Investigation of the Mating System and Reproduction in The Pasque Flower (*Anemone patens*)

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Abstract

The buttercup family (Ranunculaceae) exhibits a lot of variation in pollination methods and breeding strategies, where outcrossing (xenogamy) is typical, but self-fertilization (autogamy) has been observed. This study is an investigation of the breeding system and the pollination biology of *Anemone patens,* which exhibits both xenogamy and autogamy. Treatment groups were set up and analyzed to look at reproductive success and seed viability exhibited by both mating systems. In addition , a pollen-ovule ratio was calculated for the flower, pollen tube growth was investigated and pollen deposition as related to pollinator limitation was analyzed. Pollen tube growth was unsuccessful due to either methodological problems or the absence of pollen germination. Pollen deposition analysis indicated that pollinator activity is present in the mating system of A. Patens. However, the pollen ovule ratio obtained did not place the flower within any breeding system. Seed set, seed number and seed viability data were not obtained because all open treatments were consumed by wildlife.Thus, further investigation is necessary to understand the relationship between outcrossed and selfed seed production.

Introduction

The subtribe *Anemoninae* within the Ranunculaceae family is difficult to make reproductive generalizations about because the group is characterized by a wide range of ecological diversity (Ehrendorfer 1995). The literature characterizes the family as most often having hermaphroditic flowers that shed pollen before stigmas mature to ensure outcrossing, although in studies conducted by Kudo (1995) and Douglas and Cruden (1994) self-fertilization has been observed. In order to understand mating systems at the species level, both environmental factors and floral features that directly influence the population in question are important in understanding pollination methods.

There are several approaches that are used to analyze mating systems within angiosperms. Cruden (1977) has classified plants into groupings based on pollen ovule ratios: cleistogamous, obligately autogamous, facultative autogamous, facultative xenogamous and xenogamous. The general assumption behind his approach is that plants that self-fertilize do not need to produce as much pollen (Kearns and Inouye 1993). Another way to assess the potential for seed production is to look at pollen tube growth. Relative growth of pollen tubes from different pollination treatments can be used to compare of self and outcross pollen.

The standard approach to determine whether a plant can set seed in the absence of pollinators has been to cage flowers prior to anthesis (Kearns and Inouye 1993). To distinguish between facultatively xenogamous and facultatively autogamous plants an open-pollination should be included (Kearns and Inouye 1993). Analysis of seeds produced by such plants has important implications. In the study conducted by Douglas and Cruden (1994), reproductive success was determined in each flower by seed number per ovule number and seed viability was determined by Kudo (1995) by comparing seed weight between those flowers that outcrossed and those that selfed.

Anemone *patens,* commonly known as the Pasque Flower, is a large, showy flower with lavender sepals and numerous stamens and carpals. This flower has been found to have a mixed breeding system where both xenogamy and autogamy are involved. In order to analyze both the reproductive success of each system and the viability of seeds produced by each system, I set up treatments to determine seed set and seed weight. Preliminary data included analyzing the breeding system as indicated by the pollen-ovule ratio and observing pollen tube growth in experimentally outcrossed and selfed flowers. My null hypothesis was that there is no difference in seed set and seed weight between plants that are experimentally self-fertilized and experimentally out-crossed.

In addition, I analyzed pollen deposition in autogamous and xenogamous treatments. *A. Patens* is an early-spring ephemeral and lacks nectaries, two features that result in decreased pollinator visitation. Thus, I postulated that without autogamy, A. Patens is pollen limited for much of the growing season. I hypothesized that those plants that are experimentally manipulated to self-fertilize have higher pollen deposition than those that are experimentally cross-fertilized.

By studying the mating system of *A. patens* I hope to expand the basic knowledge of its breeding strategy and pollination biology. Further, because mating systems determine the pattern of genetic transmission in a population, analyzing these patterns in *A. patens* could be important in understanding the evolution of strategies within the species that may or may not restrict the number of available mates (Kearns and Inouye 1993).

Methodology

This study was set up on Boulder Mountain Parks land in Chatauqua Park. Four treatment groups were established within a population of A patens that included (1) flowers in cages that excluded pollinators, (2) emasculated, caged flowers that functioned to determine if apomixis (reproduction without sex) occurred, (3) open-pollinated flowers that comprised the control group and (4) emasculated, open-pollinated flowers that demonstrated whether outcrossing occurred (unless apomixis occurs).

Each treatment group consisted of 30 individuals that were marked with color-coded yarn tied around the flowering stalk. Caging and emasculation took place prior to the time when pollen was shed or the stigmas become receptive. The cages were made of bridal veil secured at the base with metal stakes. After all treatment groups were set up I checked the treatments 3-4 times a week to determine timing of seed set.

I carried out small sample studies within my examined population to obtain preliminary data. Pollen-ovule ratios were determined for five flowers suspending pollen grains from one flower in lactic acid and glycerin and then using a hemacytometer to calculate the total amount of pollen in a known volume. Each carpal contains one ovule, thus ovule numbers were obtained by counting carpals by hand. The standard error for the pollen-ovule ratio was calculated and both values were compared with Cruden's theoretical values for his five mating systems.

Pollen tube analysis was conducted by removing stigmas from had pollinated selfed and outcrossed flowers and then staining them using the standard procedure for aniline-blue epiflourescence (Kearns and Inouye 1993). Pollen deposition was analyzed within each treatment group by suspending stigmas in fuchsin jelly where pollen grains appeared pink. The ratio of stigmas with pollen grains to the total number of stigmas present was analyzed for each treatment. Analysis involved calculating average and standard error.

In the weeks following preliminary research, all open treatment flowers were consumed by wildlife. Thus seed number and seed weight data were not obtained and the relationship between autogamous and xenogamous mating systems was not analyzed.

Results

No results were obtained for pollen tube growth, seed number and seed weight. The pollen-ovule ratio as calculated to be 299.3 with a standard error of 47.5. Pollen deposition results are found in figure 1.

Discussion

The results of this study indicate that further investigation is necessary to better understand reproduction and pollination in the pasque flower. No data were obtained for seed number and seed viability because seed was not collected from open treatments. Thus, the null hypothesis can not be rejected or supported at this time.

The pollen-ovule ratio obtained has ambiguous implications. Although it does not correspond to a specific breeding system, it does fall between facultative xenogamous and facultative autogamous systems. This data does not reject the null hypothesis because neither outcrossing nor xenogamy appears to be favored. However, this data does not support the null hypothesis because no breeding system can be applied. Cruzan (1979) criticizes the P/O ratio for suggesting that pollen production is only to ensure ovuel fertilization; however, Cruden's work is still valuable as data from P/O ratio studies does provide a general sense of breeding system assignment (Kearns and Inouye 1993).

The results of pollen deposition analysis exhibit some significance. The experimentally selfed group was significantly different from the control group where there was a considerably higher pollen deposition on the control group. This suggests that the control group receives pollen from other plants. Similarly, the control group pollen deposition ratio was significantly higher from the outcrossed group. This may be explained by either a mixed mating system or perhaps the absence of anthers lowers insect visitation to emasculated flowers. The significance between the experimental apomixis group and the outcross group suggest that insect visitation is a means for pollen transfer. This comparison coupled with the control group suggests that pollen deposition is higher in functionally hermaphroditic pasque flowers. The large degree of error in the pollen deposition data can be explained by the small sample size. It is necessary to increase the sample size to ascertain whether the relative pollen depositions on either xenogamous or autogamous flowers. Until then, the data obtained currently rejects the hypothesis that selfed flowers have higher pollen deposition.

In light of pollinator visitation as indicated by pollen deposition several studies have observed that lower insect visitation is limited by temperature. Kudo (1995) found that in *Adonis ramosa* (Ranunculaceae), a spring ephemeral, activity of pollinating insects was temperature dependent where cool temperatures restricted pollinator visitation. Similarly, McCall and Primack (1992) compared three contrasting plant communities and found low temperatures to be one of the most important variables restricting insect visitation rates.

Because nectar functions solely as a reward for pollinators (Kearns and Inouye), it is not surprising that Motten (1996) found three nectarless species that received fewer insect visits than concurrently blooming, nectar-producing species. Further, the three species that lacked nectar bloomed in the early spring and were autogamous (Motten 1996). Unreliable pollinator service, due to factors such as weather and the absence of nectaries, has been invoked to explain the high proportion of autogamous species in several species with frequently inclement weather (Motten 1986), even though the majority of angiosperms seem to favor xenogamy (Stebbins 1974). From an evolutionary perspective, Richard (1986) suggests that the inefficiency of pollen transfer between individual, xenogamous plants may severely limit or prevent seed set; therefore, many of these plants favor mechanisms that lead to partial or total self-fertilization.

While self-pollination has been observed in the pasque flower, several taxonomists still view the genus *Anemone* being xenogamous (Ehrendorfer 1996). In order to determine the reproductive success and the seed viability of exhibited by both systems, seed production is necessary.

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