, 2010; Richardson et al., 2021). Many *Halictus* spp. bees were in the siring success study but only four were in the pollen removal study, so the taxon was dropped from the pollen removal analysis. Our final taxon is ‘Male *Melissodes* spp.’, which were characterized by their long antennae. At least six species of *Melissodes* visit *E. angustifolia* in our study area (Wagenius and Lyon, 2010), but visits by female *Melissodes* were rare, so our final pollinator taxon includes only the males. Although other insects visit *E. angustifolia*, including female and male *Megachile* spp., *Coelioxys* spp.,

Agapostemon spp., honey bees, bumble bees, and Lepidopterans, their visitation was very low, so they were excluded.

The table includes a brief description, the body length, the number of visits in the pollen removal and siring success experiments. Pollen removal visits are recorded for *Halictus* spp., but this taxon was not included in the pollen removal experiment due to low visitation. Other pollinators visited during these studies but were also excluded due to low visitation rates.

Pollinator taxon	Size	Description	Pollen removal visits	Plants observed July 6	Plants observed July 10-11	Plants observed July 16	Plants observed July 17
Augochlorini	5-7 mm	Small green Augochlorini bees, often <i>Augochlorella aurata</i> .	9	6 (mean 1.2 visits per plant)	6 (mean 2.4 visits per plant)	4 (1 visit per plant)	0
Small-dark bee	5-8 mm	Small black or dark-metallic colored bees usually genus <i>Ceratina</i> , <i>Lasioglossum</i> , or <i>Heterosarus</i>	57	54 (mean 3.5 visits per plant)	54 (mean 3.8 visits per plant)	19 (mean 1.5 visits per plant)	20 (mean 1.5 visits per plant)
<i>Halictus</i> spp.	7-10 mm	Dark body and light striped abdomen bees in genus <i>Halictus</i>	4	8 (mean 2 visits per plant)	36 (mean 2.5 visits per plant)	0	4 (1 visit per plant)
Male <i>Melissodes</i> spp.	10-15 mm	Male bees in genus <i>Melissodes</i> . Long antennae and no pollen-collecting features.	49	1 (1 visit)	7 (mean 1.4 visits per plant)	0	4 (1 visit per plant)
<i>Andrena helianthiformis</i>	13-15 mm	A brown med-sized coneflower-specialist bee.	18	12 (mean 1.1 visits per plant)	20 (mean 2 visits per plant)	28 (mean 1.4 visits per plant)	25 (mean 1.1 visits per plant)

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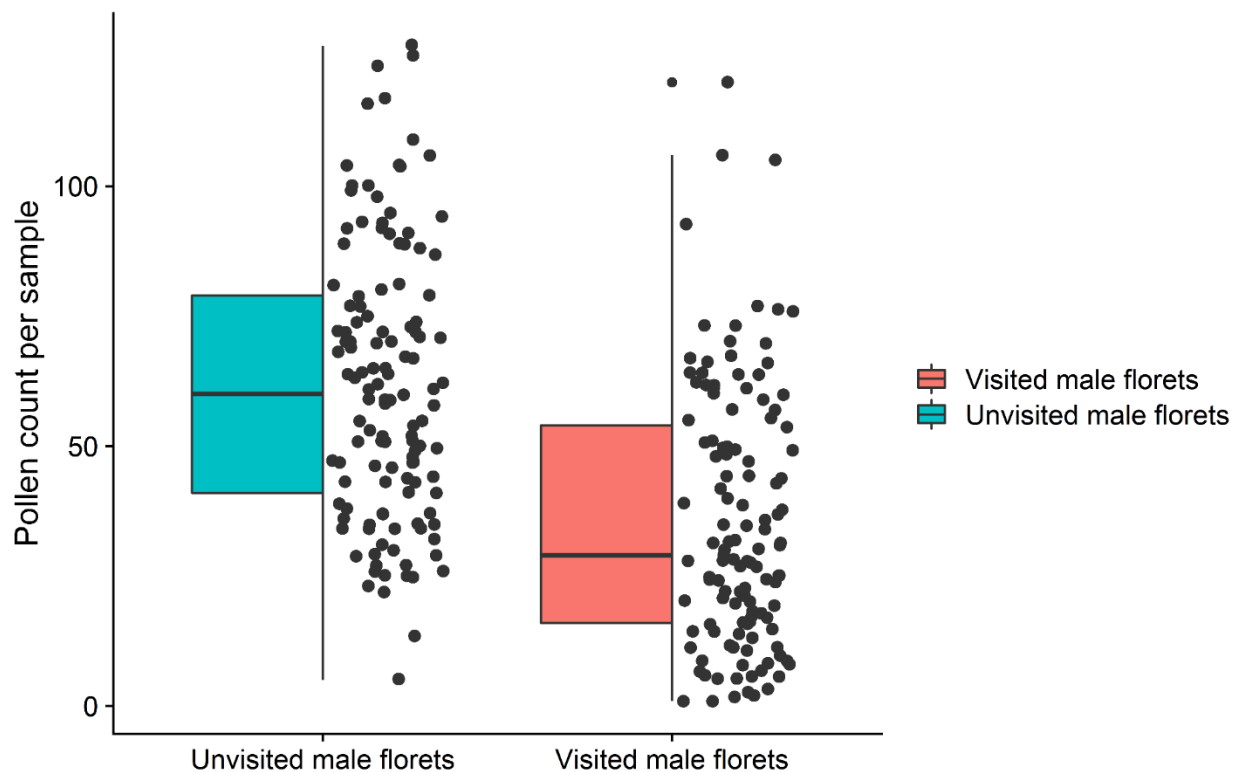
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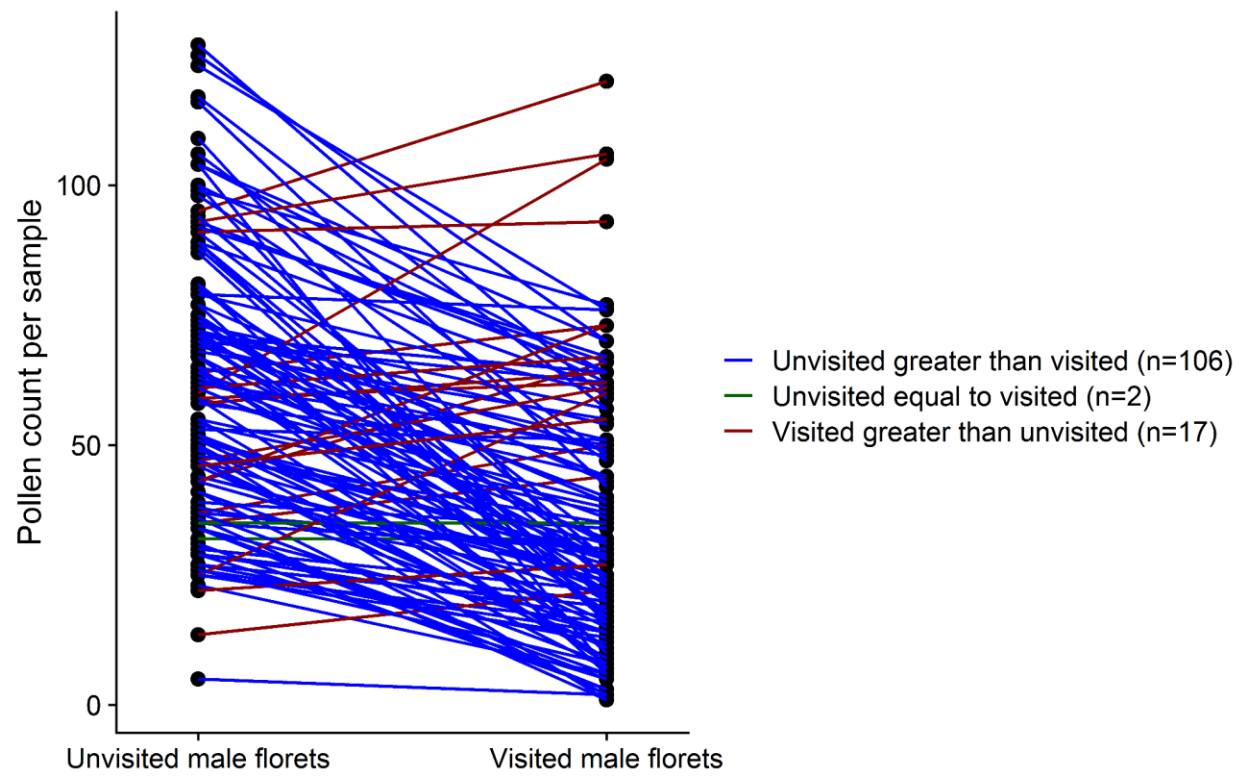
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Appendix S2. Variation in pollen count per sample between unvisited florets and florets after a pollinator visit.

We observed substantial variation in pollen counts per sample (see methods) in both unvisited florets and florets after a pollinator visit (top figure). However, nearly all (85%) of the visited florets had lower pollen count than unvisited florets collected on the same day from the same flowering head (bottom figure). This indicates that while there is variation in pollen production between florets, that there is less variation in pollen production between florets from the same flowering head at the same phenological stage compared to florets from different flowering heads, likely at different phenological stages.





Appendix S3. Likelihood ratio tests for stepwise model simplifications using backward elimination for the pollen deposition experiment. P-values are for 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 the results of model simplification. The full models included two main effects: p = pollen grain count (linear predictor), t = cross treatment (categorical predictor, with two levels crossed or not), and one interaction term. The results in line 5 compare that model to the models in lines 3 and 4, indicating no main effects were significant.

Model: Number of pollen grains deposited to set seed	Test term	Model df	Res. Deviance	Test df	Deviance	P
1. p + t + p*t		198	166.85	3	2.8839	0.4099
2. p + t	p*t	202	166.85	1	1.2423	0.265
3. p	t	202	166.85	2	2.8577	0.296
4. t	p	202	166.85	2	1.3448	0.5105
5. 1	t / p	202	168.19 / 169.70	1	1.5391 / 0.026162	0.2148 / 0.8715

Appendix S4. We examined if the number of pollinator visits predicted siring success for the three most common target pollinator taxa - *A. helianthiformis*, *Halictus* spp., and small-dark bees. The table shows the results from the likelihood ratio tests for stepwise model simplification using backward elimination for single-taxon models (*A. helianthiformis* -AND, *Halictus* spp -HAL, small-black bee-SDB). The response for these models, siring success, is quantified by the number of offspring sired using Cervus (80% confidence level) and MasterBayes (same sire assigned in at least 50% of the interactions). Siring success is modeled as a quasi-Poisson response in a generalized linear model. The maximal model included the log number of male florets shedding pollen (log male florets), the number of pollinator visits to the sire plant (total visits), and their interaction. P-values are for the test of the null hypothesis that a model simplified by excluding the focal term does not differ from the model on the above line that includes the test term. Models 2 and 3 were each compared to the null model 4 to test single predictors separately. After model simplification, the null model (no predictors) was the minimal adequate model for all AND models, HAL models using Cervus paternity assignments, and two of the SDB models). The number of pollinator visits significantly predicted siring success for plants visited by *Halictus* but only for models using the MasterBayes paternity assignments. The log number of male floret shedding pollen predicted siring success for plants visited by small-black bees in two models, but this effect was not significant with 3-SDB was tested to 4-SDB (results not shown).

Model	Model response	Residual df	Test term	Test df	Res. Deviance	P
1-AND	Cervus	76			127.69	
2-AND	Cervus	77	log male florets x total visits	1	132.21	0.137
3-AND	Cervus	78	log male florets	1	132.21	0.997
4-AND	Cervus	79	total visits	1	132.8	0.587
1-AND	MB	76			149.49	
2-AND	MB	77	log male florets x total visits	1	153.91	0.166
3-AND	MB	78	log male florets	1	154.29	0.684
4-AND	MB	79	total visits	1	154.51	0.753
1-HAL	Cervus	41			46.76	
2-HAL	Cervus	42	log male florets x total visits	1	49.21	0.139
3-HAL	Cervus	43	log male florets	1	49.43	0.666
4-HAL	Cervus	44	total visits	1	53.66	0.067
1-HAL	MB	41			51.81	
2-HAL	MB	42	log male florets x total visits	1	54.5	0.138
3-HAL	MB	43	log male florets	1	54.64	0.751
4-HAL	MB	44	total visits	1	63.37	0.011

1-SDB	Cervus	141			150.91	
2-SDB	Cervus	142	log male florets x total visits	1	151.33	0.574
3-SDB	Cervus	143	log male florets	1	157.25	0.036
4-SDB	Cervus	144	total visits	1	158.44	0.356
1-SDB	MB	141			175.97	
2-SDB	MB	142	log male florets x total visits	1	176.84	0.467
3-SDB	MB	143	log male florets	1	187.63	0.011
4-SDB	MB	144	total visits	1	187.64	0.952