Pollinator effectiveness in a composite: a specialist bee pollinates more florets but does not move pollen farther than other visitors

Maureen L. Page[1,2,*] (iD), Jennifer L. Ison[3,11,*] (iD), Alison L. Bewley[4], Keaton M. Holsinger[5], Andrew D. Kaul[6,10], Katie E. Koch[7], Kory M. Kolis[8], and Stuart Wagenius[9] (iD)

[1] Department of Entomology and Nematology, University of California–Davis, One Shields Avenue, Davis, California 95616, USA

[2] Biology Department, Scripps College, 1030 Columbia Avenue, Claremont, California 91711, USA

[3] Biology Department, College of Wooster, 1189 Beall Avenue, Wooster, Ohio 44691, USA

[4] Biology Department, Wittenberg University, 200 W. Ward Street, Springfield, Ohio 45504, USA

[5] Biology Department, Wabash College, 301 West Wabash Avenue, Crawfordsville, Indiana 47933, USA

[6] Biology Department, St. Olaf College, 1520 St. Olaf Avenue, Northfield, Minnesota 55057, USA

[7] Biology Department, Lakeland University, W3718 South Drive, Plymouth, Wisconsin 53073, USA

[8] Biology Department, Gustavus Adolphus College, 800 West College Avenue, Saint Peter, Minnesota 56082, USA

[9] Division of Plant Biology and Conservation, Chicago Botanic Garden, 1000 Lake Cook Road, Glencoe, Illinois 60022, USA

[10] Present address: Department of Ecology, Evolution, and Organismal Biology, Iowa State University, 2200 Osborn Drive, Ames, Iowa 50011, USA

[11] Author for correspondence (e-mail: jison@wooster.edu)

*These authors contributed equally.

PREMISE: Variation in pollinator effectiveness may contribute to pollen limitation in fragmented plant populations. In plants with multiovulate ovaries, the number of conspecific pollen grains per stigma often predicts seed set and is used to quantify pollinator effectiveness. In the Asteraceae, however, florets are uniovulate, which suggests that the total amount of pollen deposited per floret may not measure pollinator effectiveness. We examined two aspects of pollinator effectiveness—effective pollen deposition and effective pollen movement—for insects visiting *Echinacea angustifolia*, a composite that is pollen limited in small, isolated populations.

METHODS: We filmed insect visits to *Echinacea* in two prairie restorations and used these videos to quantify behavior that might predict effectiveness. To quantify effective pollen deposition, we used the number of styles shriveled per visit. To quantify effective pollen movement, we conducted paternity analysis on a subset of offspring and measured the pollen movement distance between mates.

RESULTS: Effective pollen deposition varied among taxa. *Andrena helianthiformis*, a Heliantheae oligolege, was the most effective taxon, shriveling more than twice the proportion of styles as all other visitors. Differences in visitor behavior on a flowering head did not explain variation in effective pollen deposition, nor did flowering phenology. On average, visitors moved pollen 16 m between plants, and this distance did not vary among taxa.

CONCLUSIONS: *Andrena helianthiformis* is an important pollinator of *Echinacea*. Variation in reproductive fitness of *Echinacea* in fragmented habitat may result, in part, from the abundance of this species.

KEY WORDS   *Andrena helianthiformis*; Asteraceae; *Echinacea angustifolia*; plant–pollinator interactions; pollen limitation; pollen movement; pollinator efficiency; tallgrass prairie.

Understanding variation in pollinator effectiveness—a visitor's per-visit contribution to plant reproductive fitness—provides valuable insights into the ecology and evolution of plant–pollinator interactions. Pollinator effectiveness estimates have been used to determine the contributions of different wild pollinator species to the pollination of agricultural crops (e.g., Rader et al., 2013; Park et al., 2016) and have provided quantitative evidence for the preeminence of bees as effective pollinators of native plants in natural areas (Ballantyne et al., 2017). Furthermore, given growing concerns about impacts of pollinator extinctions on the reproduction of rare and endangered plants (Biesmeijer et al., 2006), pollinator effectiveness estimates are increasingly used to make conservation recommendations (Gibson et al., 2006; Ne'eman et al., 2010).

Understanding variation in pollinator effectiveness may also shed light on the causes and consequences of pollen limitation, which is commonly observed in small, fragmented plant populations (Aizen

et al., 2002). Pollen limitation may provide evidence of reduced pollinator visitation because fragmentation can decrease nesting sites and floral resources, reducing the abundance of pollinators in small habitat fragments (Calvillo et al., 2010). However, different species respond differently to fragmentation, which may change the composition of floral visitor communities, but not necessarily the total abundance of visitors (Aizen and Feinsinger, 1994; Brosi et al., 2007, 2008). If insect species vary in their effectiveness as pollinators, changes in insect community composition may affect rates of pollen limitation. Indeed, reductions in the quality of visits are thought to contribute strongly to pollen limitation. For example, pollinator community shifts that lead to an overrepresentation of ineffective pollinators can increase pollen limitation by reducing compatible pollen transfer (Harder and Aizen, 2010). As such, spatial variation in pollinator community composition can contribute to spatial variation in pollen limitation (Gómez et al., 2010).

While we have broadly defined "pollinator effectiveness" as a visitor's per-visit contribution to plant reproductive success, many studies have investigated variability in pollinator performance using various definitions of pollinator "efficiency," "effectiveness," "efficacy," and "importance" (reviewed in Ne'eman et al., 2010). Despite notable variation in terminology and technique, most of these studies aimed to compare visitors' contributions to plant reproductive success. Previous studies have shown that pollinator effectiveness varies greatly both within and among taxa (Herrera, 1987; Rader et al., 2011; Benjamin et al., 2014), and effectiveness can vary for a number of reasons that are not mutually exclusive.

First, visitors vary in morphology, including size, hairiness, and location of specialized pollen-storage hairs. Large corbiculate apids (e.g., *Apis* and *Bombus*), as well as some panurgine bees, mix pollen with nectar and transport it as a dense, moist clump, making it unviable (Parker et al., 2015) and unavailable for pollination (Westercamp, 1991). By contrast, most other bees transport dry pollen in less dense scopae (Michener, 1999), and these pollen grains remain viable (Parker et al., 2015). Other taxa, including various eucerines, exomalopsines, melittids, and panurgines, pack pollen dry and then "glaze" it with nectar before returning to their nest (Portman and Tepedino, 2017). It is important to note, however, that dry scopal pollen may adhere so strongly to pollen storage hairs that it does not come off during a visit. Thus, the pollen deposited on stigmas is more likely to be from parts of a bee's body that are difficult to groom (Koch et al., 2017).

Second, visitors differ in their level of specialization. Specialists inherently display high floral constancy (Müller, 1996a, b) and may have morphological adaptations to efficiently collect and transport large quantities of pollen from their host plants. High floral constancy can increase plant fitness by decreasing stigma clogging due to heterospecific pollen deposition (Goulson, 1999) and increasing conspecific pollen deposition (Brosi and Briggs, 2013). Although generalist visitors may temporarily specialize on a particular plant species over the course of a foraging bout or day, the consistently high constancy of specialist visitors may increase their effectiveness at depositing conspecific pollen grains onto stigmatic surfaces. However, specialists may also be more effective at removing pollen, which can reduce the proportion of collected pollen that is ultimately deposited onto stigmatic surfaces (Parker et al., 2016). Indeed, some insects can be so ineffective at depositing the pollen they remove that they act as pollen thieves (Koski et al., 2018). Third, individuals vary in their foraging behavior, resulting in within-taxon variation in pollinator effectiveness (Ivey et al.,

2003; Young et al., 2007). For instance, in *Asclepias incarnata*, pollen removal, pollen deposition, and fractional pollen deposition all increased with mean flower-handling time (Ivey et al., 2003).

Pollen deposition is certainly a major component of pollinator effectiveness. However, the distance that visitors move pollen among mates may also be an important aspect of pollinator effectiveness. Indeed, the distance that pollen is transported between donors and recipients plays a key role in plant fitness and population dynamics, especially in plants with self-incompatibility systems, where near neighbors may be incompatible (DeMauro, 1996; Wagenius et al., 2007). In general, very few pollen grains are successfully delivered to plant stigmas, making pollen transport especially important for male fitness. For instance, in a community of 26 flowering plant species, only 5% of removed pollen grains successfully reached conspecific stigmas (Gong and Huang, 2014). Pre-pollination processes—such as how and where pollen is placed on visitor bodies and the ease with which visitors groom and consume pollen grains—likely play important roles in determining which pollen grains successfully reach conspecific stigmas (Minnaar et al., 2019). If pollen is layered on visitor bodies during each visit, grains in the topmost layer likely sire more seeds in subsequent visits (Harder and Wilson, 1998). If this were the case, the distances between visits may strongly influence total pollen movement distances. Examining the extent to which taxa move pollen different distances could shed light on a process that affects male fitness and the genetic structure of plant populations.

Because variation in effective transport and deposition of pollen among insect taxa may influence our understanding of plant–pollinator interactions, it is important to measure these components of pollinator effectiveness in a way that accurately quantifies per-visit contributions to plant fitness. The majority of pollinator effectiveness studies focus on species with multiovulate ovaries. However, we expect pollinator effectiveness to operate differently in plants with uniovulate ovaries. In plants with multiovulate ovaries, the number of conspecific pollen grains on a single stigmatic surface predicts the number of seeds per fruit and is generally a good indicator of seed set and a good measure of pollinator effectiveness (Ne'eman et al., 2010; King et al., 2013; Ballantyne et al., 2015). However, in the Asteraceae, one of the largest plant families, florets in the composite head are uniovulate (Anderberg et al., 2007), which suggests that the total amount of pollen deposited per style may not relate to plant fitness as well as the total number of styles with at least some pollen deposition. In *Echinacea angustifolia* (hereafter *Echinacea*), style shriveling is a visual indication of compatible pollen receipt and a strong predictor of seed set (Wagenius, 2004), and also better approximates ovule fertilization than the number of germinated pollen grains (Wist and Davis, 2013). Style shriveling is a useful and time-efficient method for measuring pollination success in *Echinacea* (and likely in other plant taxa). This method and others that quantify the number of styles pollinated per visit have great potential to measure pollinator effectiveness in uniovulate species—especially compared to measuring the amount of pollen deposited per stigma.

Here, we focus on *Echinacea*, a perennial threatened by fragmentation of its grassland habitat. As with most plant species, many biotic and abiotic factors affect reproductive success, including processes independent of pollination, such as resource availability and herbivory. However, in this system, pollination processes are particularly important. In small, isolated populations, reproduction of *Echinacea* is pollen limited (Wagenius, 2006; Wagenius and Lyon,

2010) and individual plants may be surrounded by incompatible conspecifics (Wagenius et al., 2007). Although some reproductive failure in remnant *Echinacea* populations results from isolation from potential mates, incompatible pollen of those mates, and isolation due to asynchronous flowering (Wagenius et al., 2007; Ison et al., 2014), temporal isolation and receipt of insufficient and incompatible pollen do not fully explain observed patterns of seed set and reproductive failure (Ison and Wagenius, 2014).

Additionally, while reproduction in small *Echinacea* populations is pollen limited, it is not limited by pollinator visitation. Observations during 2004, 2005, and 2016 in remnant populations revealed that population size was not closely related to insect visitation. In fact, visitation rates increased with isolation of individual plants and decreased with population size (Wagenius and Lyon, 2010; Ison et al., 2018). Research into *Echinacea* pollination and population dynamics suggests that insect visitors may differ in their effectiveness as pollinators and that variance in visit quality, rather than visit quantity, could contribute to observed patterns of reproductive failure in small populations and among isolated plants. Recent work in this system also suggests that the visitor community changes significantly over the course of a flowering season (Ison et al., 2018), and thus variation in the effectiveness of different pollinator taxa may also contribute to temporal variation in pollination success (Ison and Wagenius, 2014).

Objectives

In this four-year study, we quantified differences in single-visit effective pollen deposition, as measured by style shriveling, and in effective pollen movement, as measured by the distance pollen moved between mates, for the major insect taxa visiting *Echinacea*. We also examined the extent to which pollinator taxon, individual behavior on a flowering head, flowering phenology, and the number of available receptive stigmas at the time of the visit predicted effective pollen deposition.

MATERIALS AND METHODS

Study system

Echinacea angustifolia DC, the narrow-leaved purple coneflower (Asteraceae), is a long-lived forb widely distributed across grasslands west of the Mississippi River. In western Minnesota, most adult plants do not flower every year; in the years they do flower, they usually produce one flowering head (capitulum), although some will produce two or more in a year. Each head comprises 100–250 disk florets, each with a single ovule. *Echinacea* florets develop in circular rows sequentially from the bottom to the top-middle of the head (Fig. 1). Florets are protandrous. On day 1, anthers emerge from a single row of florets. That same day, pollen is gradually presented by the upward movement of styles. The following day, the stylar branches separate and the stigmatic surface become receptive. Nectar volume peaks when stigmas become receptive and nectar production continues for 3–5 d following anthesis (Wist and Davis, 2008). Only about half of florets contain nectar by the third day of flowering, and this proportion continues to drop through day 5. *Echinacea* exhibits a sporophytic self-incompatibility system and therefore relies on pollinators for successful reproduction (Wagenius et al., 2007). In our
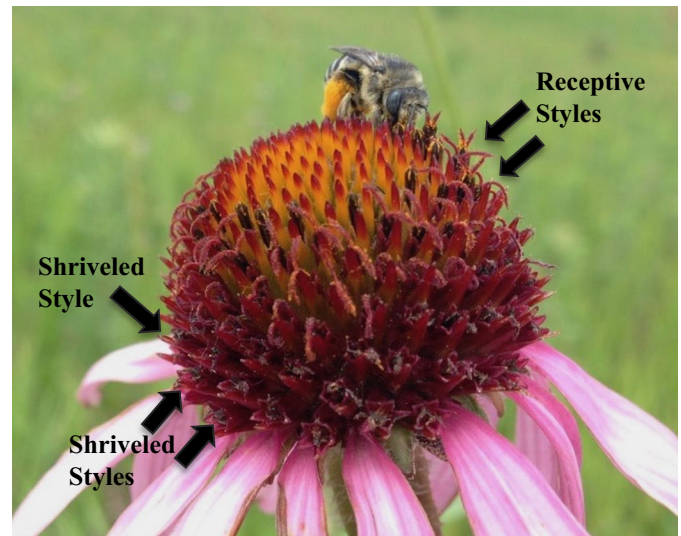

FIGURE 1. Flowering head of *Echinacea angustifolia*. Florets mature from the bottom of the head to the top in concentric rings daily. A floret that produces an anther one day produces a receptive style the following day that may persist for ≤10 d until pollinated. Once pollinated, the style will shrivel. Photo credit: J. L. Ison.

study area, *Echinacea* is visited by ≥26 species of native bees, as well as several dipterans and lepidopterans (Wagenius and Lyon, 2010; Ison et al., 2018). When compatible pollen is deposited onto the stigmatic surface, that style will shrivel into the corolla within 24–48 h (Wagenius, 2004; Wist and Davis, 2013). If no compatible pollen is received, styles will often persist unshriveled for ≤10 d (J. L. Ison and S. Wagenius, unpublished data) but may also be eaten by insects or damaged (S. Wagenius, personal observation). Although pollen is deposited on stigmatic surfaces, we use the term *style* to refer to the whole structure, such that each floret can have a "receptive style" (unshriveled) or a "shriveled style" that it is not receptive because compatible pollen was deposited or the style was damaged.

Study site

We conducted this study in western Minnesota in two experimental plots of *Echinacea* growing in a prairie restoration context. All plants were located in a 6400 ha study area that was predominantly soybean and corn fields, centered near 45°49′N, 95°43′W. We refer to the experimental plots as P1 and P2. P1 comprises many *Echinacea* plants, most of which were planted as plugs during 1996–2003. P2 was established in 2006 with 4000 plants as a 50 × 80 m plot in an old field. Within each plot, the locations of plants were randomized, thereby removing the spatial genetic structure that is common in nearby remnants populations (Wagenius et al., 2007). This allows us to examine how effective each pollinator taxon is at transferring pollen to receptive styles without the confounding effect of spatial genetic structure. Details about the other vegetation and management practices of these plots are described in Muller and Wagenius (2016). During the four years of this study (2010, 2012, 2013, 2014), within 20 m of focal plants, some 50–200 *Echinacea* plants flowered each year. Within 500 m of each experimental plot, *Echinacea* and many other species flowered abundantly in old fields, roadsides, and small prairie remnants.

Visitor observations

To ensure that each focal head received only one visit, we placed an organza-fabric pollinator exclusion bag on the head ≥24 h before the observation. All receptive styles on flowering heads were <1 wk old. We also placed a small wire cage atop each flowering head to prevent the fabric from contacting anthers and styles. While bagging plants is a standard technique in effectiveness studies, bagging plants can artificially increase nectar and pollen availability and alter nectar sugar concentration (Neff and Simpson, 1990). However, according to Wyatt et al. (1992), the use of organza fabric minimizes this concern.

We conducted observations when it was not raining and when temperatures were above 15°C. We typically observed visits between 0800 and 1200 hours. However, we occasionally conducted observations after noon if it rained or was cool in the morning but not in the afternoon. During the observation period, we removed the pollinator exclusion bag and waited for a single insect to visit the flowering head. We recorded the visit using a handheld video camera, noting the time and duration of each visit. Once the visitor flew away, we counted the number of receptive, unshriveled styles and placed the bag back over the head. We did not count the exact number of anthers available at the time of the visit, however; only one row of anthers was available for each visit. Between 24 and 48 h after the visit, we counted the number of styles that had shriveled and painted the bracts subtending shriveled styles to keep track of the visit. In 2013 and 2014, at the end of the field seasons, we harvested seed heads and removed fertilized achenes with forceps for later genetic analyses of offspring. In 2013 and 2014, the majority of our observations were conducted in P2. While we used data from all years and both plots to quantify effective pollen deposition, we used data only from P2 in 2013 and 2014 to assess effective pollen movement.

Visitor identification

Because we did not want to affect a bee's visit by capturing it, most identifications were done in the field and using the videos. Field identifications were based on a field guide for our study site. We confirmed field identifications by watching recorded videos and comparing visitors to a reference collection created for Wagenius and Lyon's (2010) study. To ensure that identifications were consistent, two researchers, M.L.P. and K.M.H., independently identified taxa in ~50% of the videos. In all but one case, the two identifications matched. Therefore, only M.L.P. watched the remaining videos. For additional confirmation, at least one insect from each observed pollinator taxon was captured, anesthetized, and brought back to our field station for identification using Discover Life (Ascher and Pickering, 2018). We also had 15 female *Andrena*, one male *Andrena*, and one female *Melissodes* professionally identified. These

bees were captured either in 2014 (during this study, at both sites) or in July 2019 (at P2 only). All *Andrena* specimens were identified as *A. helianthiformis*, and the *Melissodes* was identified as *M. druriella*. We note that a previous publication misidentified the *Andrena* as *A. rudbeckiae* (Ison et al., 2018).

To investigate differences among taxa in effective pollen deposition, we focus on six dominant taxonomic groups: Augochlorini, "small dark bees," *Agapostemon virescens*, *Andrena helianthiformis*, male *Melissodes* spp., and "medium gray bees" (Table 1). The category "small dark bees" comprised *Ceratina* sp., *Heterosarus* sp., *Lasioglossum* spp., and potentially a few other taxa. We suspect that the majority of the "medium gray bees" were female *Melissodes* sp., based on reference specimens. The "medium gray bee" that we sent to a taxonomist was identified as *M. druriella*, but several other *Melissodes* species also occur at our study site. The vast majority of the Augochlorini bees were likely *Augochlorella aurata*, but a few visits may have been from other species in the same tribe. Species-level identifcations can be difficult using video recordings, especially for smaller-bodied bees. However, based on reference specimens, we suspect that our identifications of *A. virescens* are correct nearly every time. We have occasionally seen *Agapostemon texanus* visit *Echinacea*, but they are rare compared to *A. virescens* and easy to distinguish. Several *Andrena* species are known to occur in our study region, and it is possible that one or two visits we identified as *A. helianthiformis* were actually *A. rudbeckiae*, which is also a composite specialist (Neff and Simpson, 1997). However, since every specimen we sent to the taxonomist was identified as *A. helianthiformis*, we refer to it as such. Among the taxa we observed, we were only able to confidently distinguish males of *Melissodes* spp. because of their distinct antennae length. For all other taxa, visitors had visible pollen carrying structures, suggesting that they were female.

To investigate effective pollen movement, we focused on only three dominant taxonomic groups: *A. helianthiformis*, "medium gray bees" (which includes female and male *Melissodes* spp.), and "small bees" (which includes Augochlorini, *A. virescens*, and visits from "small dark bees"; Table 1). We combined male *Melissodes* spp. and "medium gray bees" together and all of the small bees together because we lacked sufficient samples to consider these groups separately. We have 208 videos from this study, which can be viewed online at https://www.youtube.com/user/TheEchinaceaProject.

Visitor behavior on a flowering head

We watched videos of visits to quantify behavior that might predict effectiveness. M.L.P. watched all videos to minimize viewer bias. M.L.P. counted all visible anthers and noted the flowering stage before watching the entire length of the video. She estimated visit

TABLE 1. Summary table showing pollinator taxon, corresponding abbreviation (used in Fig. 2), number of observations used in the analysis, and body length for all taxa included in our analysis of effective pollen movement (2013–2014) and our analysis of effective pollen deposition (2010–2014). Body length is the range reported on Discover Life.

|	Effective pollen deposition			Effective pollen movement	
Pollinator taxon	**Abbrev.**	**No. of observations**	**Length (mm)**	**Pollinator taxon**	**No. of observations**
Andrena helianthiformis	and	92	12–16	*Andrena helianthiformis*	12
Medium gray bee	mgb	12	10–15	Medium gray bee	14
Male *Melissodes* spp.	mml	22	8–12		
Agapostemon virescens	agp	35	11	Small bee	16
Small dark bee	sdb	20	5–8		
Augochlorini	aug	18	5–8		
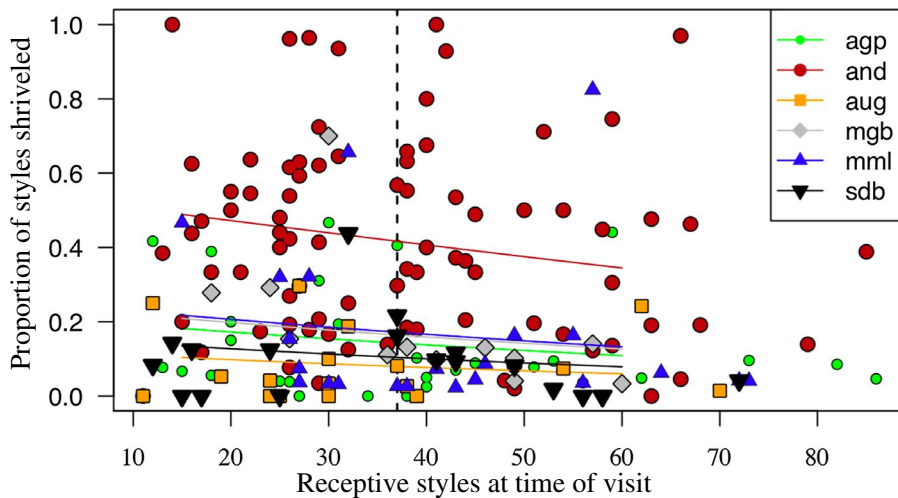
FIGURE 2. The proportion of styles shriveled per visit per taxon for all ranges of receptive styles available to the visitor. Taxon abbreviations: agp = *Agapostemon virescens*; and = *Andrena helianthiformis*; aug = Augochlorini (likely *Augochlorella aurata*); mgb = "medium gray bee" (likely female *Melissodes* spp.); mml = male *Melissodes* spp.; sdb = "small dark bee." Actual data points are shown with best-fit lines for each taxon. Estimates are based on the minimal adequate generalized linear model with a binomial response that included taxon ($F_{5,187} = 16.355$, $P < 0.001$) and the number of receptive styles available ($F_{1,183} = 6.622$, $P = 0.011$; $N = 189$ visits). The best model was selected via backward elimination of a maximal model that included five main effects and all two-way interactions. Insufficient evidence existed to keep any interactions. The other main effects tested included the number of times the visitor circumnavigated the head ($F_{1,182} = 1.007$, $P = 0.317$), the day of the visit compared to the peak day of flowering ($F_{1,176} = 2.380$, $P = 0.125$), and a two-level factor indicating the plot in which the observation occurred ($F_{1,174} = 1.835$, $P = 0.178$). The dashed vertical line at 37 styles shows the median number of receptive styles available to a visitor across the experiment.

duration, the total proportion of the head that was circumnavigated, and the number of times the individual circumnavigated the head.

Effective pollen movement field and lab methods

To determine how far pollen moved between mates and measure how far visitor taxa transported pollen, we conducted a maximum likelihood paternity analysis. We genotyped 116 offspring from 42 plants that each received single pollinator visits in 2013 or 2014 (Table 1). We germinated fertilized achenes using a protocol established by Feghahati and Reese (1994) and modified by Wagenius (2004). We grew the achenes until the first true leaf could be sampled, or a root sample was taken (~2 wk). However, not all achenes that contained an embryo germinated, and a number of seedlings died before a tissue sample could be taken. We also obtained genotypes for all flowering plants in 2013 (68) and 2014 (209) in P2, which are maternal plants and potential sires of the offspring. Some of the flowering plants had previously been genotyped by Ison et al. (2014). For this study we genotyped a total of 220 flowering plants. To extract DNA from leaf tissue samples, we used a Qiagen DNeasy Plant Kit (Qiagen, Hilden, North Rhine-Westphalia, Germany). We genotyped the offspring and flowering plants at 11 polymorphic microsatellite markers developed for *Echinacea* (Ison et al., 2013). We conducted PCR following Ison et al. (2013). We took the amplified PCR products and determined the fragment sizes using a Beckman Coulter CEQ 8000 Genetic Analysis System (Beckman Coulter, Brea, California, USA) with scoring protocols established by Ison et al. (2013, 2014). The different fragment sizes are the different alleles

for a given locus; therefore, we had a genotype for each individual at 11 loci.

Effective pollen deposition analysis

Over 4 yr, we observed and filmed 208 visits from 11 taxa, comprising three families within the order Hymenoptera (Andrenidae, Apidae, and Halictidae) and one family in the order Diptera (family Syrphidae; Table 1; Appendix S1). We excluded insect taxa with <10 visits from our dataset, leaving ≤199 visits from six bee taxa (Table 1). To assess effective pollen deposition, we counted florets with styles that had shriveled 24–48 h after the visit. We also counted the total number of florets with receptive styles at the time of the visit. Having these two counts enabled us to quantify effective pollen deposition as either a proportion of styles shriveled or just the count. Both approaches yielded practically the same results, so we elected to present proportions, which are easier to compare with other studies.

We modeled styles shriveled as a binomial response using generalized linear models (GLMs) in R (R Core Team, 2018). We tested five predictors and all possible two-way interactions using stepwise model simplification using backward elimination with a $P = 0.05$ threshold (Crawley, 2013). The residual deviance of initial models far exceeded the residual degrees of freedom, indicating overdispersion. To address this issue, we used a quasibinomial error structure (Crawley, 2013). The five predictors in our maximal model included (1) the bee taxon observed, which has six levels; (2) the number of receptive styles available; (3) the number of times the visitor circumnavigated the flowering head, which we abbreviate "circle" and which accounts for the spatial movement of the visitor and also strongly correlates with visit duration; (4) the day of the visit compared to the local median day of first flowering; and (5) the plot in which observations occurred (P1 or P2).

It is important to explain several details about four of these predictors. First, the number of receptive styles per visit ranged from 6 to 133. Only *A. helianthiformis* visitors had access to >86 receptive styles, so we removed seven visits with 89–133 receptive styles from the analysis. Removing these records did not appreciably change estimated rates of shriveling for *A. helianthiformis*. We think that *A. helianthiformis* happened to visit the heads with the most styles because it was the most frequently observed visitor. *Andrena helianthiformis* accounted for 44% of observed visits across all four years, and 53% of observed visits in 2014, the year with all of the heads presenting >86 styles. We also removed three records with six or seven receptive styles to maintain a balanced realized experimental design among visits to heads with very few receptive styles. For the main analysis, we had 189 visits with total receptive style counts 11–86.

Second, we found evidence that taxa differ in the mean number of times they circle the flowering head, according to a one-way analysis of variance (Appendices S2 and S3; $F_{5,195} = 15.590$, $P < 0.001$). This result suggests that taxon and head circumnavigations may explain

some of the same variability. Despite potential issues of collinearity, we included both taxon and head circumnavigations in models. Furthermore, variable numbers of head circumnavigations may indicate variable pollen and nectar availability. However, we partially account for variable nectar and pollen availability by including the number of available receptive styles in all models, which indicates the number of unvisited florets containing nectar and pollen rewards.

Third, we wanted to investigate the role of seasonal timing in pollinator effectiveness. We explored several options, including day of year as a continuous predictor, flowering stage as a three-level categorical variable for each flowering head (early, median, or late), week of observation, and days after median start day of flowering for each plot and year. We elected to use the last option because it captures the aspects of timing that are likely biologically relevant to plant reproductive fitness and also has the most balanced distribution across all taxa. We calculated the median start date among all plants in each plot in each year and then took the difference in days from the observed visitor.

Fourth, we made observations in two plots over 4 yr, but not in both plots in all years (Appendix S4). Our realized experimental design was not sufficiently balanced across taxa to include both plot and year as a predictor or even to include a five-level categorical plot–year predictor. We tested for effects only of plot (two levels) but note that our "day" predictor accounts for the substantial variation of peak flowering date among years and between plots.

To assess the relationship between the number of times an individual circumnavigated the flowering head and effective pollen deposition, we performed separate single-taxon GLM analyses of the relationship between style shriveling and the number of head circumnavigations for the three most frequently observed visitor taxa (*A. helianthiformis*, *A. virescens*, and male *Melissodes* spp.). As in the main model selection, maximal models included head circumnavigations, available styles, day, and plot as main effects. Similarly, we selected the minimal adequate model by stepwise backward elimination using likelihood ratio tests and a quasibinomial error structure.

Effective pollen movement analysis

We estimated the distance pollen moved between mates by conducting full probability paternity analysis on 116 offspring resulting from 42 single visits (Table 1) using the R package MasterBayes (Hadfield et al., 2006). Through a Bayesian framework, MasterBayes jointly estimates β, the peak posterior distribution for nongenetic data, and the pedigree, P. The probability of siring an offspring is modeled as an exponential decay function $e^{\beta x}$, where x is the pairwise distance between mates. We conducted a separate analysis of offspring for plants visited by each taxonomic group (*A. helianthiformis*, "medium gray bees," and "small bees") and one analysis with all offspring. For each analysis, we ran three separate chains and tested for chain convergence (for methods, see Austen and Weis, 2016). We extracted the most likely pedigree from the analysis with all offspring and estimated the total number of sires successfully transported during a visit and the mean distance between maternal and paternal plants.

RESULTS

Effective pollen deposition

Andrena helianthiformis was the most effective pollinator taxon. On average, these bees induced shriveling in more than double

the proportion of styles per visit compared to any other taxon and about five times as many as the least effective taxon (Fig. 2). The proportional differences in shriveling were slightly less pronounced when few styles were available. When 37 styles were receptive (the median number available across our experiment), *A. helianthiformis* shriveled 42% (39–45% ± 1 SE) while male *Melissodes* spp. and "medium gray bees," the next most effective taxa, shriveled 17% and 16% (14–22% and 12–23% ± 1 SE), respectively. The least effective taxon, Augochlorini, shriveled 8% (5–12% ± 1 SE) when 37 styles were available. When 60 styles were receptive (the maximum available to all taxa in our experiment), proportional differences among taxa were greater: *A. helianthiformis* shriveled 34% (31–38% ± 1 SE) while male *Melissodes* spp. and "medium gray bees" both shriveled 13% (10–17% and 9–18% ± 1 SE), respectively, and Augochlorini shriveled only 6% (4–9% ± 1 SE). When 18 styles were receptive (the minimum available to all taxa in our experiment), *A. helianthiformis* shriveled 48% (44–52% ± 1 SE) while male *Melissodes* spp. and "medium gray bees" shriveled 21% and 20% (16–27% and 14–28% ± 1 SE), respectively. With 18 styles receptive, Augochlorini shriveled 10% (6–15% ± 1 SE), which was about a fifth the rate of *A. helianthiformis*. All estimates of mean shriveling rates per visit are based on the minimal adequate generalized linear model with a binomial response that included taxon (Table 2; $F_{5, 187} = 16.355$, $P < 0.001$) and the number of receptive styles available ($F_{1, 183} = 6.622$, $P = 0.011$), for 189 visits. All model comparisons are reported in Table 2. We found no evidence that the effectiveness of taxa depends on the number of receptive styles available (taxon × styles; $F_{5, 164} = 0.619$, $P = 0.686$).

TABLE 2. Likelihood ratio tests for stepwise model simplification using backward elimination. Pollinator effectiveness, as quantified by the rate of style shriveling, is modeled as a quasi-binomial response in a generalized linear model. Deviance is the likelihood ratio test statistic. *P*-values are for the *F*-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. The maximal model included five main effect terms: bee taxon observed ("taxon" with six levels), number of receptive styles available ("styles"), number of times the visitor circumnavigated the head ("circle"), day of the visit compared to peak day of flowering ("day"), plot in which observations occurred ("plot" with two levels), and all two-way interaction terms. Models 15 and 16 were each compared to model 14 to test focal terms *styles* and *taxon*, respectively. After model simplification, the minimal adequate model (model 14) included taxon and styles. Parameter estimates are shown in Fig. 2.

Model	Residual df	Test term	Test df	Deviance	P
1	153				
2	158	taxon × plot	5	20.128	0.814
3	159	circle × plot	1	0.101	0.916
4	164	taxon × styles	5	27.756	0.686
5	165	day × plot	1	3.631	0.526
6	166	styles × day	1	2.716	0.583
7	167	circle × styles	1	2.121	0.627
8	168	styles × plot	1	6.182	0.408
9	173	taxon × day	5	48.180	0.377
10	174	plot	1	16.452	0.178
11	175	circle × day	1	27.345	0.083
12	176	day	1	6.284	0.125
13	181	taxon × circle	5	79.826	0.120
14	182	circle	1	9.026	0.317
15	183	styles	1	59.386	0.011
16	187	taxon	5	733.330	<0.001

Scant evidence exists for any of the other three factors we hypothesized to contribute to pollinator effectiveness: the number of times the visitor circumnavigated the head (Table 2; $F_{1,182} = 1.007$, $P = 0.317$), the day of the visit compared to the peak day of flowering ($F_{1,176} = 2.380$, $P = 0.125$), and the plot in which the observation occurred ($F_{1,174} = 1.835$, $P = 0.178$). Similarly, insufficient evidence exists to keep any interaction terms in our models (Table 2).

Visitor movement within a flowering head

Although we found little evidence that the number of times a visitor circumnavigated the head predicted style shriveling rates (Table 2; $F_{1,182} = 1.007$, $P = 0.320$), head circumnavigations vary by taxa (Appendices S2 and S3; $F_{5,195} = 15.590$, $P < 0.001$), and the taxa that shriveled more styles also circumnavigated the head a greater number of times. *Andrena helianthiformis* circled the head more times than all other taxa, except "medium gray bees" (Appendices S2 and S3; $P < 0.01$). "Medium gray bees" circle the head more times than male *Melissodes* spp. and Augochlorini ($P < 0.05$), and *A. virescens* circle the head more times than male *Melissodes* spp. ($P < 0.01$). For *A. virescens*, weak evidence suggests that the number of head circumnavigations slightly increases shriveling rates (Table 3; $F_{1,33} = 4.338$, $P = 0.046$). However, such evidence was not present for *A. helianthiformis* ($F_{1,88} = 0.854$, $P = 0.358$) or for male *Melissodes* spp. ($F_{1,21} = 2.072$, $P = 0.168$), indicating that this behavior does not generally explain within-taxon variation in pollinator effectiveness (Fig. 3). On another note, for *A. helianthiformis*, there is strong evidence that, as the number of available receptive styles increased, the proportion of styles that shriveled in a single visit decreased (Table 3; $F_{1,91} = 15.461$, $P < 0.001$). For *A. virescens*, there is weaker evidence that a greater proportion of styles were shriveled during single visits that occurred later in the season (Table 3; $F_{1,33} = 5.640$, $P = 0.024$). There was not sufficient evidence to keep any other main effect in the minimal adequate models for single-taxon GLMs.

Effective pollen movement

We found no evidence for differences in effective pollen movement distances among the three pollinator taxa (Table 4; Fig. 4). The probability of movement was modeled as an exponential decay function ($e^{\beta x}$), and we found that the probability decreased at a rate of $\beta = -0.059$ (95% CI: -0.082 to -0.039;) for every 0.1 m increase in pairwise distance (x; Fig. 4B). The probability of movement by *A. helianthiformis* decayed by $\beta = -0.069$ (95% CI: -0.110 to -0.035); for "medium gray bees" the decay was $\beta = -0.071$ (95% CI: -0.136 to -0.026); and for "small bees" the decay was $\beta = -0.071$ (95% CI: -0.083 to -0.013).

Using the most likely pedigree, we found that pollen moved, on average, 16 m between mates (SD = 11 m), and this distance was similar across pollinator groups (Table 4). Interestingly, the mean distances to nearest flowering conspecifics was 3.36 m (± 0.27 SE) in 2013 and 1.68 m (± 0.08 SE) in 2014, demonstraighting that bees moved pollen farther than what would be expected if they were only transfering pollen between nearest neighbors (Appendix S5). Of the 42 visits used in paternity analysis, 33 resulted in two or more genotyped offspring. By examining the most likely pedigree, we found that 32 of these 33 visits had at least two sires represented in the offspring (Table 4). Therefore, a single visitor successfully transferred pollen from more than one sire in 78% of the visits.

TABLE 3. Likelihood ratio tests for stepwise model simplification using backward elimination for single-taxon generalized linear models of the three most common taxa. Deviance is the likelihood ratio test statistic. *P*-values are for the *F*-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. The maximal model included four main effect terms: number of receptive styles available ("styles"), number of times the visitor circumnavigated the head ("circle"), day of the visit compared to peak day of flowering ("day"), and the plot in which observations occurred ("plot" with two levels). We did not have enough power to test for two-way interactions. For *Agapostemon virescens*, models 4 and 5 were each compared to model 3 to test the two focal terms that remained in the final model. After model simplification, the minimal adequate model for *Andrena helianthiformis* included styles only and the minimal adequate model for *Agapostemon virescens* included circle and day. No terms were significant predictors for model with male *Melissodes* spp. visits.

Andrena helianthiformis					
Model	Residual df	Test term	Test df	Deviance	P
1	87				
2	88	circle	1	9.235	0.358
3	89	day	1	11.223	0.311
4	90	plot	1	3.767	0.056
5	91	styles	1	167.111	<0.001

Agapostemon virescens					
Model	Residual df	Test term	Test df	Deviance	P
1	30				
2	31	plot	1	0.105	0.878
3	32	styles	1	13.654	0.088
4	33	circle	1	19.081	0.046
5	33	day	1	24.811	0.024

Male *Melissodes* spp.					
Model	Residual df	Test term	Test df	Deviance	P
1	17				
2	18	plot	1	2.576	0.685
3	19	styles	1	4.106	0.610
4	20	day	1	6.284	0.528
5	21	circle	1	31.416	0.168

DISCUSSION

The specialist *A. helianthiformis* differed from other visitors

We found that the solitary ground-nesting bee *Andrena helianthiformis* was the most effective insect visitor of *Echinacea* in our populations (Table 2; Fig. 2). However, *A. helianthiformis* are not more effective at moving pollen greater distances and are thus simultaneously exemplary and unremarkable pollinators, depending on the aspect of pollinator effectiveness. Based on Discover Life specimen records, *A. helianthiformis* appears to be a common *Echinacea* visitor, with 33 of 38 specimens collected from *Echinacea* (Ascher and Pickering, 2018). However, it has also been collected from other Asteraceae species (*Heliopsis helianthoides* and *Gaillardia* sp.) as well as two Fabaceae species (*Amorpha canescens* and *Melilotus officinalis*). Pollen load data from museum specimens suggest that *A. helianthiformis* specializes on flowers in the tribe Heliantheae (Larkin et al., 2008), which includes *Echinacea* and *Heliopsis* but not the other plant taxa associated with Discover Life specimen records. It is possible that *A. helianthiformis* is indeed a Heliantheae oligolege and that specimens collected from flowers of other tribes were nectar foraging, given that some oligoletic *Andrena* species are picky
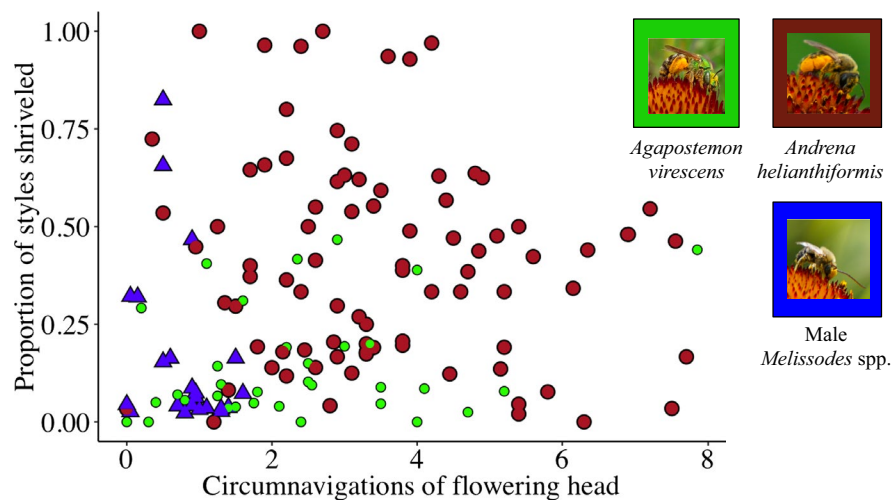
FIGURE 3. Relationship between circumnavigations of the flowering head and proportion of styles shriveled per visit for *Agapostemon virescens* (small green circles), male *Melissodes* spp. (blue triangles), and *Andrena helianthiformis* (large maroon circles). Single-taxon generalized linear model (GLM) analyses revealed that the number of times the visitor circumnavigated the head only moderately predicted shriveling by *A. virescens* ($F_{1,33} = 4.338$, $P = 0.046$) and did not predict shriveling by *A. helianthiformis* ($F_{1,88} = 0.854$, $P = 0.358$) or male *Melissodes* spp. ($F_{1,20} = 2.179$, $P = 0.158$). Photo credit: J. L. Ison.

pollen-eaters but more generalized in their pursuit of nectar (Neff and Simpson, 1997). In July 2019, two isolated *A. helianthiformis* nests were discovered near P2, indicating that, at our study sites, *A. helianthiformis* is a solitary-ground nester that does not nest in aggregate (J. L. Ison, M. Incarnato, R. Johnson, and A. Pearson, personal observation). However, as with most ground-nesting bees, we know relatively little about the nesting biology of *A. helianthiformis*.

If *A. helianthiformis* is indeed a Heliantheae oligolege, it may carry more conspecific *Echinacea* pollen on its body and also deposit more conspecific pollen grains onto more receptive styles. Using specimens from collections across the United States, Larkin et al. (2008) found that *A. helianthiformis* pollen loads contained 98% Heliantheae pollen; however, for any given specimen, pollen may have come from multiple species within this diverse tribe. When we followed marked *A. helianthiformis* individuals, they visited *Echinacea* but also *Heliopsis* and *Coreopsis* plants, sometimes during the same foraging bout (J. L. Ison, personal observation). Ultimately, because we did not collect pollen load data for any visitors, we cannot be sure that *A. helianthiformis* carried a greater quantity or proportion of conspecific *Echinacea* pollen than other visiting taxa in this system. Indeed, even generalist visitors may temporarily specialize on a particular plant species over the course of a foraging bout

(Free, 1970) and therefore carry high proportions of conspecific pollen.

In other systems, specialists are sometimes, but not always, more effective than generalist bees. In commercial sunflowers, single visits from oligolectic *A. helianthi* and *Melissodes agilis* produced 5–10 times as many seeds as single visits from polylectic honey bees, likely because oligolectic bees in this system carry more conspecific pollen grains on their bodies and forage between rows of self-incompatible varieties (Parker, 1981). In *Helianthus annuus*, specialists did not pollinate a greater proportion of florets than generalists, but large bees were more effective than small bees, and hypergeneralist bumble bees were less effective than other large bee species (Neff and Simpson, 1990). Although we cannot be sure as to why *A. helianthiformis* was the most effective pollinator, we suspect that its superior effectiveness is explained by a suite of pollinator traits that are related and not mutually exclusive, including pollinator body size, level of specialism, conspecific pollen transport, and foraging behavior both on flowering heads and among flowering plants.

While *A. helianthiformis* was the most effective pollinator in our system, in a 2 yr study of *Echinacea* in Saskatchewan, Canada, bumble bees and honey bees were the most effective pollinators (Wist and Davis, 2013). Interestingly, in Saskatchewan the observed numbers of these large Apidae bees varied greatly between years (0.3% of all flower visitors in year 1 and 12.9% of flower visitors in year 2). Throughout the 4 yr of our study, *A. helianthiformis* remained a common visitor (Appendix S1). Across all pollination biology research projects in our system since 1996, we have observed very few visits to *Echinacea* by either a bumble bee or honey bee (Appendix S1; Wagenius and Lyon, 2010; Ison et al., 2018), even though honey bees and bumble bees are prevalent in our study area.

However, across our study sites, *A. helianthiformis* are typically observed only in large populations, and only during early and peak flowering time of *Echinacea* (Ison et al., 2018). Their absence from smaller fragmented populations could contribute to observed pollen limitation in small patches. Thus, spatial and temporal variation in the abundance of larger Andrenidae specialists may help explain the observed spatial and temporal variation in *Echinacea* pollen limitation and reproductive success (Wagenius and Lyon, 2010; Ison and Wagenius, 2014). Elsewhere in *Echinacea*'s range, only

TABLE 4. Distances of effective pollen movement: mean distance that pollen moved between mates and mean number of sires per visit. Mean distance between maternal and paternal plant and mean number of sires per visit were determined by most likely pedigree. Our analysis indicates that average pollen transport distances and number of sires per visit do not vary among taxa.

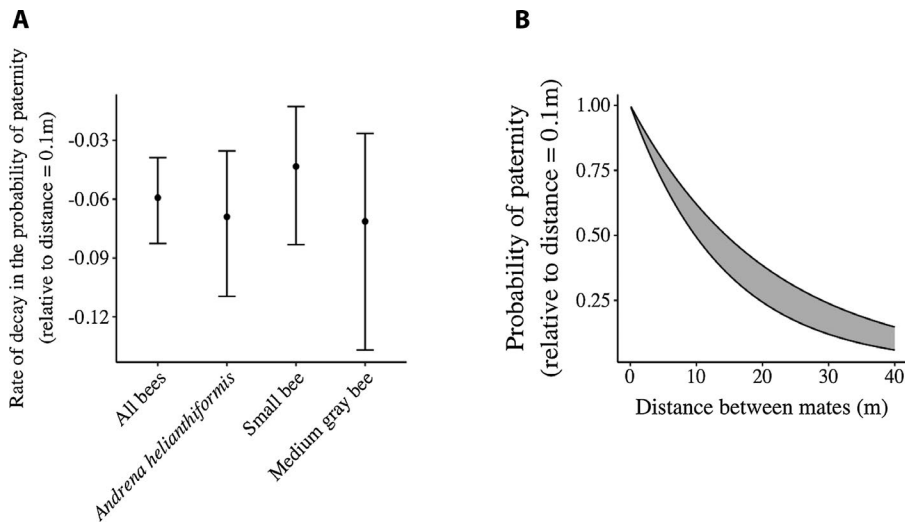| Pollinator group	No. of visits	No. of offspring	Mean genotyped offspring per visit (± SD)	Mean sires per visit (± SD)	Mean distance between mates (± SD)
Andrena helianthiformis	12	49	4.08 (2.02)	3.75 (1.86)	16.49 (10.96)
Small bee	14	29	2.07 (1.54)	1.71 (1.07)	18.07 (9.01)
Medium gray bee	16	38	2.38 (1.02)	2.31 (1.01)	13.91 (13.06)
Total	42	116	2.76 (1.72)	2.52 (1.53)	16.02 (11.24)

A

B

FIGURE 4. The probability of effective pollen movement was modeled as an exponential decay function ($e^{\beta x}$). (A) The mean rate of decay (β) with confidence intervals for each of the bee taxa (*Andrena helianthiformis*, "medium gray bees," and "small bees") as estimated by full probability paternity analysis. (B) The probability of paternity decreases as the distance between parental plants increases (using the β values for all bees; see panel A). The shaded region is bounded by lower and upper confidence intervals.

small Andrenidae bees, such as *Pseudopanurgus* sp., were observed visiting *Echinacea* and they were inefficient compared to larger bees (Wist and Davis, 2013). Taken together, our findings highlight the importance of some specialist bees for successful reproduction in native plants, even in an agricultural landscape where managed honey bee hives and commercial bumble bee colonies are prevalent.

Effects of within-flowering-head visitor behavior and phenological traits on effective pollen deposition

Previous work has hypothesized that differences in visitor movement among florets on heads could explain differences in pollinator effectiveness (Wist and Davis, 2013). While we found substantial variation in the number of times that insect taxa circumnavigate the flowering head (Appendices S2 and S3), we found a negligible relationship between this behavior and effective pollen deposition (Table 2). Within a single taxon, the number of individual circumnavigations did not predict effective pollen disposition for either *A. helianthiformis* or "medium gray bees" (Fig. 3). Since this behavior did not explain within-taxon variation in effective pollen deposition, it is therefore less surprising that it also did not explain variation among taxa. When interpreting our results, it is important to remember that, because flowering heads were bagged for ≥24 h prior to being visited, pollen and nectar levels may have been artificially high. Additionally, while we do partially account for variable nectar and pollen availability by including the number of available receptive styles in all models, individuals that circumnavigate the flowering head more times may be responding to increased nectar and pollen availability. Such behavior was observed in insect visits to *Helianthus annuus*—visitors spent more time on bagged heads than they did on unbagged heads (Neff and Simpson, 1990).

Other studies have suggested that flower-handling time and related metrics of visit duration are positively correlated with pollen deposition (e.g., Robertson, 1992; Ivey et al., 2003). However, our

study suggests that there is not always a clear relationship between insect behavior on the flowering head and effective pollen deposition. The difference between our results and those of others may hinge on whether visitors are foraging for pollen, nectar, or both. Most pollinator observation studies focus on bees foraging for nectar. Although *Echinacea* produces nectar (Wist and Davis, 2008) and bees likely imbibed nectar while visiting flowering heads, most of the bees we observed, with the exception of male bees, appeared to be primarily foraging for pollen. Another difference between most other studies and ours is that a single visit in many systems comprises a visit to a single flower. By contrast, in each visit to one head, our bees visited 11–86 flowers (i.e., florets). Making generalization about visitor behavior and effective pollen deposition will benefit from more studies of multiple types of flowers and inflorescences, especially in uniovulate species, which are less frequently studied.

While behavior on the flowering head did not predict the proportion of styles shriveled, the number of receptive styles available for pollination at the time of a visit was a good predictor (Table 2; Fig. 2). As the number of available receptive styles increased, the proportion of styles shriveled in a single visit decreased. However, the magnitude of this effect was relatively small, suggesting that taxon is a much better predictor than the availability of receptive styles. If plants are visited by less effective pollinators, more visits would be required to pollinate all receptive styles. For instance, our results suggest that a flowering head visited four times by Augochlorini should have fewer pollinated styles than a flowering head visited only once by *A. helianthiformis*. Our finding complements earlier work in this system (Wagenius and Lyon, 2010), which demonstrated that pollen limitation in *Echinacea* is not correlated with insect visitation rates.

Previous work has shown that late-flowering *Echinacea* produce fewer seeds than early-flowering plants (Ison and Wagenius, 2014). Yet, the day that we observed a visit—our proxy for individual flowering phenology relative to population-level peak flowering—did not explain variation in the proportion of styles shriveled per visit (Table 2). We found weak evidence suggesting that a greater proportion of styles were shriveled during single visits from *A. virescens* that occurred around peak bloom as compared to visits that occurred earlier in the season (Table 3). However, we observed few visits from *A. virescens* late in the flowering season and late in an individual plant's flowering period (Appendices S6 and S7). Previous work has found that, over the course of *Echinacea*'s flowering season, the total number of insect visits decreases and the inefficient Augochlorini make up an increasingly greater proportion of *Echinacea*'s flower visitors (Ison et al., 2018).

Effective pollen movement did not vary among visitors

On average, pollen moved 16 m between paternal and maternal plants, and pollen transport distances ranged from 1 to 50 m

(Fig. 4). However, a few of the genotyped offspring had low paternity confidence, which may indicate that pollen was transported from an un-genotyped individual outside of our experimental plot. Thus, we cannot say with certainty that pollen was never sourced from plants >50 m away. Interestingly, we also found that pollen moved much farther than the mean nearest-flowering-neighbor distances, which were 3.36 m and 1.68 m in 2013 and 2014, respectively (Appendix S5). This could indicate that bees are flying farther than the nearest-flowering-neighbor distance when moving in between flowers or that bees are transporting pollen from less recently visited plants.

In small populations of *Echinacea*, individual plants are often surrounded by incompatible neighbors (Wagenius et al., 2007), which means that studies of effective pollen deposition distances in those areas may not reflect actual pollen movement distances, because the movement of pollen to an incompatible mate might not produce a seedling. By contrast, because we planted multiple diverse genotypes together when establishing our experimental plots, our plots lack genetic structure and it would be very unusual for plants flowering in close proximity to be incompatible. Thus, our estimates of effective pollen movements likely reflect true pollen transport distances, regardless of compatibility. In other forb species, pollen transport distances are much shorter than 16 m (Evans et al., 2017; Thavornkanlapachai et al., 2018; Valverde et al., 2019), with pollen sometimes moving <1 m between mates (Evans et al., 2017, Valverde et al., 2019).

Mean pollen transport distances did not differ among our three taxa (Table 4; Fig. 4). Previous studies have speculated that, because larger bees forage across greater distances (Gathmann and Tscharntke, 2002; Greenleaf et al., 2007), they will also transport pollen greater distances among plants. However, despite substantial size variation among *A. helianthiformis*, "medium gray bees," and "small bees," we found negligible differences in estimates of mean transport distances. Although the bees in our system vary in size, they are also primarily solitary. In honey bees, and potentially other social apids, workers from the same colony often brush against one another in between foraging bouts, picking up pollen grains in the process (B. Johnson, UC Davis, personal communication). Although speculative, it is possible that this socially mediated pollen transfer could promote pollen movement among isolated plant populations. Social bees are hypothesized to transport pollen farther than solitary bees because individual foragers make riskier trips to search for isolated patches (Real and Caraco, 1986). For instance, scout honey bees often make such risky trips and, when successful, communicate results to other foragers (Ratnieks and Shackleton, 2015). It would be interesting to investigate whether the social honey bees and bumble bees that visit Canadian populations of *Echinacea* (Wist and Davis, 2013) would transport pollen farther than solitary bees.

Interestingly, visitors in this system carried and deposited a high diversity of conspecific pollen. For example, 78% of the single visits that resulted in at least two offspring had at least two sires and, in some cases, visitors delivered pollen from six or more sires. This suggests that, in this system, pollen is deposited from multiple plants visited by an insect during its foraging bout and not just the most recently visited plant or the topmost layer of pollen on insect bodies. Because many small *Echinacea* populations have high rates of pollen incompatibility between plants (Wagenius et al., 2007), the observation that bees carry a diverse conspecific pollen load is potentially good news for these fragmented populations.

Conservation implications

Echinacea populations are small and isolated due to the conversion of prairie habitat to agriculture—and losses continue (Samson et al., 2004; Gage et al., 2016). Like many plants threatened by habitat destruction and fragmentation, reproduction in *Echinacea* is highly variable and pollen limitation is prevalent. Variability in pollen limitation is sometimes thought to result from variable pollinator visitation rates. However, pollinator visitation rates are consistent across small isolated and larger non-isolated *Echinacea* populations (Wagenius and Lyon, 2010; Ison et al., 2018), suggesting that visitation rates are not the primary cause of variation in reproductive success. While asynchronous flowering and mate incompatibility also both contribute to pollen limitation (Wagenius et al., 2007; Ison and Wagenius, 2014), here we explored how much variation in pollinator effectiveness might also contribute to variability in *Echinacea* reproductive success.

Our results build on the results of previous studies in our study system, described above, to suggest that variation in pollinator community composition—and, more specifically, the abundance of highly effective pollinators—likely contributes to temporal and spatial variation in pollen limitation and reproductive success of plants in fragmented habitats. If certain visitor taxa are more effective pollinators, their loss and altered distribution will have disproportionately large impacts on plant pollination. As such, the effects of pollinator species losses may have greater ecological consequences than previously predicted by simulation models (e.g., Memmott et al., 2004; Memmott et al., 2007), depending on the order in which pollinator species are lost. Therefore, there is a pressing need to better understand how disturbances that alter pollinator community composition affect pollen limitation and plant reproduction. For *Echinacea*, the local abundance of *Andrena helianthiformis* may strongly predict reproductive success. Future research should investigate the distribution of *A. helianthiformis*, assess why it is absent in many parts of our study area, and improve our understanding of what limits its local abundance. Beyond the intrinsic value of conserving native bee species, understanding how to best conserve *A. helianthiformis* may allow for better conservation of *Echinacea* and other Heliantheae in tallgrass prairie habitat.

AUTHOR CONTRIBUTIONS

All authors conceived and designed the experiment. M.L.P., J.L.I., K.M.H., A.D.K., K.E.K., and K.M.K. collected field data. J.L.I. and A.L.B. collected lab data. M.L.P., J.L.I., and S.W. analyzed the data. M.L.P. wrote the initial draft of the manuscript. All authors contributed to manuscript revisions.

DATA AVAILABILITY

Data for this study are available at http://echinaceaproject.org/datasets/pollinator-effectiveness/. Our 207 videos are online at https://www.youtube.com/user/TheEchinaceaProject.

SUPPORTING INFORMATION

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

APPENDIX S1. Number of visitor observations for all taxa during each year of the study.

APPENDIX S2. Tukey post hoc results for pairwise taxa comparisons of head circumnavigations.

APPENDIX S3. Number of head circumnavigations for the most frequently observed taxa.

APPENDIX S4. Total number of visitor observations for each year of the study.

APPENDIX S5. Histogram of nearest neighbor distances for P2 in 2013 and 2014.

APPENDIX S6. Number of visitor observations for the most frequently observed taxa during each week of the study.

APPENDIX S7. Number of observed visits to early-, mid-, and late-blooming plants.

LITERATURE CITED

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.

Aizen, M. A., and P. Feinsinger. 1994. Habitat fragmentation, native insect pollinators, and feral honey bees in Argentine 'Chaco Serrano'. *Ecological Applications* 4: 378–392.

Anderberg, A. A., B. G. Baldwin, R. G. Bayer, J. Breitwieser, C. Jeffrey, M. O. Dillon, P. Eldenäs, et al. 2007. Compositae. *In* J. W. Kadereit and C. Jeffrey [eds.], The families and genera of vascular plants, vol. 8, Flowering Plants · Eudicots, 61–588. Springer-Verlag Berlin, Heidelberg, Germany.

Ascher, J. S., and J. Pickering. 2018. Discover Life bee species guide and world checklist (Hymenoptera: Apoidea: Anthophila). http://www.discoverlife.org/mp/20q?guide=Apoidea_species.

Austen, E. J., and A. E. Weis. 2016. The causes of selection on flowering time through male fitness in a hermaphroditic annual plant. *Evolution* 70: 111–125.

Ballantyne, G. K., C. R. Baldock, L. Rendell, and P. G. Willmer. 2017. Pollinator importance networks illustrate the crucial value of bees in a highly speciose plant community. *Scientific Reports* 7: 8389.

Ballantyne, G. K., C. R. Baldock, and P. G. Willmer. 2015. Constructing more informative plant–pollinator networks: visitation and pollen deposition networks in a heathland plant community. *Proceedings of the Royal Society B - Biological Sciences* 282: 14–22.

Benjamin, F. E., J. R. Reilly, and R. Winfree. 2014. Pollinator body size mediates the scale at which land use drives crop pollination services. *Journal of Applied Ecology* 51: 440–449.

Biesmeijer, J. C., S. P. M. Roberts, M. Reemer, R. Ohlemüller, M. Edwards, T. Peeters, A. P. Schaffers, et al. 2006. Parallel declines in pollinators and insect-pollinated plants in Britain and the Netherlands. *Science* 313: 351–354.

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* 110: 13044–13048.

Brosi, B. J., G. C. Daily, and P. R. Ehrlich. 2007. Bee community shifts with landscape context in a tropical countryside. *Ecological Applications* 17: 418–430.

Brosi, B. J., G. C. Daily, T. M. Shih, F. Oviedo, and G. Durán. 2008. The effects of forest fragmentation on bee communities in tropical countryside. *Journal of Applied Ecology* 45: 773–783.

Calvillo, L. M., V. M. Ramírez, V. Parra-Tabla, and J. Navarro. 2010. Bee diversity in a fragmented landscape of the Mexican neotropic. *Journal of Insect Conservation* 14: 323–334.

Crawley, M. J. 2013. The R book. Wiley, Chichester, West Sussex, United Kingdom.

DeMauro, M. M. 1996. Relationship of breeding system to rarity in the lakeside daisy (*Hymenoxys acaulis* var. glabra). *Conservation Biology* 7: 542–550.

Evans, T. M., S. Cavers, R. Ennos, A. J. Vanbergen, and M. S. Heard. 2017. Florally rich habitats reduce insect pollination and the reproductive success of isolated plants. *Ecology and Evolution* 7: 6507–6518.

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

Free, J. B. 1970. The flower constancy of bumblebees. *Journal of Animal Ecology* 39: 395–402.

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. University of Nebraska Press. Retrieved May 31, 2019, from Project MUSE database.

Gathmann, A. and T. Tscharntke. 2002. Foraging ranges of solitary bees. *Journal of Animal Ecology* 71: 757–764.

Gibson, R. H., I. L. Nelson, G. W. Hopkins, B. J. Hamlett, and J. Memmott. 2006. Pollinator webs, plant communities and the conservation of rare plants: Arable weeds as a case study. *Journal of Applied Ecology* 43: 246–257.

Gómez, J. M., M. Abdelaziz, J. Lorite, A. J. Muñoz-Pajares, and F. Perfectti. 2010. Changes in pollinator fauna cause spatial variation in pollen limitation. *Journal of Ecology* 98: 1243–1252.

Gong, Y. B. and S. Q. Huang. 2014. Interspecific variation in pollen–ovule ratio is negatively correlated with pollen transfer efficiency in a natural community. *Plant Biology* 16: 843–847.

Goulson, D. 1999. Foraging strategies of insects for gathering nectar and pollen, and implications for plant ecology and evolution. *Perspectives in Plant Ecology, Evolution, and Systematics* 2: 185–209.

Greenleaf, S. S., N. M. Williams, R. Winfree, and C. Kremen. 2007. Bee foraging ranges and their relationship to body size. *Oecologia* 153: 589–596.

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.

Harder, L. D. and M. A. Aizen. 2010. Floral adaptation and diversification under pollen limitation. *Philosophical Transactions of the Royal Society of London, Series B* 365: 529–543.

Harder, L. D., and W. G. Wilson. 1998. Theoretical consequences of heterogeneous transport conditions for pollen dispersal by animals. *Ecology* 79: 2789–2807.

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

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., and S. Wagenius. 2014. Both flowering time and spatial isolation affect reproduction in *Echinacea angustifolia*. *Journal of Ecology* 102: 920–929.

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.

Ivey, C. T., P. Martinez, and R. Wyatt. 2003. Variation in pollinator effectiveness in Swamp Milkweed, *Asclepias Incarnata* (Apocynaceae). *American Journal of Botany* 90: 214–225.

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

Koch, L., K. Lunau, and P. Wester. 2017. To be on the safe site—Ungroomed spots on the bee's body and their importance for pollination. *PLoS ONE* 12: e0182522.

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.

Larkin, L. L., J. L. Neff, and B. B. Simpson. 2008. The evolution of a pollen diet: Host choice and diet breadth of *Andrena* bees (Hymenoptera: Andrenidae). *Apidologie* 39: 133–145.

Memmott, J., P. G. Craze, N. M. Waser, and M. V. Price. 2007. Global warming and the disruption of plant–pollinator interactions. *Ecology Letters* 10: 710–717.

Memmott, J., N. M. Waser, and M. V. Price. 2004. Tolerance of pollination networks to species extinctions. *Proceedings of the Royal Society B-Biological Sciences* 271: 2605–2611.

Michener, C. D. 1999. The corbiculae of bees. *Apidologie* 30: 67–74.

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.

Müller, A. 1996a. Convergent evolution of morphological specializations in Central European bee and honey wasp species as an adaptation to the uptake of pollen from nototribic flowers (Hymenoptera, Apoidea and Masaridae). *Biological Journal of the Linnean Society* 57: 235–252.

Müller, A. 1996b. Host-plant specialization in Western Palearctic Anthidine bees (Hymenoptera: Apoidea: Megachilidae). *Ecological Monographs* 66: 235–257.

Muller, K. E. and S. Wagenius. 2016. *Echinacea angustifolia* and its specialist ant-tended aphid: a multi-year study of manipulated and naturally-occurring aphid infestation. *Ecological Entomology* 41: 51–60.

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.

Neff, J. L. and B. B. Simpson. 1990. The roles of phenology and reward structure in the pollination biology of wild sunflower (*Helianthus annuus* I. Asteraceae). *Israel Journal of Botany* 39: 197–216.

Neff, J. L. and B. B. Simpson. 1997. Nesting and foraging behavior of *Andrena* (Callandrena) *rudbeckiae* Robertson (Hymenoptera: Apoidea: Andrenidae) in Texas. *Journal of the Kansas Entomological Society* 70: 100–113.

Park, M. G., R. A. Raguso, J. E. Losey, and B. N. Danforth. 2016. Per-visit pollinator performance and regional importance of wild *Bombus* and *Andrena* (Melandrena) compared to the managed honey bee in New York apple orchards. *Apidologie* 47: 145–160.

Parker, A. J., J. Tran, J. L. Ison, J. D. Bai, and J. D. Thomson. 2015. The effect of grooming on pollen quality on corbiculate and non-corbiculate bees. *Arthopod-Plant Interactions* 9: 197–203.

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.

Parker, F. D. 1981. How efficient are bees in pollinating sunflowers? *Journal of the Kansas Entomological Society* 54: 61–67.

Portman, Z. M. and V. J. Tepedino. 2017. Convergent evolution of pollen transport mode in two distantly related bee genera (Hymenoptera: Andrenidae and Melittidae). *Apidologie* 48: 461–472.

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.

Rader, R., J. Reilly, I. Bartomeus, and R. Winfree. 2013. Native bees buffer the negative impact of climate warming on honey bee pollination of watermelon crops. *Global Change Biology* 19: 3103–3110.

Ratnieks, F. L. W. and K. Shackleton. 2015. Does the waggle dance help honey bees to forage at greater distances that expected for their body size? *Frontiers in Ecology and Evolution* 3: 31.

R Core Team. 2018. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

Real, L. and T. Caraco. 1986. Risk and foraging in stochastic environments. *Annual Review of Ecology and Systematics* 17: 371–390.

Robertson, A. W. 1992. The relationship between floral display size, pollen carry-over and geitonogamy in *Myosotis colensoi* (Kirk) Macbride (Boraginaceae). *Biological Journal of the Linnean Society* 46: 333–349.

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

Thavornkanlapachai, R., P. G. Ladd, and M. Byrne. 2018. Population density and size influence pollen dispersal pattern and mating system of the predominantly outcrossed *Banksia nivea* (Proteaceae) in a threatened ecological community. *Biological Journal of the Linnean Society* 124: 492–503.

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

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. 2006. Scale dependence of reproductive failure in fragmented *Echinacea* populations. *Ecology* 87: 931–941.

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.

Westerkamp, C. 1991. Honey bees are poor pollinators — why? *Plant Systematics and Evolution* 177: 71–75.

Wist, T. J. and A. R. Davis. 2008. Floral structure and dynamics of nectar production in *Echinacea pallida* var. *angustifolia* (Asteraceae). *International Journal of Plant Sciences* 196: 708–722.

Wist, T. J. and A. R. Davis. 2013. Evaluation of inflorescence visitors as pollinators of Echinacea angustifolia (Asteraceae): comparison of techniques. *Journal of Economic Entomology* 106: 2055–2071.

Wyatt, R., S. B. Broyles, and G. S. Derda. 1992. Environmental influences on nectar production in milkweeds (*Asclepias syriaca* and *A. exaltata*). *American Journal of Botany* 79: 636–642.

Young, H. J., D. W. Dunning, and K. W. von Hasseln. 2007. Foraging behavior affects pollen removal and deposition in *Impatiens capensis* (Balsaminaceae). *American Journal of Botany* 94: 1267–1271.