



Effects of Self Versus Outcross Pollination on Pollen Tube Growth in *Platanthera leucophaea*

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Abstract

Faced with fragmented habitats and low self seed viability, the Eastern Prairie Fringed Orchid, *Platanthera leucophaea*, has been placed on the federally threatened species list. While several experiments (see, for instance, Bowles, 2002) have examined the viability of self versus outcross seed, there is a serious need to understand why this phenomenon occurs in order to create the most effective conservation strategy.

This study compares the effects of self versus outcross pollination on pollen tube growth in *P. leucophaea* and attempts to explain the observed lower viability of selfed seeds. Eighty plants from a population in Iowa were randomly chosen for the experiment, forty of which were self-pollinated and the other half outcrossed. Individual flowers were fixed in FAA at one of four randomly assigned collection times, stained with aniline blue, and then examined under fluorescence microscopy.

In addition to qualitative data that illustrate several irregularities in self pollen tube growth as compared to outcross tube growth, the quantitative results of both an Analysis of Variance test and a logistic regression show that self pollen tubes grow at a significantly slower rate than outcross tubes. These observations suggest that there could be a complex system of self-incompatibility in *P. leucophaea*.

Introduction

Platanthera leucophaea, the Eastern Prairie Fringed Orchid, is just one of many species whose continued survival is dependent upon the conservation of the tall grass prairie. While *P. leucophaea* inhabits prairie remnants across the mid-western portion of the United States, low pollination rates have placed it on the federally threatened species list (Bowles, 1983). Like many members of Orchidaceae, *P. leucophaea* exhibits a floral morphology that is highly adapted to a specific pollinator (Bowles, 1983). Research by Sheviak & Bowles (1986) indicates that one or more species of hawkmoth is likely the natural pollinator of *P. leucophaea*.



Research to date suggests that pollinators limit fruit production in this facultative outcrosser (Bowles, 1983). Recent research by Bowles et al. (2002) suggests that while *P. leucophaea* does set seed when self-pollinated, seed viability is significantly higher in fruits produced via outcrossing. This phenomenon is especially troubling in small populations, which may experience a higher rate of self pollination relative to large populations. In populations with low natural pollination rates, it is especially important to understand the effects of selfed versus outcrossed pollination in order to further conservation efforts.

Figure 1: *Platanthera leucophaea*.

This study attempts to explain the observed incomplete self-incompatibility in *P. leucophaea* by examining pollen tube growth of selfed vs. outcrossed individuals. Pollen tube growth is expected to be irregular or incomplete in selfed individuals, while a majority of pollen tubes are expected to reach the ovary in those outcrossed.

Methods

Part One: Field Work

- >20 plants of *Platanthera leucophaea* with more than 6 flowers in bloom were chosen for hand pollination and randomly assigned to either a selfing or outcrossing treatment.
- >4 flowers from each individual were hand pollinated and randomly assigned a collection time of 8, 12, 24 or 48 hours after treatment.
- >Flowers were fixed in FAA upon collection.

Part Two: Laboratory Preparation

- >Each flower was removed from FAA and rinsed with DI water before removing the petals and bracts, leaving only the column.
- >Each specimen was softened in a 1M NaOH bath at 60° C for one hour, then soaked in DI water for one hour to ensure that excess NaOH was removed.
- >Specimens were then transferred to a .1% solution of aniline blue solution.

Methods (cont'd)

Part Three: Fluorescence Microscopy

- >Stigmas were examined at 50x magnification 24-52 hours after being transferred to aniline blue using a Leica DMIRBHC microscope with a UV filter under epifluorescence at near UV (~370 nm) excitation.
- >Detailed photographs were obtained using a Kodak DC290 Zoom digital camera.
- >The cover slides were marked to denote the length of the longest pollen tubes.
- >Digital images of each specimen were examined to determine the approximate number of pollen tubes that successfully entered the ovary.
- >Samples were placed in one of four categories: **A**=no pollen tubes entering the ovary (0), **B**<20, **C**=20-100, and **D**>100 tubes.

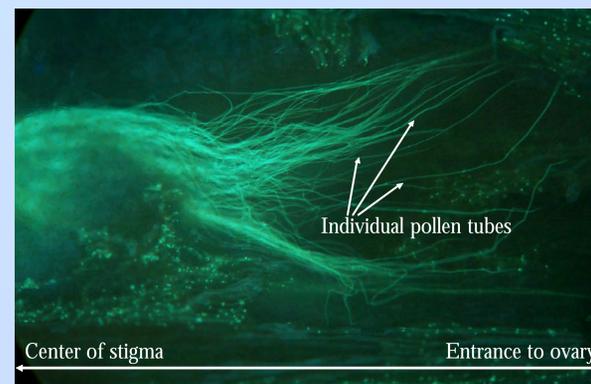


Figure 2: Pollen tubes entering the ovary (enlarged to 200%).

Results

A logistic regression was performed on the number of pollen tubes reaching the ovary for self versus outcross pollen. The results (shown in Table 1 below) show that time (from pollination to fixation of the flower in FAA) has a significant effect on the number of pollen tubes that successfully reach the ovary.

There is also a significant effect of treatment (self pollination or outcrossing) on the number of tubes reaching the ovary, regardless of fixation time. Self crosses had significantly fewer pollen tubes reaching the ovary at both 8 and 12 hours after pollinia application, suggesting that self pollen tubes grow more slowly than do outcross pollen tubes. The model becomes saturated after fitting three time parameters and is unable to estimate the individual effect for the 48 hour fixation time.

The data were also tested for significant differences in pollen tube growth rate using Analysis of Variance, which shows a significant difference between self pollen tube growth rates and outcross growth at 8 hours, though not at any other fixation time. Pollen tube length at each of the four fixation times is presented at right (Figure 3).

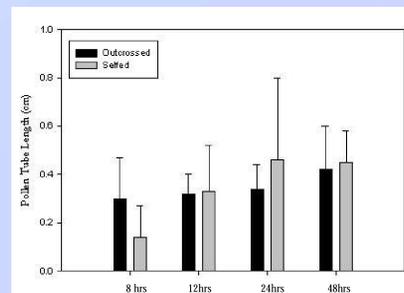


Figure 3: Pollen Tube Length.

Table 1: Nominal Logistic Fit for # Tubes Reached Ovary Self Versus Out Cross Effect of Time.

Whole Model Test (N=84)				
Model	-LogLikelihood	DF	ChiSquare	Prob>ChiSq
Difference	14.83992	6	29.67985	<.0001
Full	97.26892			
Reduced	112.10885			
Effect Wald Tests				
Source	Nparm	DF	Wald ChiSquare	Prob>ChiSq
Time	3	3	14.62199	0.0022
Self/Out	3	3	8.6880255	0.0337
Effect Likelihood Ratio Tests				
Source	Nparm	DF	L-R ChiSquare	Prob>ChiSq
Time	1	3	21.9920371	0.0001
Self/Out	1	3	9.8184501	0.0202

Discussion

Efforts to recover the federally threatened orchid, *Platanthera leucophaea*, have included hand pollination by volunteers to increase seed set. It is important that these efforts are conducted with a thorough understanding of the reproductive biology of this species. Several researchers have noted lower seed viability in self versus outcross seeds, and it has become increasingly apparent that seed viability is affected by habitat fragmentation and small population size. Current research focuses on the mechanisms of self incompatibility in *P. leucophaea*. Though the total number of pollen tubes reaching the ovary in self pollinations is greater than expected, the results suggest that outcross pollen tubes grow at a faster rate than do self pollen tubes. Additionally, many self pollen tubes exhibit irregular growth patterns, including multiple small tube clusters, large numbers of aborted pollen tubes, and pollen tube growth away from the ovary. In contrast, most outcross pollen tubes follow a discreet and direct path from the pollinia to the ovary, generally including only one large tube mat with fewer aborted tubes overall. All of these observations suggest that *P. leucophaea* is able to self-pollinate when other pollination mechanisms are not present.

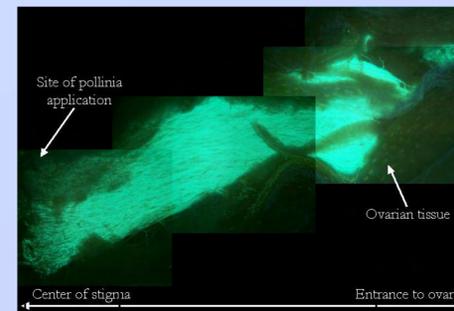
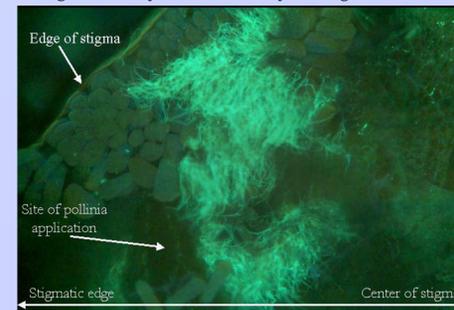


Figure 4: Outcross pollen tubes entering the ovary.

Figure 5: Self pollen tubes growing toward stigmatic edge and away from the ovary (enlarged to 225%).



Our results support our initial hypothesis that self pollen tubes exhibit irregular growth as compared to outcross pollen tubes, but further research is needed to determine the nature of the incompatibility system present in *P. leucophaea*. Examining pollen tube growth at shorter time intervals would provide greater insight into rates of pollen germination and early tube growth.

Also, the process of squashing the stigmas often led to a layer of tissue covering the site of pollinia application which made it difficult to clearly distinguish the pollen tubes growing on the stigma. Thinly slicing the tissue would allow a better view of the pollen tubes as they travel through the stigma and enter the ovary.

The results of this experiment support research to date that suggests that *P. leucophaea* exhibits some form of partial self-incompatibility. The slower growth rate exhibited by self pollen tubes in *P. leucophaea* may be due to an incomplete self-incompatibility system that prevents self pollen tubes from growing as quickly as outcross tubes.

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