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Reproduction of *Echinacea angustifolia* in fragmented prairie is pollen-limited but not pollinator-limited

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Abstract. Pollen limitation of plant reproduction occurs in many plant species, particularly those in fragmented habitat; however, causes of pollen limitation are often unknown. We investigated the relationship between pollen limitation and pollinator visitation in the purple coneflower, *Echinacea angustifolia* (Asteraceae), which grows in the extremely fragmented tallgrass prairie of North America. Previous investigations showed that pollen limitation of *E. angustifolia* increases with plant isolation and decreases with population size. We observed insect visitation to *E. angustifolia* over two flowering seasons and estimated pollen limitation of observed plants, using seed set as a proxy measure in 2004 and persistence of receptive style rows in 2005. We analyzed spatial patterns of bee visitation and pollination at two spatial scales: individual isolation, as measured by the distance to their kth nearest flowering neighbors ($k = 1 - 15$), and population size. Our results indicate that *E. angustifolia* is pollinated by over 26 species of native bees, with 70–75% of visits by halictid bees. Surprisingly, in both years, bee visitation increased with isolation of individual plants and did not vary significantly with population size. As expected, plant isolation increased pollen limitation and lowered seed set. There was no effect of population size on seed set in 2004, and pollen limitation decreased nonsignificantly with population size in 2005. We conclude that pollen receipt limits reproduction in *E. angustifolia,* but pollinator visitation does not. Remarkably, isolated plants simultaneously have increased rates of pollinator visitation by pollinators and decreased reproduction. We discuss alternative explanations of pollen limitation that are consistent with this apparent discrepancy, including a decline in the availability of compatible conspecific pollen with increased plant isolation.

Key words: density; Echinacea angustifolia; *habitat fragmentation; isolation; pollen limitation; pollinator limitation; population size; prairie remnants, western Minnesota, USA; purple coneflower; spatial scale; style persistence.*

INTRODUCTION

Pollen limitation occurs when a plant's reproduction is limited by the quantity or quality of pollen received (Byers 1995, Aizen and Harder 2007). Pollen limitation is widely observed in fragmented habitats (Aizen et al. 2002); however, pollen limitation is not universal, and observed effects of fragmentation vary considerably among studies (Burd 1994, Larson and Barrett 2000, Knight et al. 2005, Aguilar et al. 2006). Investigators have attempted to identify factors responsible for this variation, which may include plant breeding system (e.g., self-incompatibility), mode of pollination, and, among animal-pollinated plants, the degree of specialization in plant–pollinator relationships. Understanding causes of pollen limitation will improve prediction of its consequences for plants in fragmented habitats. Such consequences may include population decline and altered population genetic dynamics (Haig and Westoby 1988, Kearns et al. 1998, Ashman et al. 2004).

When pollen limitation is observed in fragmented habitat, it is often interpreted as evidence for insufficient visitation by pollinators (pollinator limitation of reproduction). Two general lines of evidence support the broad assumption that plants in fragmented habitat have fewer visitors. First, fragmentation threatens pollinator populations (Jennersten 1988, Rathcke and Jules 1993); small habitat remnants may lack sufficient nest sites or other resources to support resident pollinator populations (Sih and Baltus 1987, Steven et al. 2003). Second, pollinators have been shown to respond strongly to the local abundance of flowering plants (Kunin 1997*b*), and small remnants may not attract pollinators. More specifically, two expectations emerge from the hypothesis that low pollinator visitation causes pollen limitation in fragmented habitat: (1) pollinator visitation is expected to decrease with fragmentation, and (2) pollen limitation is expected to decrease with pollinator visitation. Few studies have directly tested these expectations (e.g., Jennersten 1988). Alternative causes of pollen limitation in fragmented

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habitat have been posited that are not related to changes in pollinator visitation rate, such as loss of compatible mates and altered pollinator behavior that reduces pollen quality but not quantity (Byers and Meagher 1992, Ashman et al. 2004, Aizen and Harder 2007).

The processes that are hypothesized to cause pollen limitation likely operate at different spatial scales. Some studies have focused on landscape scales by measuring plant population size, mean population density, or interpopulation isolation (reviewed in Kunin [1997*a*]) to test, for example, whether larger habitat remnants support more pollinators. Other studies focus on within-population variation in floral resources by measuring the local abundance of individual plants (e.g., Allison 1990, Roll et al. 1997) to test, for instance, whether foraging pollinators visit denser or less isolated patches of flowers. The diversity of spatial scales in pollen-limitation studies may hamper our ability to make generalizations about the effects of fragmentation on plant reproduction (Aizen et al. 2002, Ghazoul 2005), if fragmentation effects differ in strength or direction from one spatial scale to another (Levin 1992).

We investigated the relationship between pollinator visitation and the previously well-documented spatial patterns of pollen limitation and reproductive failure in *Echinacea angustifolia* (Asteraceae), hereafter *Echinacea,* a widespread, perennial prairie species. The tallgrass prairie of central North America is extremely fragmented: <1% of this habitat remains (Samson and Knopf 1994), and prairie plants now reside in habitat patches that are much smaller than they were prior to European settlement. During a previous three-year study, pollen limitation in *Echinacea* consistently increased with isolation of individual plants and decreased with population size (Wagenius 2006). Mean seed set was inversely related to isolation, consistently ranging from over 40% to under 10% for the least and most isolated plants, respectively.

Echinacea exhibits characteristics typical of many plants native to the prairie, including self-incompatibility, reproduction strictly by seed, and lack of specialized insect pollinators and seed dispersers (Leuszler et al. 1996). These characteristics vary in their expected effects on the susceptibility of plants to fragmentation-related reproductive failure: fragmentation is expected to increase pollen limitation in self-incompatible plants (Larson and Barrett 2000, Aguilar et al. 2006), whereas it may have little effect on pollen limitation in generalized plant–insect interactions (Waser et al. 1996, Aizen et al. 2002, Ashworth et al. 2004).

Here we describe a two-year study of pollinator visitation to *Echinacea* in 21 prairie remnants in an agricultural landscape. We investigated the role of pollinators in generating previously observed spatial patterns of pollen limitation. Specifically, (1) we identified floral visitors and likely pollinators of *Echinacea* within our study area, (2) we tested the hypothesis that pollinator visitation to *Echinacea* increases with the local abundance of flowering *Echinacea* plants at population and individual spatial scales, and (3) we tested the hypothesis that plant reproduction increases with pollinator visitation.

METHODS

Study site and population mapping

The study area comprises 6400 ha of rural western Minnesota, USA (centered near 45°49′ N, 95°43′ W). Before European settlement began in the 1870s, the entire area, except for lakes and wetlands, was potential *Echinacea* habitat. *Echinacea* and other prairie plants now persist in remnant populations on hillsides too steep for agricultural production, in fence corners inaccessible to farm machinery, along road and railroad rights-of-way, and on abandoned pastureland. For this study we selected 21 prairie remnants of three size classes from 29 remnants where *Echinacea* reproductive biology has been studied previously (Wagenius 2006). For the second year of this study (2005), we randomly eliminated 5 of the 21 populations to observe each population on more days.

In each year all flowering *Echinacea* plants were counted and mapped at each population except the largest, a 45-ha virgin Nature Conservancy prairie. There, we estimated population size by counting flowering plants within randomly placed, 10-m-wide belt transects that spanned the preserve. We mapped only those plants on a 600 m long, 5-m-wide transect. We mapped flowering plants using a total station (Topcon GTS-303; Topcon Positioning Systems, Livermore, California, USA) and high-precision (±3 cm) surveying procedures. The resulting maps enabled us to characterize the local abundance of flowering plants at two spatial scales: at the population scale we used population size, and at the individual scale we calculated individual isolation, defined as the distance (in meters) to the kth nearest flowering plant, $k < 16$.

Observing insect visitors

Our goal was to observe plants in each population four times in 2004 and five times in 2005. We observed populations during the flowering season on rain-free mornings when at least 25% of that population's reproductive individuals were shedding pollen. Each day we chose six populations: we visited each population within three days of it meeting the 25% criterion, and randomly selected the remaining sites for observation. On a given day, each population was randomly assigned an observer, who counted the number of plants presenting pollen that day, randomly selected five of those plants, and observed them in random order. Observers sat approximately 1.5 m north of each target plant to avoid casting a shadow over it. All observations occurred between 08:30 and 12:00.

In 2004 each plant observation began with five minutes of passive observation. We recorded each insect's arrival and departure time, a tentative identification, and any pollinating behaviors (e.g., collecting pollen, touching styles). After five minutes, we collected all visitors that landed on a flower head during the next five minutes using a net. If a specimen was an individual that had been recorded during the observation period we noted it to avoid double-counting visits. In 2005 we attempted to collect every visitor to each *Echinacea* plant. All failed captures and tentative identifications were recorded. After 10 minutes of observing/collecting, we moved to the next target plant and repeated the process. In both 2004 and 2005, after all five target plants had been observed once, we conducted a second round of 10-minute observation of the plants in the same order. All specimens were identified to species using Mitchell (1960), Michener et al. (1994), and Droege et al. (2008); nomenclature follows the Integrated Taxonomic Information System database (as of 12 March 2009) (*available online*).[5] Voucher specimens will be deposited in the University of Minnesota Entomology Collection (photos of specimens are *available online*).[6]

Quantifying pollen limitation

In 2004 we estimated seed set in a sample of the plants that we observed during the summer. On 15, 16, and 25 September we attempted to sample one seedhead from each of the observed plants. If a majority of achenes had already dispersed or were not ready to harvest, or if the head was damaged, grazed, or missing, then the head was excluded from analysis. At least one of these circumstances occurred in 33% or more of the plants observed in 11 populations and in 50% or more of the plants from 6 populations. The chance of exclusion was not associated with the isolation of a plant according to a generalized linear model with exclusion (yes/no) modeled as a function of distance to 3rd nearest neighbor ($n = 229$ plants, $P = 0.27$). We weighed 30 randomly selected achenes from each collected head. The distribution of achene masses was bimodal with a mode at 3 mg indicating full achenes (seeds) and the other mode at <1 mg indicating empty achenes (unfertilized florets). A prior germination experiment indicated that dividing a sample of achenes at a critical mass of 1.3–2.1 mg yields a batch consisting primarily of empty achenes (0–8% germination) and a batch consisting primarily of viable seeds (97–100% germination) (J. Ison and S. Wagenius, *unpublished data*). We define "seed set" as the proportion of achenes above the critical mass of 2.1 mg. All reported relationships involving seed set were similar whether we used 1.3 or 2.1 mg as the critical mass. Seed set in *Echinacea* closely reflects fertilization by compatible pollen and is inversely related to pollen limitation. We expected that seed set, per individual and per population, would increase with pollinator visitation rate and with the local abundance of flowering plants.

In 2005 we estimated pollen limitation of every plant that we observed each day by quantifying the persistence of receptive style rows. On an *Echinacea* head one row of florets emerges each day, sheds pollen for one day, then presents styles. Receptive *Echinacea* styles persist from emergence until they receive compatible pollen, at which point they shrivel within 24 hours. Style persistence has been verified to indicate a lack of receipt of compatible pollen (Wagenius 2004). We define our "index of pollen limitation for each plant on each day" as the number of persistent style rows divided by all rows that had emerged, averaged over all heads on a plant. The index ranges from 0, all style rows shriveled (i.e., no evidence of pollen limitation), to 1, no style rows shriveled. We expected that the index of pollen limitation, per individual and per population, would decrease with pollinator visitation rate and with the local abundance of flowering plants.

Of the 470 *Echinacea* plants observed in this study, 348 plants (74%) had one head (capitulum), 75 plants (16%) had two heads, 26 plants (6%) had three, and the others (4%) had 4–10 heads. We found no evidence that head count in these plants was related to plant isolation or population size (not shown), which is similar to a previous study that found no relationship between floret count per plant and isolation (Wagenius 2006).

Data analysis

We tested two related hypotheses: (1) pollinator visitation increases with population size, and (2) pollinator visitation is greater in less-isolated plants. Each plant is characterized by its isolation (distance to kth nearest flowering plant, $k < 16$) and the size of its population (number of flowering plants). Each of these spatial measures was log-transformed to use as an explanatory variable. Because bee behavior is very sensitive to wind, sky cover, and temperature, and because there was no difference between years and no linear changes within a season, we modeled observation day as an unordered factor. To control for the differences in the duration of observations (10 of 769 observations were not 20 minutes), the number of minutes observed per plant per day was modeled as a known linear predictor (offset). An initial analysis using generalized linear models with the response bee count per plant per day was overdispersed and could not be modeled with a Poisson error distribution owing to the many zeros (73% of observations experiment-wide) and a bee count as high as 8 individuals. As described by Cane (2001), this problem is typical of insect-visitation studies. We chose to model bee visitation per plant per day as a binomial response (yes/no) to avoid blatantly violating model assumptions. Although we gain a model that is statistically defensible, we do not use all of the count data we collected. We emphasize that our measure of bee visitation captures meaningful variation—the difference

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[5] ⟨http://www.itis.gov⟩

[6] ⟨http://echinacea.umn.edu/⟩

PLATE 1. *Agapostemon virescens* collecting pollen from *Echinacea angustifolia*. Photo credit: Gretel Kiefer.

between being visited or not during 20 minutes. We used backward elimination to select generalized linear models with a binomial response and used explanatory variables observation day and a spatial measure at either the population or individual level. We note that the main conclusions are the same whether the response is modeled as Poisson or binomial. Quantitative results are shown graphically as logistic regression lines, one line for each observation day, with the slope revealing the spatial effect. We picked distance to the 3rd nearest neighbor ($k = 3$) to show in figures. We report qualitative results for individual isolation measures of distances to all kth nearest neighbors $1 < k < 15$.

We conducted linear regression analyses to investigate relationships between measures of annual reproduction (seed set and index of pollen limitation) and isolation and population size. Our index of pollen limitation was not well distributed because it had many 0 and 1 values, indicating no style persistence or maximal style persistence, respectively. All the reported relationships were qualitatively the same even if 0's, 1's, or both 0's and 1's were excluded.

We related bee visitation per plant to both measures of annual reproduction. In each year some plants, particularly those in small populations, were observed on more than one day. To account for differing number of days we observed each plant, we classified bee visitation per plant per season into three categories: plants that had no bee visitors on any day observed, plants with a bee on at least one, but not all, days observed, and plants with a bee visit on every day. Pollen limitation per plant per season was divided into three categories: persistent styles not present on any day observed (no evidence of pollen limitation), style persistence observed some days, style persistence observed on all days. Because both variables were categorical, we used a contingency table analysis to relate bee visitation to pollen limitation. We used an analysis of variance to relate bee visitation to seed set. If plant reproduction were pollinator limited, then plants in categories with increasing bee visitation would be expected to have higher mean seed set in 2004. Also, such plants would be expected to be associated with fewer days of persistent style rows present (i.e., less pollen limitation) in 2005. All analyses were conducted using R (R Development Core Team 2008).

To assess the extent to which bee community composition is related to plant population size and reproduction, we performed ordination of bee visitors per year per population using nonmetric multidimensional scaling (Kruskal 1964). We used the metaMDS function with default settings in the vegan package (Oksanen et al. 2008) with R. We performed analyses with all bee taxa included, with specimens not identified to species excluded, and with rare species excluded. We tested for relationships between key population variables (number of flowering plants, mean bee visitation, mean seed set, and mean index of pollen limitation) and community composition using function envfit in the vegan package and using linear regressions of ordinations and key variables. We also tested for relationships

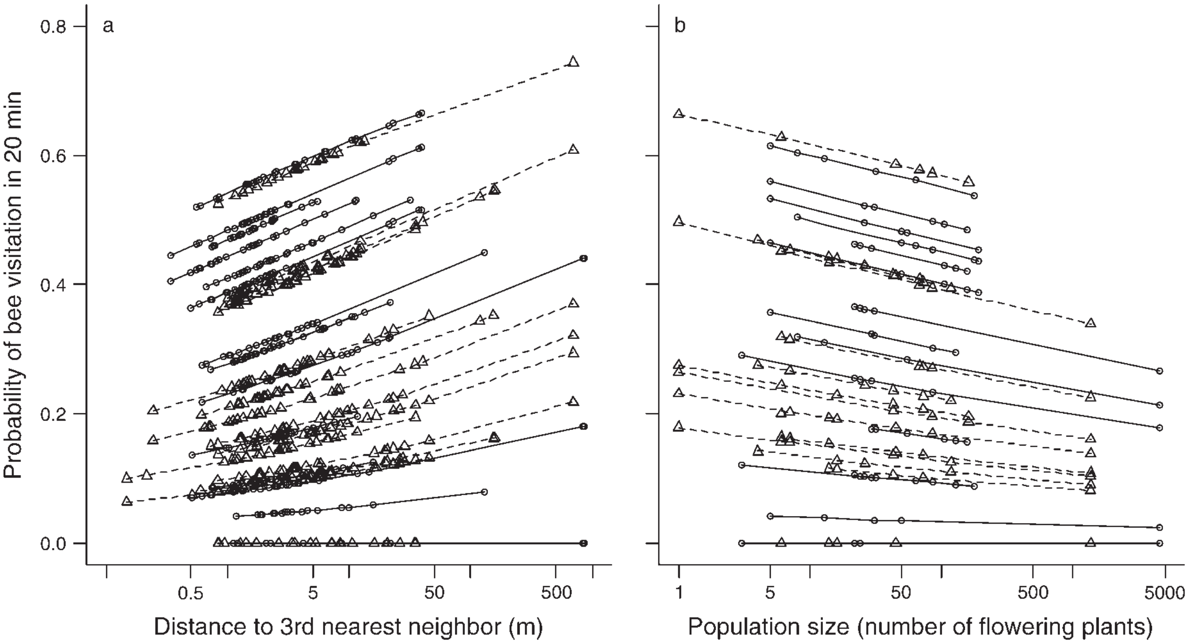

FIG. 1. Probability of bee visitation in a 20-min interval in relation to (a) a plant's isolation from flowering neighbors and to (b) a plant's population size. The logistic-regression lines are solid with circles for days in 2004 and dashed with triangles for days in 2005. Circles and triangles represent either (a) individual plants or (b) populations. Graphs show predicted values based on a generalized linear model with a binomial response and two explanatory variables: observation day and either isolation or population size (Appendix B). The effect of day is significant in both the population- and individual-based models, but there is no evidence that the spatial effect varies among days (Appendix B). The population size effect on bee visitation is not significant ($P = 0.14$). The trend of greater visitation in more isolated plants is consistent for all kth nearest-neighbors, $k = 1$–15, with marginal significance for nearest neighbors, $k = 2$, 4–8, and 13–15 ($0.05 < P \leq 0.17$), and significance for nearest-neighbors, $k = 3$, 9–12 ($P < 0.05$). When the distance to the kth nearest-neighbor was unknown, plants were excluded from the analysis (individual-based analysis, n ranged from 729 observations for $k = 1$ to 711 observations for $k = 15$; population-based analysis, $n = 164$ day-population observations).

between indices of bee diversity (Shannon-Weaver, Simpson, inverse Simpson, Pielou's evenness, and species richness) and key population variables.

RESULTS

We observed at least 26 species of native bees visiting *Echinacea* (Appendix A). Almost all species exhibited behavior that would lead to pollination, e.g., collecting pollen, contacting anthers, contacting styles. All of these species are known to visit species other than *Echinacea* (Mitchell 1960), though *Andrena rudbeckiae* is likely a composite specialist. We noted several bees flying from flowers of other plants or arriving with non-*Echinacea* pollen. *Augochlorella aurata* was the most common bee visitor to *Echinacea*, comprising 34% of bee visits (Appendix A). Based on abundance and behavior, *A. aurata* is likely an important pollinator of *Echinacea* in our study area. *Agapostemon virescens* was another potentially important pollinator, based on behavior and pollen loads (see Plate 1). The abundances of certain visitor taxa varied substantially between years: *Ceratina calcarata*/*dupla* and *Lasioglossum* spp. (at least nine species) were much more common in 2004 than 2005.

There was no evidence that bee visitation increased with population size or with the abundance of flowering *Echinacea* plants surrounding an individual plant (Fig. 1; Appendix B); rather, the trend was in the opposite direction. Bee visitation rates did vary considerably from day to day: for mid-sized populations or moderately isolated plants, model predictions of daily visitation ranged from 0% to about 60% (Fig. 1). Models with an interaction term including observation day and either isolation or population size were not significantly better than models lacking the interaction term, meaning that there was no evidence that the spatial effect varied among days (Appendix B). Thus the slopes of the logistic-regression lines within each panel of Fig. 1 are the same (on a logit scale). Greater individual isolation was associated with higher visitation rates for distances to all kth nearest neighbors, $k = 1$–15 ($n = 729$ observations for $k = 1$ decreasing to $n = 711$ observations for $k = 15$), but the effect was not significant for nearest neighbors, $k = 2$, 4–8, and 13–15 ($0.05 < P \leq 0.17$). For individual isolation with $k = 3$, as shown in Fig. 1a, the probability of bee visitation in 20 minutes on a day with moderate visitation is predicted to vary from 22% to 44% for the least and most isolated plants, respectively, according to the additive generalized linear model (Appendix B). Population size did not predict bee visitation ($P = 0.14$, $n = 34$ population sizes, df = 1;

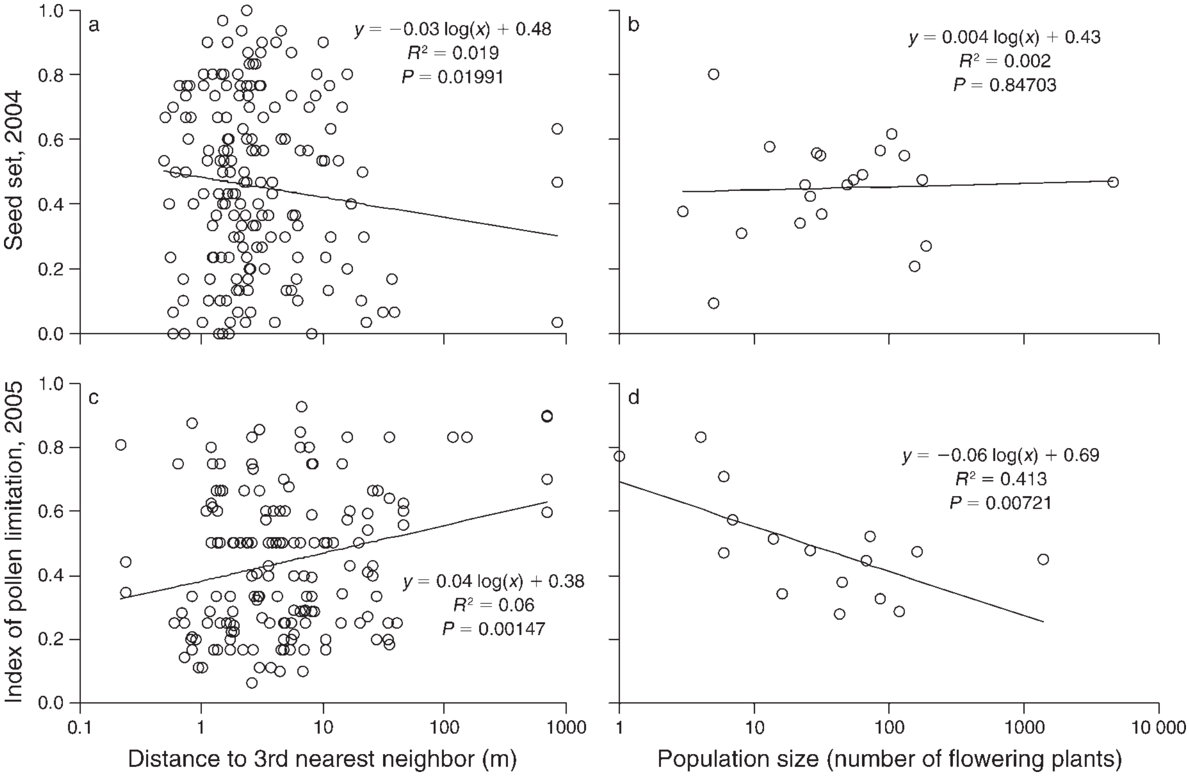

FIG. 2. The relationship between two measures of annual reproduction and two measures of fragmentation. Population size is the count of flowering plants in 21 populations in 2004 and 16 populations in 2005. Isolation is measured as the distance to the 3rd nearest flowering plant. In 2004 (a) seed set decreased consistently with individual plant isolation for all kth nearest neighbors, k = 1–15, but significantly so only for $k = 3$ and 4, and (b) seed set was not significantly related to population size. In 2005 the index of pollen limitation, estimated during the flowering season by the proportion of persistent receptive style rows, (c) increased significantly with individual plant isolation for all kth nearest neighbors, k = 1–15, and (d) decreased significantly with population size (2004, $n = 283$ plants, 21 populations; 2005, $n = 312$ plants, 16 populations).

Fig. 1b), but the trend was decreasing visitation with population size. Only the effect of observation day on visitation was significant for the population-based generalized linear model (Appendix B).

Spatial patterns of reproductive failure were evident in the pollen limitation observed during 2005 at both spatial scales and in the seed set estimated during 2004 at only the individual scale (Fig. 2). These patterns are similar to those observed in the study area in 1996–1998 (Wagenius 2006). The index of pollen limitation in 2005 had a strong spatial pattern as revealed in both the population- and individual-based analyses (Fig. 2c, d). The index of pollen limitation decreased significantly with population size and increased significantly with individual plant isolation for all kth nearest neighbors, k = 1–15 ($n = 312$ plants). Qualitatively similar results emerged if pollen limitation indexes of 0, 1, or both 0 and 1 were excluded from the analysis. The index of pollen limitation as predicted by linear regressions ranged from ~30% for the largest populations and the least isolated plants to ~70% for the smallest populations and the most isolated plants. In 2004 seed set was not significantly related to population size according to a linear regression analysis (Fig. 2b). Seed set decreased consistently with individual plant isolation for all kth nearest neighbors, k = 1–15, but significantly so only for $k = 3$ and 4. For $k = 3$, seed set dropped from 50% in the least isolated plants to 30% in the most isolated plants ($n = 283$ plants) (Fig. 2a).

Bee visitation per plant per season was not associated with high seed set in 2004 or low pollen limitation in 2005 (Appendix C). In contrast to expectations, high bee visitation per plant was associated with greater evidence of pollen limitation in 2005 (Fisher exact test of 3 × 3 contingency table, $n = 159$ plants, $P = 0.013$). In 2004 there was no association between bee visitation per plant per season and seed set (ANOVA, $F_{2,176} = 0.0004$, $P = 0.99$). There is little evidence that the bee community varied consistently with population size or any measure of population mean reproduction. We found no significant correlation between any key population variable (size, mean bee visitation, mean seed set, and mean index of pollen limitation) and any ordination of bee community composition (all $P > 0.13$). Indices of bee species diversity were not strongly or consistently related to any key population variable. In 2005, all diversity indices increased with population size but not significantly so (all $P > 0.06$). Also, in 2004, mean bee visitation increased significantly ($P < 0.05$) with bee species richness and Shannon-Weaver diversity. Neither pollen limitation or seed set was related to any diversity index (all $P > 0.4$).

Discussion

We tested the hypothesis that pollen limitation of plant reproduction in isolated *Echinacea* plants is caused by reduced pollinator visitation. Because spatial patterns of reduced seed set differ depending on whether they are examined at the level of populations or individuals (Wagenius 2006), we investigated pollinator visitation at those two levels. During two flowering seasons we observed many species of generalist bees visiting *Echinacea*. If low bee visitation caused pollen limitation, then two results were expected: (1) Bee visitation would be higher in plants that had greater reproduction, which we quantified as seed set in 2004 and via pollen limitation in 2005. (2) Bee visitation would increase with population size and decrease with plant isolation. We did not reject the null hypothesis in tests of these expectations. In fact, we found the opposite trends in most cases.

Pollen limitation vs. pollinator limitation

Pollen limitation is often interpreted as evidence for insufficient visitation by pollinators (pollinator limitation of reproduction). There are studies that show such a relationship between pollinator visitation and reproduction (Jennersten 1988, Waites and Ågren 2004). Our present study excludes the hypothesis that reproduction in *Echinacea* is pollinator limited, while reaffirming that it is pollen limited. We offer little explanation for the surprising findings that bee visitation is higher among isolated and among pollen-limited plants, except to note that they may be separate phenomena with distinct confounding variables. Instead, we focus on hypothesized causes of pollen limitation that are consistent with the findings of this study. Many alternative causes of pollen limitation have been hypothesized and documented (e.g., Knight et al. 2005). We discuss four of them.

First, bees may move a sufficient quantity of pollen, but the pollen may be incompatible. In self-incompatible plants, like *Echinacea*, pollen does not fertilize ovules with which it shares an S-allele, thus some pairs of plants are incompatible as mates (Byers 1995). Wagenius et al. (2007) investigated effects of population size on mate compatibility in 20 *Echinacea* remnants, including some in this study. They found that predicted population mean mate compatibility between pairs of nearby plants increased with population size from 62% to 92%, with a minimum mate compatibility of 25% in a small remnant. In light of the current findings about bee visitation, mate incompatibility among nearby potential mates remains a plausible explanation of the observed spatial patterns of pollen limitation.

Second, some bee species may not move and deposit a sufficient quantity of pollen. Variation in pollination effectiveness surely exists among species in terms of pollen load sizes, amounts of pollen deposited, and the number of anthers contacted per visit (Motten et al. 1981, Thomson and Thomson 1992, Ivey et al. 2003). We did not quantify any component of pollination effectiveness in this study. We found no evidence that pollinator community composition correlates with pollen receipt and reproductive success in *Echinacea* in this study, but without information on pollination effectiveness of the bee taxa, this is an imprecise test of variation in community-wide pollination effectiveness. To test the hypothesis that reproduction in *Echinacea* is limited by effective pollinators, we should quantify pollination effectiveness of *Echinacea* pollinators and determine the extent to which effective taxa visit non-isolated plants.

Third, even if *Echinacea*-pollinating species do not differ in inherent pollination effectiveness or in their visitation to isolated plants, their realized effectiveness may vary with local conditions (Herrera 2005). In particular, the identity and density of local co-flowering plants often influences pollinator foraging behavior and pollen movement, i.e., competition for pollination (Mitchell et al. 2009). Low density of co-flowering plants has been called "population purity" (Kunin 1997*a*, Ghazoul 2005). Kunin (1993) experimentally demonstrated that seed set in *Brassica kaber* plants decreased with isolation, and the effect was exacerbated when congeneric plants flowered nearby. The pollinators exhibited low fidelity to *B. kaber* when other species were flowering nearby. We did not quantify the fidelity of *Echinacea* visitors to *Echinacea* plants or the density of co-flowering plants, but we have observed bees flying to *Echinacea* plants with white pollen loads (*Echinacea* pollen is yellow) and flying between *Echinacea* and non-*Echinacea* flowers, such as those of *Coreopsis palmata*, *Cirsium flodmanii*, *Melilotus officinalis*, and *Carduus acanthoides*. Reduced seed set in isolated *Echinacea* plants may result from reduced deposition of *Echinacea* pollen per pollinator visit due to decreased *Echinacea* fidelity per pollinator flight. This hypothesis warrants further investigation.

Fourth, flowering phenology in *Echinacea* is not entirely synchronous within populations and early or late-flowering plants may be more pollen limited. We avoided off-peak observations by observing when at least 25% of that year's reproductive individuals at that population were flowering. However, our measure of isolation may not capture the effective isolation experienced by a plant. For example, consider a plant in the middle of a patch with many nearby flowering neighbors. Such a plant would have low isolation with our measure. But if that plant flowered later than its neighbors, then its actual isolation while flowering would be high. Pollinator visitation rates and plant reproduction may increase with flowering synchrony

(Gross and Werner 1983). Current investigations in experimental *Echinacea* plots shows that reproduction increases with flowering synchrony, but we have not yet quantified the relationship between synchrony and bee visitation.

All four of these hypothesized causes of pollen limitation may influence reproduction in *Echinacea* and contribute to the observed spatial patterns of reproductive failure and bee visitation. There is compelling evidence for the first mechanism (Wagenius et al. 2007), experimental evidence for the fourth (J. Ison and S. Wagenius, *unpublished data*), and we plan to investigate the others. Investigating causes of pollen limitation is difficult because fragmentation simultaneously affects many ecological and evolutionary processes, thereby directly and indirectly influencing bee communities, local abundance of flowering plants, flowering phenology, and mate availability (Ghazoul 2005). In spite of these challenges, the effort to elucidate the mechanisms of pollen limitation in fragmented habitat, and elsewhere, is warranted (Aizen et al. 2002).

It is important to identify causes of pollen limitation (Ashman et al. 2004, Ghazoul 2005). How one interprets the evidence of widespread pollen limitation and what one infers about its causes bears directly on current debates about evolution of floral traits, ecology of pollination, and conservation priorities for plants and pollinators. For example, there is controversy about whether widely observed pollen limitation reflects an equilibrium condition in most plants or circumstances related to environmental change (Thomson 2001, Ashman et al. 2004). Knowledge about the recent and enormous impacts of habitat fragmentation in the previously vast and interconnected prairie and great plains (Samson and Knopf 1994), combined with our consistent evidence of a population size effect on pollen limitation in *Echinacea*, suggests that we are observing disequilibrial dynamics resulting from habitat fragmentation, and thus equilibrial predictions may be inappropriate (e.g., Haig and Westoby 1988). Additionally, as habitat fragmentation induces pollen limitation, new sets of traits may confer increased seed set and thus be under selection. *Echinacea* examples may include attractiveness compared to co-flowering species, synchronous flowering time, recessive S-alleles, or alleles that promote self-compatibility. Furthermore, identifying actual causes of pollen limitation is critical to developing appropriate conservation strategies for pollen-limited plants in fragmented habitat. Conservation strategies may include such diverse and potentially conflicting goals as maintaining bee nest sites, managing co-flowering species, managing effective population size to maintain S-allele diversity, and increasing density and synchrony of flowering plants (Glémin et al. 2008). Reconciling the diverse studies that investigate pollen limitation to infer broad patterns about its causes will prove a challenge because most studies to date focus on identifying or quantifying pollen limitation and do not distinguish causes.

Pollen limitation decreases with local abundance of flowering plants in many species, but some may not be pollinator limited (Knight et al. 2005). In a review of plant reproduction in fragmented habitat Ghazoul (2005), identified 22 species for which pollinator visitation was modeled as a function of local abundance of flowering plants. Only one of these studies found a negative relationship (in one of three years studied) and reproduction was not related to population size (Alexandersson and Ågren 1996). Similar decreases of pollinators with population size in non-pollen-limited plants have been documented (Yates and Ladd 2005, Campbell and Husband 2007, Lopes and Buzato 2007). Other studies have observed reproductive declines with population size but a weak or positive relationship with pollinators (Bosch and Waser 1999, Vázquez and Simberloff 2004). No study besides ours has found pollinator visitation and pollen limitation decreasing with local abundance of flowering plants.

Few studies are available that directly examine the effects of two or more ecological conditions on the magnitude of pollen limitation. We echo Bosch and Waser's (1999) and Knight et al.'s (2005) calls for ecologists and conservation biologists to explore the role of pollinator visitation and pollen limitation together in the context of habitat fragmentation, so that we may clearly identify causes of pollen limitation and begin to make generalizations about the consequences of pollen limitation for evolutionary and ecological processes.

Inference of effects of density on pollen limitation depends on spatial scale

We characterized both population size and individual plant isolation in our study of pollinator visitation because we previously observed different patterns of pollen limitation at each spatial scale. In the previous study (Wagenius 2006) pollen limitation, seed set, and fecundity were strongly related to individual plant isolation and less strongly related to population size. Investigating pollinator visitation at multiple scales is warranted because studies have found that pollinators respond to variation in floral density at a variety of spatial scales (reviewed in Caruso [2002]). Additionally, pollinator limitation could vary among spatial scales for reasons other than the distribution of floral resources (Steffan-Dewenter et al. 2002). For example, if bee nests are distributed independently of *Echinacea* habitat remnants, then the plants closest to nests are likely to be isolated plants in small populations or on the edge of large populations. Thus isolated plants might get more visits solely due to their proximity to nests.

Our results reveal that patterns of pollinator visitation do vary among spatial scales, but the effect is in the same direction—pollinator visitation decreased with increasing local abundance of flowering plants. At individual spatial scales, visitation usually increased significantly with isolation. At the population scale, the trend was not significant, but given that the individual and population trends are in consistent directions, our failure to detect a significant population size effect could result, in part, from insufficient statistical power. Similarly, patterns of pollination and reproduction varied among spatial scales. In both years of this study we observed reduced reproduction in isolated plants. This pattern is consistent with observations we have made in all four other years in which we have made such individual-based observations (Wagenius 2006; S. Wagenius, *unpublished data*). At the population scale, we have observed increasing reproduction with population size in all years except 2004. The differing results that emerge when investigating pollen limitation and pollinator limitation at the two spatial scales may reflect different underlying mechanisms operating at each scale. An alternate explanation is that one fine-scale mechanism operates and "scales up" to the population scale due to correlations of local abundance at different scales. The results of our present study provide insight into scale-dependent processes in the ecology, evolution, and conservation of *Echinacea,* a model prairie plant with pollen-limited reproduction.

Conclusion

We have demonstrated that reproduction in *Echinacea angustifolia* is pollen limited but not pollinator limited. The most isolated plants reproduced the least, yet they were visited the most frequently by pollinators. Importantly, individual-based analyses were required to detect these contrasting relationships. If we had focused only on the population-level spatial scale, we would have failed to detect fine-scale patterns and would have drawn misleading conclusions. No studies we know of have found plant abundance negatively associated with insect visitation and positively associated with reproduction, yet such investigations are scarce, and this pattern may be prevalent among self-incompatible plants with generalist pollinators.

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LITERATURE CITED

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

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

Aizen, M. A., and L. D. Harder. 2007. Expanding the limits of the pollen-limitation concept: effects of pollen quantity and quality. Ecology 88:271–281.

Alexandersson, R., and J. Ågren. 1996. Population size, pollinator visitation and fruit production in the deceptive orchid *Calypso bulbosa*. Oecologia 107:533–540.

Allison, T. D. 1990. Pollen production and plant density affect pollination and seed production in *Taxus canadensis*. Ecology 71:516–522.

Ashman, T.-L., T. M. Knight, J. A. Steets, P. Amarasekare, M. Burd, D. R. Campbell, M. R. Dudash, M. O. Johnston, S. J. Mazer, R. J. Mitchell, M. T. Morgan, and W. G. Wilson. 2004. Pollen limitation of plant reproduction: ecological and evolutionary causes and consequences. Ecology 85:2408–2421.

Ashworth, L., R. Aguilar, L. Galetto, and M. A. Aizen. 2004. Why do pollination generalist and specialist plant species show similar reproductive susceptibility to habitat fragmentation? Journal of Ecology 92:717–719.

Bosch, M., and N. M. Waser. 1999. Effects of local density on pollination and reproduction in *Delphinium nuttallianum* and *Aconitum columbianum* (Ranunculaceae). American Journal of Botany 86:871–879.

Burd, M. 1994. Bateman's principle and plant reproduction: the role of pollen limitation in fruit and seed set. Botanical Review 60:83–139.

Byers, D. L. 1995. Pollen quantity and quality as explanations for low seed set in small populations exemplified by *Eupatorium* (Asteraceae). American Journal of Botany 82: 1000–1006.

Byers, D. L., and T. R. Meagher. 1992. Mate availability in small populations of plant species with homomorphic sporophytic self-incompatibility. Heredity 68:353–359.

Campbell, L. G., and B. C. Husband. 2007. Small populations are mate-poor but pollinator-rich in a rare, self-incompatible plant, *Hymenoxys herbacea* (Asteraceae). New Phytologist 174:915–925.

Cane, J. H. 2001. Habitat fragmentation and native bees: a premature verdict? Conservation Ecology 5(1):3 [online]. ⟨http://www.consecol.org/vol5/iss1/art3/⟩

Caruso, C. M. 2002. Influence of plant abundance on pollination and selection on floral traits of *Ipomopsis aggregata*. Ecology 83:241–254.

Droege, S., S. Kolski, J. Ascher, and J. Pickering. 2008. Apoideae *in* Discover Life website. ⟨http://www.discoverlife.org/mp/20q?search=Apoidea⟩

Ghazoul, J. 2005. Pollen and seed dispersal among dispersed plants. Biological Reviews 80:413–443.

Glémin, S., C. Petit, S. Maurice, and A. Mignot. 2008. Consequences of low mate availability in the rare self-incompatible species *Brassica insularis*. Conservation Biology 22:216–221.

Gross, R. S., and P. A. Werner. 1983. Relationships among flowering phenology, insect visitors, and seed-set of individuals: experimental studies on four co-occurring species of goldenrod (*Solidago*: Compositae). Ecological Monographs 53:95–117.

Haig, D., and M. Westoby. 1988. On limits to seed production. American Naturalist 131:757–759.

Herrera, C. M. 2005. Plant generalization on pollinators: species property or local phenomenon? American Journal of Botany 92:13–20.

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.

Jennersten, O. 1988. Pollination in *Dianthus deltoides* (Caryophyllaceae): effects of habitat fragmentation on visitation and seed set. Conservation Biology 2:359–366.

Kearns, C. A., D. W. Inouye, and N. M. Waser. 1998. Endangered mutualisms: the conservation of plant–pollinator interactions. Annual Review of Ecology and Systematics 29:83–112.

Knight, T. M., J. A. Steets, J. C. Vamosi, S. J. Mazer, M. Burd, D. R. Campbell, M. R. Dudash, M. O. Johnston, R. J. Mitchell, and T.-L. Ashman. 2005. Pollen limitation of plant reproduction: pattern and process. Annual Review of Ecology, Evolution, and Systematics 36:467–497.

Kruskal, J. B. 1964. Nonmetric multidimensional scaling: a numerical method. Psychometrika 29:115–129.

Kunin, W. E. 1993. Sex and the single mustard: population density and pollinator behavior effects on seed-set. Ecology 74:2145–2160.

Kunin, W. E. 1997*a*. Population biology and rarity: on the complexity of density dependence in insect–plant interactions. Pages 150–173 *in* W. E. Kunin and K. J. Gaston, editors. The biology of rarity. Chapman and Hall, London, UK.

Kunin, W. E. 1997*b*. Population size and density effects in pollination: pollinator foraging and plant reproductive success in experimental arrays of *Brassica kaber*. Journal of Ecology 85:225–234.

Larson, B. M. H., and S. C. H. Barrett. 2000. A comparative analysis of pollen limitation in flowering plants. Biological Journal of the Linnean Society 69:503–520.

Leuszler, H. K., V. J. Tepedino, and D. G. Alston. 1996. Reproductive biology of purple coneflower in southwestern North Dakota. Prairie Naturalist 28:91–102.

Levin, S. A. 1992. The problem of pattern and scale in ecology. Ecology 73:1943–1967.

Lopes, L. E., and S. Buzato. 2007. Variation in pollinator assemblages in a fragmented landscape and its effects on reproductive stages of a self-incompatible treelet, *Psychotria suterella* (Rubiaceae). Oecologia 154:305–314.

Michener, C. D., R. J. McGinley, and B. N. Danforth. 1994. The bee genera of North and Central America (Hymenoptera: Apoidea). Smithsonian Institution Press, Washington, D.C., USA.

Mitchell, R. J., R. J. Flanagan, B. J. Brown, N. M. Waser, and J. D. Karron. 2009. New frontiers in competition for pollination. Annals of Botany 103:1403–1413.

Mitchell, T. B. 1960. Bees of the Eastern United States. North Carolina Agricultural Experiment Station Technical Bulletin number 141.

Motten, A. F., D. R. Campbell, D. E. Alexander, and H. L. Miller. 1981. Pollination effectiveness of specialist and generalist visitors to a North Carolina population of *Claytonia virginica*. Ecology 62:1278–1287.

Oksanen, J., R. Kindt, P. Legendre, B. O'Hara, G. L. Simpson, and M. H. H. Stevens. 2008. Vegan: community ecology package. R package version 1.15-0. ⟨http://vegan.r-forge.r-project.org⟩

R Development Core Team. 2008. R: A language and environment for statistical computing. Version 2.8.0. R Foundation for Statistical Computing, Vienna, Austria. ⟨http://www.R-project.org⟩

Rathcke, B. J., and E. S. Jules. 1993. Habitat fragmentation and plant–pollinator interactions. Current Science 65:273–277.

Roll, J., R. J. Mitchell, R. J. Cabin, and D. L. Marshall. 1997. Reproductive success increases with local density of conspecifics in a desert mustard (*Lesquerella fendleri*). Conservation Biology 11:738–746.

Samson, F. B., and F. L. Knopf. 1994. Prairie conservation in North America. Bioscience 44:418–421.

Sih, A., and M.-S. Baltus. 1987. Patch size, pollinator behavior, and pollinator limitation in catnip. Ecology 68:1679–1690.

Steffan-Dewenter, I., U. Münzenberg, C. Buerger, C. Thies, and T. Tscharntke. 2002. Scale-dependent effects of landscape context on three pollinator guilds. Ecology 83:1421–1432.

Steven, J. C., T. P. Rooney, O. D. Boyle, and D. M. Waller. 2003. Density-dependent pollinator visitation and self-incompatibility in upper Great Lakes populations of *Trillium grandiflorum*. Journal of the Torrey Botanical Society 130: 23–29.

Thomson, J. D. 2001. Using pollination deficits to infer pollinator declines: Can theory guide us? Conservation Ecology 5(1):6 [online]. ⟨http://www.consecol.org/vol5/iss1/art6⟩

Thomson, J. D., and B. A. Thomson. 1992. Pollen presentation and viability schedules in animal-pollinated plants: consequences for reproductive success. Pages 1–24 *in* R. Wyatt, editor. Ecology and evolution of plant reproduction: New approaches. Chapman and Hall, New York, New York, USA.

Vázquez, D. P., and D. Simberloff. 2004. Indirect effects of an introduced ungulate on pollination and plant reproduction. Ecological Monographs 74:281–308.

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.

Waites, A. R., and J. Ågren. 2004. Pollinator visitation, stigmatic pollen loads and among-population variation in seed set in *Lythrum salicaria*. Journal of Ecology 92:512–526.

Waser, N. M., L. Chittka, M. V. Price, N. M. Willians, and J. Ollerton. 1996. Generalization in pollination systems, and why it matters. Ecology 77:1043–1060.

Yates, C. J., and P. G. Ladd. 2005. Relative importance of reproductive biology and establishment ecology for persistence of a rare shrub in a fragmented landscape. Conservation Biology 19:239–249.

APPENDIX A

A table of pollinating bee species observed on *Echinacea angustifolia* heads (*Ecological Archives* E091-053-A1).

APPENDIX B

Results of analysis of deviance for a generalized linear model with binomial response (*Ecological Archives* E091-053-A2).

APPENDIX C

A table and figure showing the relationship between bee visitation per plant per season and two measures of plant reproduction (*Ecological Archives* E091-053-A3).