How pollen age, style age, and self-incompatibility influence pollination in fragmented populations of *Echinacea angustifolia*

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Abstract

The tallgrass prairie of North America was once a vast continuous habitat but is now fragmented into small, isolated remnants. Fragmentation affects pollinator dynamics resulting in pollen limitation in many prairie species with inbreeding avoidance mechanisms like self-incompatibility. The common perennial *Echinacea angustifolia*, the narrow-leaved purple coneflower, experiences an increase in pollen limitation as remnant size decreases. We tested the hypotheses that the duration of pollen viability and style receptivity, and the mechanics of self-incompatibility, play a role in pollen limitation in *E. angustifolia*. We conducted three experiments: one with aging pollen and fresh styles, another with aging styles and fresh pollen, and one where we examined pollen tube growth in four pollination treatments at specific times after pollination. We found that freshly emerged pollen fertilized in 50% of ovules but by day 7 only 27%. Similarly, we found that style receptivity decreased over time. As styles aged, seed set decreased from 76% on day 1 to 36% on day 9. We found all pollen produces pollen tubes but compatible pollen did so at a significantly higher rate and set significantly more seeds than incompatible pollen. These results show that pollen viability and style receptivity decrease over time, thus limiting the opportunity for plants in fragmented populations to receive compatible pollen.

Background

Fragmentation of the once vast, continuous North American tallgrass prairie is a serious concern. Fragmentation affects pollinator dynamics resulting in pollen limitation for many prairie species with inbreeding avoidance mechanisms like self-incompatibility (SI). SI occurs in approximately 60% of flowering plants. The most common form of SI is gametophytic self-incompatibility (GSI) in which the haploid genotype of the pollen determines compatibility with the style. In GSI, pollen tubes may be arrested at the surface of the sigma or after significant growth into the style. The other type of SI system is sporophytic self-incompatibility (SSI). In SSI, the diploid genotype of the anthers determines compatibility with the style. Most research on SSI has been done on *Brassica* and a single multi-allelic locus has been identified. In *Brassica* pollen tubes rarely form, or if they do, they are soon arrested and do not penetrate the stigma tissue (Ockendon 1972). Researchers originally thought that SSI systems in all families worked similar to what had been found in *Brassica,* but new studies indicate that this is not the case (Hiscock 2000, Kowyama *et al.* 2000). Hiscock found that incompatible pollen tubes develop much further, many times even penetrating the style in *Senecio squalidus* (Asteraceae) (2000).

Study species

Echinacea angustifolia (Asteraceae), the narrow-leafed purple coneflower (Fig. 1), experiences pollen limitation due to self-incompatibility and habitat fragmentation: limitation increases as remnant size decreases (Wagenius 2000). *E. angustifolia* is an herbaceous perennial, and has a sporophytic self-incompatibility system. The inflorescence of *E. angustifolia* is typically made up of between 100-200 disk florets, each of which is subtended by a bract. The florets are arranged in regular circular rows that develop inwards. Anthers of an entire row present pollen on the same day. The styles emerge through anthers the day after anthesis, thus self pollen gets on the styles unless all pollen is removed. A fresh style of *E. angustifolia* remains turgid for up to 10 days unless pollinated with compatible pollen or damaged (Wagenius In press). This allows us to determine pollen compatibility via style shriveling.

Figure 1: *E. angustifolia* in a remnant population

Figure 2: *E. angustifolia*, with painted bracts used to denote crossings.

General protocol

❖All pollination work was done in July 2003 in an experimental garden in western Minnesota with plants grown from seeds collected in naturally occurring populations.

❖We placed pollinators exclusion bags on all experimental plants.

❖We painted each floret's subtended bract with acrylic paint on the day pollen was shed in order to denote the emerging style for later pollination (Fig. 2).

❖We applied pollen to the stigma lobes using a clean toothpick.

❖The day after pollination we determined whether or not the styles had shriveled.

❖We harvested all seedheads in the fall and the achenes were removed and weighed.

❖Based on mass, we assigned achenes as either containing an embryo "full" (≥ 3.0 mg) or "empty" (≤1.0 mg). We previously found fertilization rates of 79% for achenes categorized as "full."

❖Intermediate masses usually did not clearly indicate fertilization status (1.1-2.9 mg).

❖We classified achenes using two cut-offs for predicted fertilization(>3.0 mg) and (>1.1mg), in no case did the choice of cut-off mass change the test result of a hypothesis.

❖We tested all hypotheses using a chi-square contingency table and a test of independence.

Pollen age experiment

❖We collected pollen on a single day from five plants and kept it in a vial at room temperature.

❖We chose five different plants as maternal plants and pollinated them with the aging pollen every other day using the method described in the general protocol.

❖We replicated each pollination combination on a least 6 freshly emerged styles.

❖After 10 days, the maternal plants finished flowering so we chose a new set of 5 maternal plants.

❖To verify the receptivity of the second set of maternal plants we also collected pollinated control styles with fresh pollen.

❖The control mix applied to the second set of maternal plants shriveled at a rate of 96% and had 57% seed set

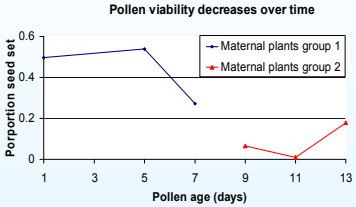

Figure 3: We found that rate of shriveling (not shown) and seed set in both sets of maternal plants are related to age of pollen ($p<0.05$).

❖There are many random pollination events which could bring pollen from one fragmented population to another. We found pollen viability decreases over time thus limiting the opportunity for a fragmented population to receive outside pollen.

❖Pollen was stored inside under optimal constant conditions. This may have changed how the pollen aged, even slowing down the aging effect compared to natural conditions.

❖We found at 13 days the seed set was greater than at 11 day after anthesis. There was more rain on the day of this pollination than on any other day during the experiment. This may have lead to an increase in contamination or affected the plants in some unforeseen way. Still the level of set seed even when pollen was 13 days old is much lower that observed when the pollen was 1-5 days old.

Style receptivity experiment

❖This experiment was conducted on five maternal plants.

❖We painted subtending bracts every other day with the method described in general protocol (Fig. 2).

❖We allowed styles to age 1, 3, 5, 7 and 9 days.

❖We used a mix of fresh pollen from the five different plants to pollinate the maternal plants.

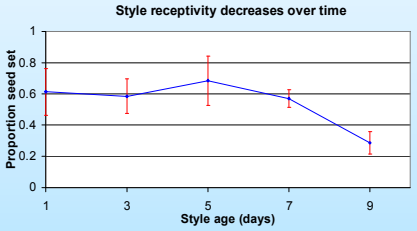

Figure 4: We found style shriveling (not shown) and seed set are related to style age ($p<0.05$). Error shown is due to achene masses that did not clearly indicate fertilization status.

❖Styles in fragmented populations stay open longer than styles in larger populations (Wagenius In press). Most unpollinated styles do not stay turgid past day 10. Styles appear to drop in receptivity shortly before their natural death but not much before this point. This limits the opportunity for successful fertilization in fragmented populations

❖We found many more intermediate achenes (1.1-2.9mg) in this experiment than in either the pollen age or pollen tube experiments. While this does not change the test result of the hypothesis (Fig. 4) we plan to germinate the achenes to determine if they contain embryos.

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Pollen tube experiment

❖We painted 18 bracts on each of 20 maternal plants.

❖We crossed eight plants with compatible pollen from a single donor, five plants with self pollen, five plants with incompatible pollen from a related plant, and two plants had no pollen manually applied. Anthers on all plants were left intact.

❖We pulled one randomly chosen style from each plant at time 1, 2, 4, 8, 22, and 27 hours after pollination and placed it in an individual vial of FAA (70%EtOH: glacial acetic acid: formalin).

❖We left the remaining styles and allowed them to fully develop.

❖For viewing we softened the styles in FAA with 8N NaOH for 26 hours and dyed them with Aniline Blue for 2 hours (Martin 1959).

❖We scored styles for pollen tubes using two fluorescence microscopes.

❖We found the compatible crosses shriveled at a rate of 92% and had a 67% seed set, the three types of incompatible crosses shriveled at rates between 3%-9% and had between 4%-6% seed set ($p<0.001$). (Data not shown).

❖We found pollen tubes in all treatments. The proportion of pollen tubes formed out of total pollen observed on each style differed between treatments. In compatible crosses 34% of observed pollen produced pollen tubes (Fig. 6), incompatible (non-self) crosses 13%, self pollen crosses 10%, and styles with no pollen treatment 5% ($p<0.001$). (Data not shown).

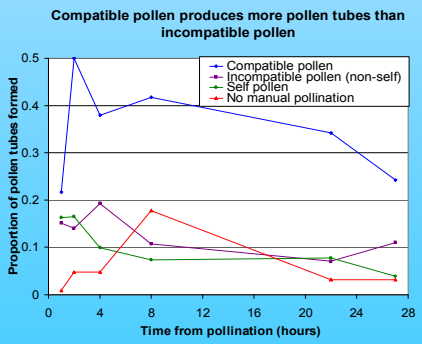

Figure 5: We found significant differences in the proportion of pollen tubes formed by the time from pollination for each treatment ($p<0.001$).

❖Fragmentation limits the number of potential mates within a population especially for species that are self-incompatible.

❖We found pollen tubes penetrating the stigma in all treatments indicating that SSI in *E. angustifolia* does not appear to operate the same as in *Brassica*. Pollen tubes that do penetrate the sigma must be arrested in the style since we observed extremely low seed set in achenes that were allowed to develop using the same pollination treatments.

❖For *S. squalidus* Hiscock found that incompatible pollen that enters the stigma is inhibited early and can be characterized by a clump of callose at the base of the pollen tube (Hiscock 2000). In our initial observations we did not detect any pattern in clumps of callose. We have observed a pattern of what appears to be unattached pollen tubes in the incompatible crosses (Fig. 7). *E. angustifolia* is closely related to *S. squalidus* and initially it does seem to have, while not identical, similar patterns for pollen tubes of incompatible pollen. The next step in deciphering SSI in *E. angustifolia* is to see exactly where pollen tubes are arrested in incompatible crosses.

Figure 6: Pollen tubes penetrating the stigma in a compatible cross.

Figure 7: An incompatible pollen tube has germinated but not penetrated the stigma.