



# The effects of flowering phenology and spatial distances on gene dispersal in *Echinacea augustifolia*

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## Introduction

The 0.1% of pre-settlement prairie that still exists today has undergone great fragmentation. Species present in these fragments are especially prone to reduced fitness because the lack of gene flow can reduce genetic variation, causing populations to drift or become inbred. With the possibility of genetic isolation due to spatial distances determining how far pollen can travel, flowering phenology becomes more important for the success of greater gene dispersal in these specialized prairie species. The start time, duration, intensity, and synchrony of flowering of all plants within a population determine when, or even if, the individual plant will be pollinated. In order to better implement effective, efficient methods of genetic conservation to preserve genetic variation within these isolated, specialized ecosystems, understanding the flowering phenology and pollen movement trends of these prairie species are vital.

**Research objective:** Determining how gene dispersal is affected through flowering phenology and pollination distances by running a paternity analysis on a number of progeny of a model prairie plant.

## Study species: *Echinacea augustifolia*

(narrow-leaved purple coneflower)

- Model prairie species
- Self-incompatible
- No specialized seed dispersal



Fig. 1 Close-up of an *E. Augustifolia* head

## Materials & methods

### Study site:

- Common garden in western Minnesota
- Seeds collected from remnant populations (within 5 km)

### Experimental design:

- 224 flowering plants in 2005
- Followed daily flowering phenology
- Mapped and took tissue samples
- Collected seeds
- Chose 17 focal maternal plants
- Germinated 10-20 seeds of focal plants and took tissue samples of offspring
- Assigned paternity to all offspring of focal plants

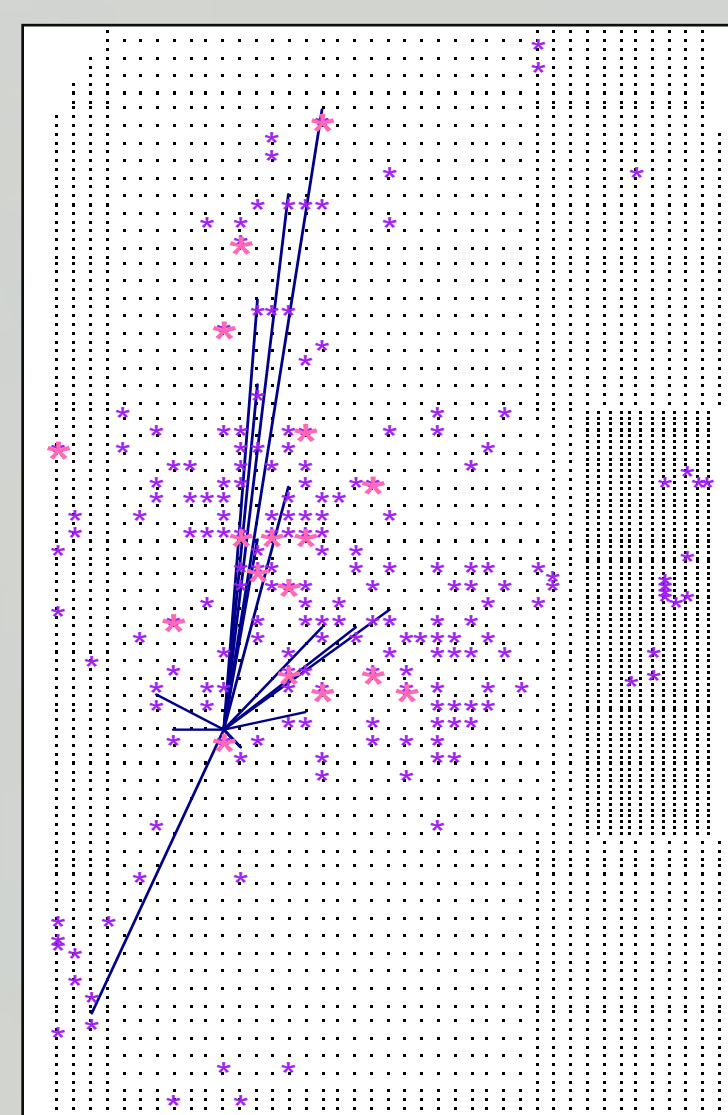


Fig. 2 Example of one focal mom paternity analysis of 2005 common garden. Purple dots: flowering plants. Pink dots: focal maternal plants

## Materials & methods (cont.)

### Assignment method:

- 8 microsatellites (5, 11, 13, 13Z, 15, 36, 37, 46) were developed for *E. augustifolia* using the protocol by Glenn and Schable (2003).
- Parental and offspring DNA were extracted using either the protocol by Chaundry et al. (1999) or with the Qiagen DNeasy Plant Mini Kit.
- Extracted DNA were run on PCR with fluorescently-labeled forward primers
- PCR results were ran on a gel
- Parentals and progeny were genotyped and analyzed on Beckman Coulter CEQ 8000 (9.0.25)
- Paternity was assigned using Cervus 3.0.3 (Kalinowski et al. 2007) with 85% strict and 70% relaxed confidence intervals
- All data were analyzed using R 2.6.0 (2007 CRAN)

## Data analysis



Fig. 3. Average pollen movement in meters between parental matches in an individual population on a given day, organized by when the maternal's styles became receptive. Last 8 days of flowering were grouped into 2 because of lack of data points to generate average values. (GLM,  $p=0.359$ )



Fig. 4. Average pollen movement in meters between parental for all pollinations. Date organized by when the maternal's styles became receptive. (GLM,  $p<0.01$ )

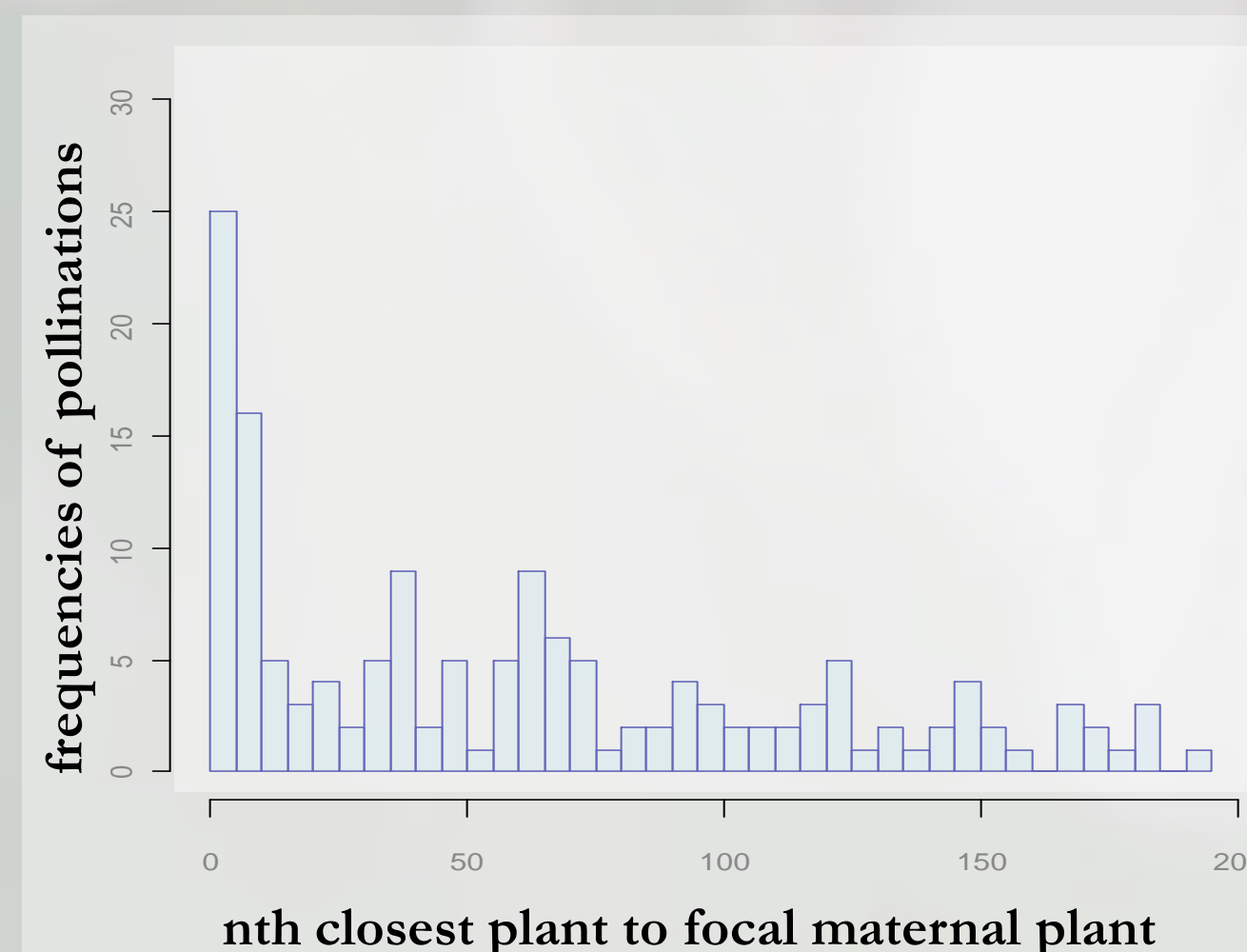


Fig. 5. Frequency of pollinations on focal maternal plants that were pollinated by the nth closest plant. Distances not taken into account. 25.8% of pollinations to focal maternal plants occurred from their 1st to 10th closest neighbors.

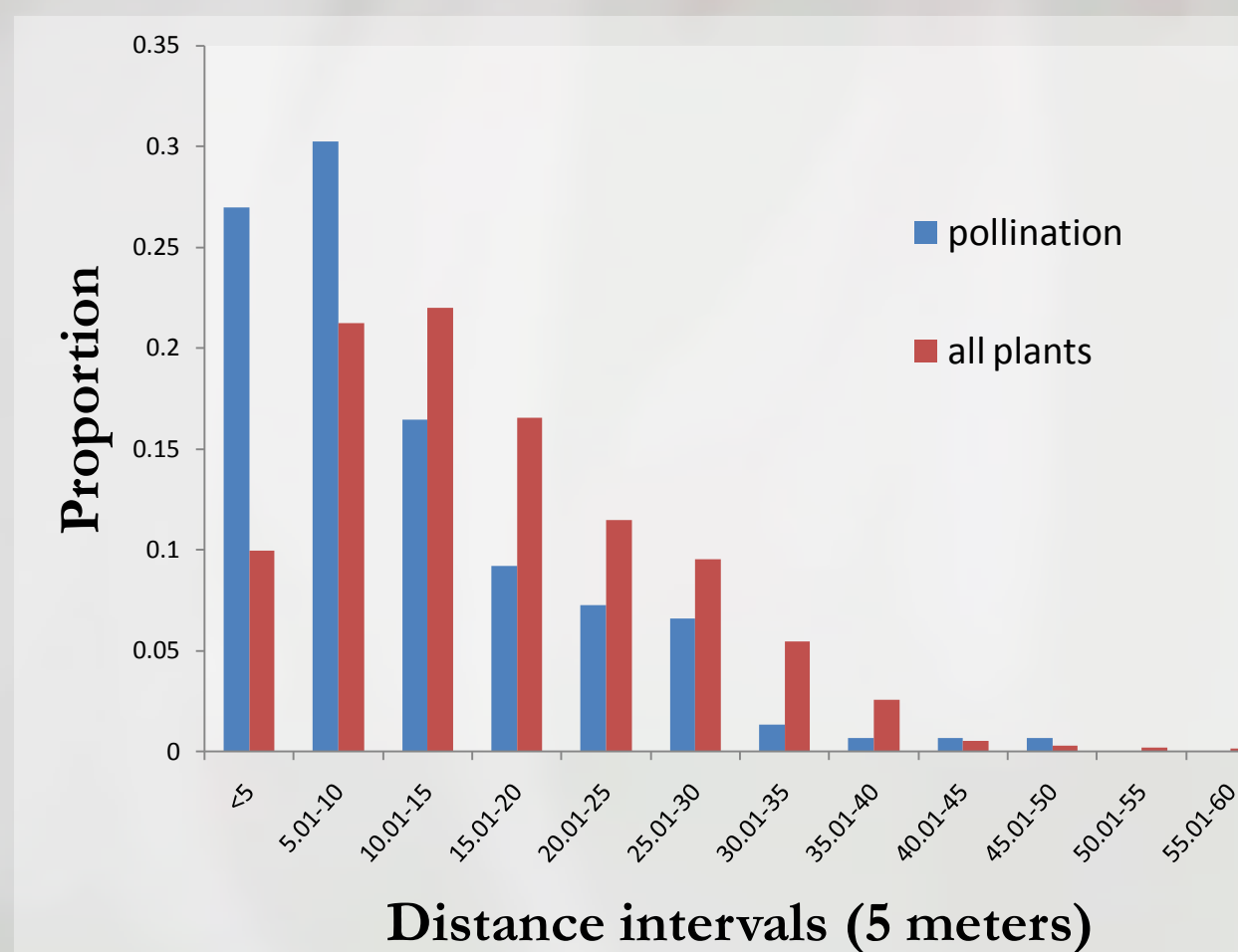


Fig. 6. Comparison of the proportion of all flowering plants within 5m intervals to focal maternal plants (red) to the proportion of within population pollinations at 5m intervals from each focal maternal plant (blue). (Kolmogorov-Smirnov test,  $p<0.01$ )

## Results

### Spatial and phenological:

- No significant correlation between time of flowering and pollen movement distances within a population (fig. 3,  $p=0.359$ )
- Significant decrease in distance of pollen movements in late-flowering plants than early flowering plants for all pollinations (fig. 4,  $p<0.01$ )
- Almost all long-distance pollen movements (4 out of 5) occurred within the first three days of flowering (fig. 4)

### Spatial:

- 25.8% of plants received pollen from their 1st to 10th closest neighbor (distance not taken into account) (fig. 5)
- 57.2% of plants were pollinated by plants 10 meters or closer (fig. 6)

## Conclusion

57.2% of pollinations were from plants 10 meters or closer. But only 25% of pollinations occurred within the 10 closest neighbors, indicating that a near-neighbor advantage is not significant. Early flowering plants have the greatest advantage of gene dispersal, when pollen traveled between populations. Understanding pollinator behavior during different stages of the flowering season can help to better grasp how far pollen travels and how to increase gene flow in prairie fragments. If specific species only begin to pollinate *E. augustifolia* after a certain density of flowering plants is reached, then how plants get pollinated can differ between early, typical, and late stages of the flowering season. Fragmentation can also change pollinator behavior and movement. If fragmentation is not advantageous to pollinator movement, there could be a trend towards more inbreeding, or population drifts, as is observed in over 50% of pollinations that occurred in the focal maternal plants.

## Citations

- Chaudhry, B, A Yasmeen, T Husnain, S Riazuddin (1999) mini-scale genomic DNA extraction from cotton. *Plant Molecular Biology Reporter* 17: 1-7.
- Glenn, T C and M Schable. 2003. Microsatellite Isolation with Dynabeads. Retrieved December 20, 2004, from "[http://www.uga.edu/srel/DNA\\_Lab/protocols.htm](http://www.uga.edu/srel/DNA_Lab/protocols.htm)."
- Kalinowski, ST, Taper, ML & Marshall, TC (2007) Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. *Molecular Ecology* 16: 1099-1006.

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- A. Abt, M. Ashley, K. Carim, C. Dumoulin, E. Ellis, J. Fant, K. Feldheim, K. Hereid, G. Kiefer, W. Stutz, S. Wagenius, and B. Wallin
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