

Reproductive ecology and pollination biology of *Geum triflorum* (prairie smoke)

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Abstract

Understanding plant reproductive strategies is important for interpreting population dynamics and species resilience. We investigated the mating system, pollen limitation, and pollinator interactions of *Geum triflorum* (prairie smoke), a subalpine wildflower. Experiments took place at the Rocky Mountain Biological Laboratory in Gothic, CO. Using a randomized block design, we applied five pollination treatments: hand outcross, open outcross, hand self, autogamy, and control. We measured seed set, floral traits, and pollinator identity and activity in 26 naturally occurring *G. triflorum* rosettes. However, due to severe drought stress, widespread herbivory, and issues with pollinator exclusion bags, only three flowers produced seeds across all treatments. This precluded statistical analysis of reproductive success. Despite these limitations, results suggest *G. triflorum* does not reproduce via autonomous self-pollination and may be capable of some amount of self-fertilization via geitonogamy. Natural pollination was observed, though seed production was minimal. These findings, coupled with low visitation rates and reduced reproductive output in dry years support the hypothesis that *G. triflorum* may be pollinator-dependent. Future research should explore drought effects on reproduction more directly and use improved methods to reduce herbivory and bag failure.

Introduction

Plants use a variety of reproductive strategies that play a key role in their population persistence and ability to adapt to changing environments. Most plants reproduce sexually, through abiotic or biotic pollination (Barrett and Harder 2016). Sexual reproduction can occur via outcrossing between individuals, via pollen transfer between flowers on the same plant (geitonogamy), or via selfing within a single flower (autogamy). Asexual reproduction may involve the formation of seeds without fertilization (apomixis) (Barrett and Harder 2017). Breeding systems can influence broad evolutionary outcomes, including speciation and extinction risk (Cascante-Marín & Núñez-Hidalgo, 2023). Furthermore, these reproductive modes shape population-level processes like gene flow, genetic diversity, and resilience to environmental stress. For example, pollinator behaviours and movement patterns can affect levels of genetic connectivity and inbreeding (Marín & Núñez-Hidalgo, 2023). Thus, understanding the diversity of plant reproductive strategies is important for interpreting ecological and evolutionary dynamics.

Identifying the extent to which plant seed production is limited by pollen receipt – pollen limitation – helps us understand how mating systems and pollinator behavior interact to affect plant reproduction. Pollen limitation occurs when a plant doesn't receive enough pollen to fully fertilize its ovules, resulting in fewer fruits or seeds than it could produce under ideal conditions (Knight et al, 2005). The extent of pollen limitation in natural populations can vary depending on both the plant's evolutionary history and current ecological conditions (Ingold et al., 2024).

Studying a species' pollen limitation can provide insight into what is limiting its reproductive success and potentially its population viability (Knight et al., 2005).

While these general patterns of pollen limitation and reproductive ecology are well documented in many plant species, little is known about how they apply to *Geum triflorum*, including its specific mating system, reliance on pollinators, or whether its reproduction is limited by pollen availability. It is known that several *Geum* species are able to persist through clonal propagation alongside seed reproduction (Gajewski 1959). Seeds are wind dispersed, but there is little information on *G. triflorum*'s regenerative processes, seedling establishment, and asexual reproduction (USGS). Holm et al. (2014) describes *G. triflorum* as buzz pollinated by bumble bees, sweat bees, and visited by nectar thieves like ants. Previous research at The Rocky Mountain Biological Laboratory suggests that *G. triflorum* matures a high proportion of its flowers into fruits, but pollination events appear to be rare compared to other species (Iler, unpublished). One potential explanation is that *G. triflorum* is capable of autonomous self-pollination. However, a pilot experiment conducted in 2024 showed that flowers with pollinator exclusion bags did not produce seeds (Iler, unpublished). It is also unclear whether the species experiences pollen limitation.

We therefore ask the following questions: (1) What is the mating system for *G. triflorum*? (2) Is reproductive success limited by pollen receipt (i.e., pollen limitation)? (3) What are the pollinators/visitors of *G. triflorum*? We expect to see solitary bees and bumblebees based on the literature for other *Geum* species (Holm 2014). Based on unpublished observations, we hypothesize that *G. triflorum* is self-incompatible and relies primarily on outcrossing for successful reproduction, or that it is self-compatible but requires pollinators to set seed (Iler, unpublished). Because pollen limitation is widespread among flowering plants (Knight et al., 2005; Bennett et al., 2020), we predict that *G. triflorum* will also experience pollen limitation, particularly if pollinator visitation is low or pollen transfer between genetically distinct individuals is limited.

Methods

Study Site

This study was conducted in the Research Meadow at the Rocky Mountain Biological Laboratory (RMBL). This is a subalpine meadow with known *Geum triflorum* populations. A total of 26 experimental blocks have been set up across the meadow to capture genetic and environmental variation. Blocks are spatially distributed to minimize genetic similarity through rhizomatous growth. They are about 1 square meter.

Experimental Design

The plots contained naturally growing *G. triflorum* individuals assigned to the following five treatments: (1) Hand outcrossed: Flowers were hand-pollinated with pollen from individuals outside the plot and bagged before and after to exclude ambient pollen. (2) Open outcrossed: Flowers were left unbagged to receive ambient pollination from surrounding plants and hand

pollinated from individuals outside the plot. (3) Hand self-pollinated: Flowers on the same stalk were used to pollinate one another, with exclusion bags applied to exclude ambient pollen. (4) Autogamy (spontaneous self-pollination): Flowers were bagged and left unmanipulated to test for autonomous self-fertilization. Bags were always placed on flowers prior to their opening, for all treatments containing pollinator exclusion bags. (5) Control: Flowers were left unbagged and unmanipulated, allowing natural pollination to occur. Each treatment was replicated within all 26 blocks to account for environmental changes in a randomized block design, using multiple flower stalks per block. Each treatment was applied to one flowering stalk emerging from a single *G. triflorum* rosette. It is possible that the treatments will be assigned to the same individual per block, depending on the extent of clonal growth. Delimiting distinct individuals was beyond the scope of our project. The number of leaves, number of flowers, and length of the longest leaf will also be recorded for each rosette, as covariates that might affect seed production in addition to the treatments.

On July 11, 2025, all blocks were revisited to replace exclusion bags that had fallen off due to wind or herbivory. As a result, some bagged treatments may have received ambient pollen before replacement. To address plant stress, watering began on July 17, as some individuals showed signs of drying or wilting. All plants were watered in the evenings (around 5–6 p.m.) twice per week, with equal water volume per plant (~400mL), continuing through July 29.

Hand Pollination Procedure

Hand-pollination was conducted twice during the flowering season. Applications were repeated to ensure pollen was delivered during potential stigma receptivity windows. Clean brushes were used to transfer pollen. Flowers were then bagged immediately after pollen treatments to prevent contamination. Pollen for outcrossing was obtained from *G. triflorum* individuals located outside the experimental blocks to minimize genetic similarity due to clonal growth.

Pollinator Observations

Timed observations of floral visitors were conducted on sunny days. Insect visitors were identified to the lowest taxonomic level possible in the field. Identity of visitors and visitation rate (visits per flower per unit time) were recorded. Observations were conducted for 5 minutes per area. Areas for observation were defined based on the number of flowers that could be reliably observed.

Reproductive Success

At the end of the flowering season, fruit set and seed set per flower per treatment was measured. These were compared across treatments to assess the mating system, presence of pollen limitation, and potential for autonomous selfing

Statistical Analysis

We used an ANOVA to determine whether the different pollination treatments differed from one another. Leaf length was analyzed with a linear mixed effects model (LMM) and leaf number and flower number with a generalized linear mixed effects model (GLMM) with a Poisson error distribution. Treatment was a fixed effect and block was a random intercept term.

Results

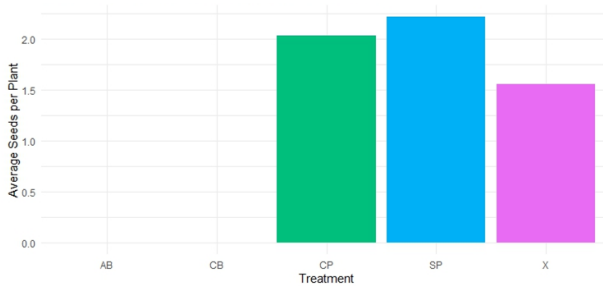


Figure 1. Average seed set per plant across five mating system treatments in *C. ovata*.

Out of all flowers in the study, only three flowers produced mature seeds, in three different treatments: the control, self pollination treatment, and the open outcross treatment (Figure 1). No seeds were produced in the autogamy or outcrossed and bagged treatments. In total, 52 plants experienced herbivory, resulting in the complete removal or destruction of the flowers before fruiting could occur. An additional 24 plants experienced issues with the pollinator

exclusion bags, such as bags falling off or tearing, which may have compromised treatment integrity and allowed unintended pollen exposure.

Because of the extremely low number of seed-producing flowers, statistical comparisons of reproductive success across treatments were not possible. These limitations prevented conclusions about treatment effects, pollen limitation, or the mating system based on seed set.

We were, however, able to check for bias across pollination treatments. We compared three measures of plant size: leaf length, number of leaves, and number of flowers (Figure 1). None of these traits varied significantly among treatments (leaf length: $F = 1.64$, $p = 0.17$;

number of leaves: $X^2 = 6.40$, $p = 0.16$; number of flowers: 0.83 , $p = 0.83$). Overdispersion ratios were close to 1, indicating appropriate model fit for GLMMs.

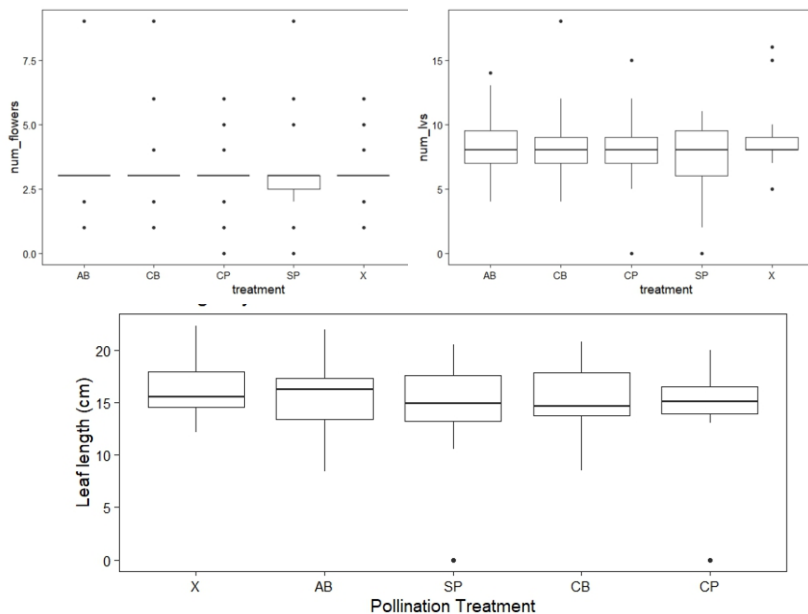


Figure 2. Vegetative and floral traits did not differ significantly among pollination treatments. (A) Number of flowers (B) number of leaves, and (C) leaf length are shown across five pollination treatments. These results show that initial differences in plant size or floral output did not bias

Discussion

This study set out to investigate the mating system, potential pollen limitation, and pollinator interactions of *Geum triflorum* by applying five different pollination treatments in a subalpine meadow. However, only three flowers produced mature seeds across all treatments. Many of the plants were affected by herbivory or issues with the pollinator exclusion bags. This made it difficult to fully carry out the experiment as planned. More importantly, it was a very dry season because the snow melted earlier than average. Almost all of the control plants failed to go to seed, which confirms that difficult environmental conditions affected plant reproductive success. Many plants throughout the meadow showed signs of wilting or stress. We started watering plants in mid-July, but this was after pollination treatments. It is possible that stress from drought affected flower development or fertilization before water treatments. Future studies may benefit from starting a watering treatment earlier in the season if the study is being conducted when snowmelt date is earlier than average.

Although we were unable to draw strong conclusions from the results, some patterns still offer useful early insights into the species reproductive biology. No seeds were produced in the autogamy (bagged and unmanipulated) treatment, which supports the idea that *G. triflorum* does not reproduce through autonomous self-pollination (Figure 2). This result is consistent with a 2024 study that also found no seed set in bagged flowers (Iler, unpublished). At the same time, one flower in the hand self-pollination treatment did produce seed. Since there were no exclusion bag issues on that flower, it is unlikely that the seeds resulted from outcrossing. It is possible that geitonogamy may sometimes result in successful fertilization. This challenges the initial hypothesis that *G. triflorum* is self-incompatible, though more data are needed to confirm this result.

Seed production in both the open outcross and control treatments shows that natural pollination is possible. This is consistent with unpublished data from a nearby demography project, where *G. triflorum* plants have regularly produced fruits from 2021 – 2024 (Iler, unpublished). Similar to our results, fruit set was very low in this project during summer 2025.

It is unclear whether *G. triflorum* experiences pollen limitation through these results. Earlier observations from RMBL have noted that this species has a high fruit set when pollination happens but that pollinator visitation rate is low (Iler, unpublished), which matches what we saw in this study. Still, we were unable to determine whether the limitation is caused by a lack of visits or problems with the pollen itself.

Additionally, herbivory had a large impact on the experiment, with 53 plants losing their flowers completely. A total of 24 flowers had issues with their exclusion bags falling off, tearing, or otherwise failing. This made it unclear what kind of pollen they may have received, but none of these made fruits, so we were unable to compare plants with bags that did not malfunction. Future studies can use white exclusion bags to deter grasshopper herbivory, or stronger bagging materials.

Despite the lack of reproductive output, this study provides a foundation for understanding the reproductive ecology of *Geum triflorum*. The early signs point to a reproductive strategy that likely depends on pollinators, with some potential for self-compatibility. Further research is needed to confirm these findings.

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