Effects of Color and Plot Size on Pollination of Sweet Alyssum (*Lobularia maritima*)

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Abstract

Pollination is essential for the proliferation of plants that require biotic interactions to reproduce sexually. In the forest understory, animal pollination is common and competition for flower visitors is often fierce. Flowers use traits such as color and smell to attract potential pollinators. Inevitably, some flower phenotypes draw in higher rates of visitation by pollinators than others. This paper investigates the effects of variation in patch size and color of Sweet Alyssum (*Lobularia maritima*) on pollination rates. We analyzed field data regarding the number of pollinator visits to large and small patches of both white and purple Alyssum. We observed twelve individual patches for ten minutes, three times a day, for eight days, providing 48 total hours of observation. The results showed a significant difference between mean visits of small and large patches as well as white and purple patches, with large patches and white patches both attracting more pollinators. The results of this analysis suggest that pollinators do prefer flowers in the forest understory based on the flowers' color and patch size. These results are important as they can be used to influence future studies of pollination and increase understanding of understory competition dynamics.

Introduction

The process of pollination is used to facilitate the production of seeds, enabling plants that are pollinated to generate offspring. Pollination is the act of transferring pollen grains from the male anther of a flower to the female stigma. Plants that cannot reproduce asexually rely on one of two primary pollination methods: biotic and abiotic. Abiotic pollination is facilitated by wind or water, while biotic pollination involves living organisms, often insects, that aid in the movement of pollen grains (USDA, 2017). In temperate zones, 78% of species are known to use biotic pollination processes (Ollerton et al., 2011). Pollination is especially important because it helps diversify the gene pool of future seeds by increasing gene flow across distances and ensuring recombination. A diverse gene pool makes plant populations more robust and prevents inbreeding (Loveless & Hamrick, 1984). Pollination can take place within a single plant, when one flower receives pollen from another flower; or between plants, when pollen is transferred from one individual of a species to another. Alternatively, plants can utilize outcrossing, another method of pollination that involves introducing unrelated genetic material to plant populations which is generally better for increasing genetic diversity (Hamrick et al., 1992).

Attracting pollinators is crucial to the survival and fitness of biotically-pollinated plant species that undergo sexual reproduction. In order to attract pollinators and thereby maintain a stable population, plants must be identifiable to pollinators (Sargent, 2008). Most flower visitors innately prefer a particular color and scent, using these as cues for flower recognition and selection. As a result, many flowers have evolved specific mechanisms to effectively attract pollinators, including production of flowers, nectar, fragrance, and color (Ômura, 2005). In this mutualistic relationship, plants benefit when pollinators aid in their successful fertilization and many pollinators (e.g. insects, hummingbirds, and bats) may benefit by gaining access to substantial food sources that the plants provide (Harbourne, 2001).

This study was conducted at the University of Michigan Biological Station (UMBS), located in Pellston, MI, which provides habitats for a wide variety of flowering species. The UMBS is a temperate environment containing large tracts of forests that are primarily made up of second-growth aspens, northern hardwoods, and conifers (Heinen & Vande Kopple, 2003). Pollination in the region is typically generalized, meaning that a variety of pollinators visit a variety of plant species (Wasser et al., 1996). This contrasts with specialized pollination syndromes, which are typically seen in plants that rely on only one or a few pollinator species and are often the product of coevolution between these plants and their pollinators (Johnson, 2000).

During the spring, plants in the forest understory at UMBS produce flowers that are predominantly white. Common white flowering plants include lilies like Canada Mayflower (*Maianthemum canadense)*, Starry False Solomon's Seal (*Maianthemum stellatum)*, starflowers (*Trientalis borealis*), blueberries (*Vaccinium angustifolium*), trillium (*Trillium grandiflorum*), and bunchberries (*Cornus canadensis*). It is possible that these plants have evolved to produce white flowers because white flowers confer an evolutionary advantage. For example, white flowers may be more visible to pollinators in the understory. Additionally, some of these flowers grow in large patches, while others are found growing in small patches or as lone individuals. Perhaps pollinators can more easily spot flowers in large patches, while flowers in small patches are more easily overlooked.

Several studies have investigated pollinator preferences for color and patch size. Pollinators often use visual cues in order to find flowers to visit (Chittka & Raine, 2006). The visual resolution of bees–some of the most common pollinators–is significantly lower than that of humans, which means that they must be very close to flowers in order to identify their color. Insects use the light receptors in their eyes to distinguish and hone in on certain flowers by comparing them to the overall appearance of the background (Chittka & Menzel, 1992). As a result, it is more likely that insects will see flowers that contrast with the surrounding area. Insect vision can be mimicked by looking at plants under UV lighting (Chittka & Raine, 2006; Figure 1). Although white flowers may not be easy to distinguish with human eyes, almost all white flowers are UV-absorbing and are therefore equally reflective in the visual spectrum of the insects (Kevan, Giurfa & Chittka, 1996). A study conducted on adult Asian Admiral butterflies (*Vanessa indica)* showed that the butterflies preferred odorless yellow flowers significantly more than scented purple flowers, which further demonstrates the importance of color to pollinators (Ômura, 2005).

Patch size (or flower density) is also a significant variable in pollinator decision-making, as pollinator visitation rates have been found to be significantly higher for flowers in denser groups (Kunin, 1997). In lower light conditions, which are common in the understory, it becomes more difficult for pollinators to see well. Therefore, large patches may be more likely to attract the attention of pollinators (Kilkenny & Galloway, 2008).

For this project, we focused on the parameters of color and patch size. We hypothesized that pollinators would prefer white flowers over purple flowers in the forest understory. We also hypothesized that pollinators would have higher visitation rates at large patches of flowers over smaller patches of flowers. We formulated these hypotheses based on two assumptions. First, we assumed that white contrasts to the backdrop of the understory more than purple, and is therefore more likely to attract pollinators. Second, we postulated that a larger patch size would be more visible to a pollinator than a smaller patch size, thereby making it more likely that pollinators would choose larger patches over smaller patches.

Methods

To test our hypotheses, we conducted an observational experiment to examine pollinator visitation rates using Alyssum (*Lobularia maritima).* Four flats of Alyssum flowers (192 total flowers) that contained equal numbers of white and purple plants were purchased. We chose to use Alyssum because it has small white flowers similar to many of the wild plants found in the understory near UMBS. We chose Grapevine trail–which runs north on an incline along the west side of Douglas Lake's South Fishtail bay on UMBS property–as the location for this study due to its proximity to the main camp as well as its relatively consistent forest understory conditions, which are representative of the surrounding ecosystem. Starflower (*Trientalis borealis)* and several species of lilies (*Maianthemum)* are common in the understory around the trail, along with seedlings of Striped Maple (*Acer pensylvanicum)* and Sugar Maple (*Acer saccharum)*. The forest canopy is primarily comprised of American Beech (*Fagus grandifolia),* with populations of Aspen (*Populus)* and Sugar Maple (*Acer saccharum*), among others.

We divided the flowers into twelve separate plots consisting of three plots in each test group (small white, large white, small purple, and large purple). Small plots contained four plants, while large plots each had 24 plants. We spaced the plots randomly between five and ten meters apart, with six along each side of Grapevine trail. Special consideration was given to light levels at each plot site and a lux meter was used to ensure relative uniformity of light conditions at each plot. We conducted ten minute observation periods three times a day (at 7 am, 11:30 am, and 5:00 pm) for eight separate days. During these observation periods, we recorded both the number of pollinator visits and pollinator types at each site. If the same pollinator visited the same plot twice during an observation period, it was counted twice. Other information collected during each observation period included weather conditions, light level, and any other factors that may have affected pollinator visitation rates. Observers rotated plots each day in an attempt to remove bias from the data.

Results

A Chi-Squared Test of Independence was conducted to determine whether there was a difference between the distribution of pollinator visitation to plots based on patch size or color. The results of this analysis were statistically significant ($p=0.0687$). This test indicates that large and small samples, or purple and white samples, are independent of each other. The results support our hypothesis by indicating that the distributions of pollinator visits to flowers of distinct colors and sizes are not the same.

To interpret the difference between mean pollinator visits per observation period in large patches compared to small patches, a non-parametric Mann-Whitney U test was used. The results showed that average pollinator visits to large patches of flowers was significantly higher than the average number of visits to small patches ($p<0.0001$) and supports the hypothesis that large patches of flowers would receive more pollinator visits than small patches (Table 1). A second Mann-Whitney U test was conducted to determine whether there was a significant difference between mean pollinator visits per observation period for white and purple patches. This test demonstrated that white patches of flowers, on average, do attract significantly more pollinators than purple patches (p=0.01), corroborating our hypothesis (Table 1).

Table 1: The mean number of visits by pollinators for all large, small, purple, and white plots during any ten minute observation period.

Plot type	Mean # of Pollinator Visits per Observation Period
Small	0.1701
Large	0.8298
Purple	0.3962
White	0.6037

A linear regression model was used to interpret the relationship between pollinator visits and time of day in large, small, purple, and white patches. The resulting R-squared values (Table 2) suggest that 0.6% of the variation in pollinator visits to small patches, 12% of variation in visits to large patches, 5.7% of variation in visits to purple patches, and 3.8% of variation in visits to white patches, can be attributed to temperature. All of these correlations are statistically significant ($p<0.05$), except for the relationship between visitation to small patches and temperature (Table 2). The associated scatter plots show an upward-sloping trendline, with more pollinator visits at higher temperatures.

Table 2: R-squared values and p-values from a linear regression analysis of the relationship between temperature and pollinator visits. Values provided for each patch size and color at a significance level of 0.05.

Treatment	**R^2**	**Significance**
Small	0.006	0.373
Large	0.120	<0.0001
Purple	0.057	0.003
White	0.038	0.013

Since we consistently collected data at the same times every day, we were also able to test whether there was a difference in pollinator visits in the morning (7-7:30am), mid-day (11:30am-12pm), and evening (5-5:30pm). A Kruskal-Wallis test showed that there was a difference in mean number of pollinator visits during these three times ($p=0.01$). Across eight days, we observed a total of 28 pollinator visits in the morning, 290 visits at midday, and 111 visits in the evenings (Figure 2). The midday observation period had over twice as many pollinator visits as the evening period, and over 10 times as many as the morning period.

Figure 1: Purple flowers (black) and white flowers (front) under UV light

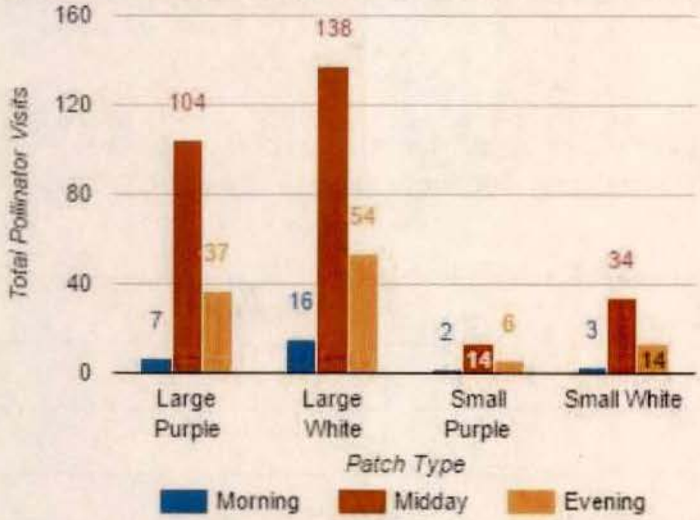

Figure 2: Total pollinator visits over the course of the 8-day study for specific patch types and times of day.

Discussion

Our findings supported our hypotheses that more pollinators would visit large patches and white flowers over small patches and purple flowers. We also found that mid-day was the peak time for pollinator visits. A similar study conducted on pollinator visitation over a wider variety of flower colors confirms preferential pollination rates of white over purple as pollinators as seen on the UV light spectrum (Reverté et al., 2016; Figure 1). Colors that appear blue to bees and UV-blue flowers (seen as blue and violet to humans) are colors typically associated with large bee pollinators. Blue-green and green (yellow and white to humans) are colors often linked to higher rates of fly pollination (Arnold et al., 2009). Pollinators have relatively short ranges of vision, so they are more likely to be able to identify large patches of flowers while overlooking small patches of flowers. Additionally, data which indicate peak pollinator rates during afternoon viewing periods may be related to amount of sunlight but not temperature. A similar study conducted in 2013 found that high pollination rates correlate with more sunlight exposure, which is typical of the afternoon viewing period when the sun is highest (Bastain et al., 2013).

Several sources of error may have impacted our results in this observational study. First, since there were twelve different plots, not every individual could be present for every observational period. Instead, each individual observed three plots per day. While we tried to account for possible variations in how each individual observed or classified pollinators, individual bias may have impacted our results. Different individuals have different eyesights and the majority of the pollinators we observed were small and often hard to see. It is also possible that our physical presence affected the behavior of the pollinators. For instance, it is possible that the pollinators were attracted to us due to our clothing, heat, scent, or other factors. Although we tried to stay as still and quiet as possible, we may have scared insects away from pollinating the flowers or acted as a barrier between pollinators and flower patches. Additionally, in an effort to standardize soil quality among the flower patches, we kept the plants in the original plastic packaging from the flower flats. This meant that the flowers were slightly above ground, and while flying pollinators such as bees and flies were able to easily access the flowers, other types of pollinators (such as ants) may have had a harder time accessing the flower patches. Lastly, Alyssum are not native flowers to this region, which may have impacted their rates of pollination. Studies have shown that native pollinators are better at pollinating native plants than non-native plants (Pardee, 2014).

We also encountered a number of confounding variables that we were unable to control. While we controlled for distance between plots, some patches may have had more exposure to humidity and wind because they were closer to the lake. Furthermore, although we initially chose locations for our plots that had similar exposure to light, the amount of sunlight that each patch received still varied throughout the day. Finally, since we conducted the experiment in the understory, other wild white flowers were not evenly distributed around our patches. If a patch was located near a number of other wild white flowering species, pollinators may have been able to identify our plants more easily.

Conclusion

Our results supported both of our hypotheses. First, pollinators in the forest understory at UMBS did prefer white flowers over purple flowers. Additionally, pollinators were more likely to visit flowers that were in large patches as opposed to small patches. A future study that compares pollinators' visitation rates to white non-native plant species versus their visitation rates to flowers that are native to the ecosystem surrounding UMBS would provide more insight into the behaviors of pollinators. In our study, we classified ants that visited our patches as pollinators, although there is scientific debate over whether they do, in fact, pollinate flowers. Further study might look specifically at whether ant behavior can be classified as effective pollination. While our results indicate that larger patches of flowers receive higher rates of pollinator visitation, future studies could investigate whether large patches of flowers also receive more pollinators per inflorescence. Finally, we recommend further study into what patch size results in peak pollinator visitation, which would provide insight into what conditions provide the highest likelihood that flowering plant species will be pollinated.

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