Assessing the Effects of Co-Flowering Species on Pollinator Behavior Towards *Echinacea angustifolia*

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Abstract

Fragmentation of prairies creates smaller populations and reduced gene pools. Plants that are self-incompatible face even lower reproductive fitness because they cannot mate with close relatives. *Echinacea angustifolia* is a self incompatible, long-lived perennial native to North American prairies and is pollinated by generalist, solitary bees. Pollinator behavior toward *Echinacea* could potentially limit its reproductive fitness further. This study aims to better understand pollinator constancy towards *Echinacea* and assess heterospecific pollen loads throughout the flowering season in order to understand whether *Echinacea* will persist in a fragmented landscape.

Introduction & Background

Echinacea angustifolia (hereafter *Echinacea*) is an herbaceous perennial native to North American prairies. Found primarily in the eastern and central U.S. and southern Canada, *Echinacea* is a member of the Asteraceae family, known for their head units that are comprised of multiple, small florets. *Echinacea* exhibits self-incompatibility and is pollinated by many generalists. These traits make *Echinacea* an excellent study species to understand the survival of many plant species within endangered prairie systems. *Echinacea* also experiences pollen limitation. Pollen limitation occurs when there is a deficiency in the amount of pollen that fertilizes ovules on a plant such that the plant does not produce a full seed set, thus lowering the plant's reproductive fitness. Pollen can be limited during any of the steps of reproduction. Plants can release a low amount of pollen, pollen transportation can be disrupted or prevented, pollen can be poorly deposited, or failure in germination or fertilization can occur (Wagenius, 2004). Pollen limitation can be a result of many factors, including self-incompatibility, which is seen in *Echinacea*, and competition for pollination (Byers and Meagher, 1992; Ison et al., 2014). It has been shown that *Echinacea* is pollen limited but not because of a limited density of pollinators (Wagenius and Lyon, 2010). There is another factor causing *Echinacea's* pollen limitation other than the density of pollinators, but the exact factor is not entirely understood. One hypothesis is that *Echinacea* is pollen limited because of the limited number of compatible mates because *Echinacea* is self incompatible, it cannot reproduce with relatives that share the S allele. Any reduction to an individual's reproductive fitness can have drastic effects on the total population size in a fragmented ecosystem. Thus, it is important to understand what is limiting *Echinacea's* reproduction.

It has been shown that *Echinacea* is not pollinator limited; in fact, *Echinacea* is pollinated by 26 species of primarily solitary bees. A significant pollinator of *Echinacea* is *Andrena rudbeckiae*, which is a potential composite specialist (Wagenius and Lyon, 2010). Specialist pollinators are dependent on very few plants, sometimes only one species of plant, as a food source, as opposed to generalist pollinators which are capable of using many plant species as food sources (Ashworth et al., 2004). Specialist pollinators are often higher quality pollinators because plants do not have to compete for visitation and pollen is not contaminated with heterospecific pollen, or lost on a heterospecific style. *Augochlorella aurata* is one of the more common visitors of *Echinacea* and is a generalist pollinator (Wagenius and Lyon, 2010).

Echinacea is visited by an array of pollinators and is not reproductively limited by their density or visitation rate, but it could be limited by pollinator foraging behavior.

Pollinators can often have preferences over where they tend to forage. When a pollinator prefers one species over another this can reduce the number of pollinator visits to the less preferred species (Campbell and Motten, 1985). Because *Echinacea* is pollinated by generalist pollinators that visit several plant species *Echinacea* pollen could be travelling to heterospecific stigmas. Interspecific pollen movement could be causing the pollen limitation, in addition to asynchronous flowering. Interspecific pollen movement is when pollen moves from one plant to a plant of a different species and is either lost or becomes contaminated and incapable of fertilizing the original species (Campbell and Motten, 1985). Competition for pollination often occurs when species are phenotypically similar and flower synchronically. It is therefore important to understand whether co-flowering species exhibit similar phenotypes or overlapping flowering seasons, or if the flowering seasons are staggered as a mechanism to avoid competition (Sargent and Ackerly, 2008). Because *Echinacea* is pollen limited, but not pollinator limited, the next step in research is to study the pollinators' foraging behaviors and pollen load composition to understand what is happening to *Echinacea* pollen when it is moved. When pollinators switch between plant species, pollen can be lost on interspecific stigmas (Waser and Fugate, 1986). Plants can either benefit from neighboring species when pollinator visitation increases, or experience reduced fitness if the transfer of heterospecific pollen affects the quality or quantity of conspecific pollen (Sargent and Ackerly, 2008). In Le Petrin on the island of Mauritius, it was found that the declining, endemic plant, Trochetia blackburniana, had a higher reproductive success when located closer to dense patches of Pandanus plants. This was because Pandanus are a preferred microhabitat of the gecko Phelsuma cepediana that pollinates T. blackburnian (Hansen et al., 2007). This example of facilitation shows how plant-plant interactions can have positive indirect effects within an ecosystem. Plants may also suffer fitness declines due to pollinator facilitation if the pollen is contaminated or deposited on heterospecific plants rather than conspecifics. The intricacies of co-flowering plant interactions vary between species and better understanding the costs and benefits of such interactions can help to better explain population declines. It is crucial to understand how *Echinacea's* pollen is moving throughout the flowering season in order to better predict its ability to survive in fragmented landscapes. Understanding how co-flowering plants are interacting with each other during the flowering season as well as how plants and pollinators affect each other's behavior can help create conservation plans for endangered prairies.

Goals of research

The focus of this research is on the relationship between flowering communities and pollinator foraging behavior in order to better understand the reproductive fitness of *Echinacea* in fragmented landscapes. **Aim 1 will examine pollinator behavior in a community setting**. Pollinators will be observed throughout the flowering season to determine which plants they choose after an *Echinacea* visit. Pollen samples will be collected from pollinators and identified by species under a microscope. Pollen loads will be categorized based on amount of *Echinacea* pollen present, and presence of non-*Echinacea* pollen. This data will be evaluated temporally over the flowering season. This information will help better understand the quality of pollinators in the prairies, as it has been established that *Echinacea* is pollen limited rather than pollinator limited. **Aim 2 will examine the flowering times of co-flowering species over *Echinacea's* flowering season**. Ten random plots will be checked throughout the flowering season to determine what species are simultaneously flowering with *Echinacea* when pollinators are sampled. This data will be used to understand what flowers are available to pollinators as compared to the species found in pollen loads.

This research will help understand which pollinator taxa carry the most *Echinacea* pollen at different times of the flowering season and averaged across the entire summer. I am predicting that *Andrena* will carry the most *Echinacea* pollen out of all the taxon because it has been suggested to be a composite specialist and the most efficient *Echinacea* pollinator. I am also predicting that most taxa will carry more *Echinacea* pollen in the early flowering period because as *Echinacea* begins to flower it may provide pollinators with proteins not previously available from other plant species.

Research plan

Aim 1 will examine the pollinator constancy behavior towards *Echinacea* in a community context. Pollinator behavior and pollen load will be analyzed during the summer. Pollinator flower preference and pollen load composition will be compared to better understand the flower constancy of local pollinator taxa and the implications on *Echinacea's* reproductive fitness. This information will give better insight into the quality and effectiveness of pollinators within the prairies. It will also help explain why *Echinacea* is pollen limited but not pollinator limited; pollinators may be providing pollen to *Echinacea* but this pollen may no longer be viable if it has been mixed with heterospecific pollen too much, or lost on heterospecific stigmas.

Experiment #1 Pollinator behavioral observations. Pollinators will be observed throughout the flowering season to note which plant species pollinators land on after foraging *Echinacea*. Pollinators to be observed will be: *Agapostemon, Andrena, Augochlorella, Melissodes, Lasioglossum, Halictid.*

Expected Outcomes. I predict that *Andrena* will visit *Echinacea* most frequently because it has been suggested that *Andrena* could be a specialized pollinator of *Echinacea*, though this is still uncertain. I also predict *Augochlorella* will exhibit the least flower constancy toward *Echinacea* because it has been shown to be the least effective at fertilizing *Echinacea* out of the group. Its smaller body and lower effectiveness may result in decreased flower constancy and more generalist behavior in order to collect more pollen throughout the day.

Experiment #2 Obtaining and analyzing pollen loads. Pollinators will be caught within 20 seconds of landing on *Echinacea* plants over the course of the flowering season. Pollinators will be captured in Aanenson, Landfill, and East Elk Lake Road remnants on days not designated for observations. If pollinators cannot be captured within 20 seconds they will not be used for pollen analysis but can still be used for pollinator behavior observations. Pollinators to be captured will be off the same taxa observed in experiment #1, *Agapostemon, Andrena, Augochlorella, Melissodes, Lasioglossum, Halictid.* Pollinators captured will be cooled for one hour on ice and brought back to the lab. Pollinators will be wiped with a tooth pick to collect the pollen from body and scopa separately which will be mounted on a slide with fuchsin jelly (Adler and Irwin, 2006; Huang et al., 2015). Special slides with graphs printed on will be used. Up to 10 random squares will be randomly selected to analyze pollen loads. Selected squares containing pollen will first be identified as containing *Echinacea* pollen or not, and as containing heterospecific pollen or not. Squares will then be categorically counted as 0% (no *Echinacea* pollen present), 0-25%, 25%-50%, 100% (exclusively *Echinacea* pollen). I will identify heterospecific pollen species using my own pollen reference collection I will make throughout the summer and the

Diersen 2011 catalogue[1]. Data will be used to analyze the heterospecific pollen movement and rank pollinator taxa based on amount of *Echinacea* pollen carried (Herrera, 1987). A repeated 2-way ANOVA and analysis of binomial distribution will be run to compare which pollinator taxa carry the most *Echinacea* pollen, how heterospecific pollen loads change over their respective flowering windows, and how *Echinacea* pollen loads change within each pollinator family over the course of the flowering season.

Expected Outcomes. Based on preliminary data collected by the Echinacea team I expect *Andrena* to carry the highest percentage of *Echinacea* pollen (Ison, Wagenius unpublished). I also expect pollen loads of *Echinacea* to remain relatively high within *Andrena* than other pollinators regardless of time within flowering season.

Potential Problems and alternative approaches. Bee behavior is very dependent on weather and an extremely rainy summer could result in few pollinators. This can be fixed by collecting more samples on clear days to compensate for rainy days. It has also been suggested that pollinator type changes over the course of the flowering season (Ison, Wagenius unpublished). Therefore, I may have to adjust the pollinator taxa I study when I am in the field. If pollen is unidentifiable under a microscope due to bee saliva I will only use body pollen and exclude scopa pollen.

Aim 2 will examine the flowering times of co-flowering species over *Echinacea*'s flowering season. Preliminary data will be gathered to help analyze data collected in aim 1. This aim provides crucial background information for Aim 1 and the ecosystem in general. By understanding the flowering windows of heterospecifics[2] it can give better insight in pollinator behavior and the reproductive fitness of *Echinacea angustifolia* (here after *Echinacea*). This preliminary information can give insight into competition for successful pollination within the prairies and overall the reproductive fitness of *Echinacea*.

Experiment #1 Monitoring co-flowering densities.

In the beginning of the season I will set up 10 random 1x1 meter plots throughout my remnants (ANN, EELR, LF). Random *Echinacea* will be selected to act as the southwest corner of my plot. Plots will be assessed for heterospecific flowers within one day of collecting pollinators in order to capture the community accurately. I will record any species flowering in the plot and tally the total plots flowers were found in (example: 8 out of 10 plots may contain flowering alfalfa).

Expected Outcomes. I am expecting most plants to flower synchronously with *Echinacea* to create a plant-pollinator network rather than flowering asynchronously and potentially losing pollinators that visit heterospecifics (Sargent and Ackerly, 2008). However, if flowering windows vary extensively this could explain results in Aim 1.

Significance

Fragmentation of ecosystems is a major cause of extinction because it not only destroys habitats but also prevents gene flow by isolating populations into smaller groups. In 1994 the U.S. already had reported fifty-five grassland species as threatened or endangered, and land development of prairies has only increased since then (Samson and Knopf, 1994). Smaller

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[1] Diersen pollen library is found at http://pollen.wikispaces.com/

[2] Heterospecific is defined here as plants whose flowering windows overlap with *Echinacea angustifolia* but are not *Echinacea angustifolia.*

populations of plants are also at risk of pollen limitation, when an individual plant does not receive enough compatible pollen to fertilize all ovules available, thus lowering its seed set and reproductive fitness. Prairies are also home to many species of pollinator, a vital "life-support service" to both plants and humans. Pollination services have been estimated to be worth $112 billion, and up to $40 billion for agriculture (Carol A. Kearns et al., 1998). When fragments become small and more isolated from other fragments, pollinators may avoid them entirely. An excellent method to better understand the effects of fragmentation is to study a single species' survival and reproductive fitness within the ecosystem in order to extrapolate research for the bigger picture. Studying the reproductive success of *Echinacea* in fragmented prairies can bring a better understanding of the overall ecosystem and ultimately formulate better conservation plans.

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Timeline for the proposed research

Pollinators will be sampled 4 times throughout July, or flowering season, during early flowering, peak, late flowering, and the tail end of flowering. Co-flowering plots will be checked within one day of pollinator sampling. Pollen samples will be analyzed continuously throughout the season as time permits.

Data management plan

Data sheets will be created for pollinator catches, pollen loads, and co-flowering plots. Data on both pollen loads and co-flowering species will be collected; only pollen loads will be analyzed. When the last pollinator catch has been completed, plots will be removed from fragments.

Environmental impacts (of the proposed research)

Pollinators will be released the same day they are captured to ensure populations remain constant and their broods are not left unattended for too long. Walking through co-flowering plots will be avoided to maintain plants in that square. Paths around the plot may occur and will inadvertently mat down neighboring plants.