

Effect of Native and Non-native Flower Species on Pollinator Behavior

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Abstract

The effect that introduced flower species may have on the pollination events for native species is important to understand because a species' reproductive success depends on the pollination dynamics of the plant. Introduced species are often seen as have a stronger floral signal and hardiness, especially since they are leaving the area where their natural predators are and require the attention of pollinators in order to reproduce.

Therefore, we hypothesized that since introduced species in an early successional field in the Great Lakes area have a wide field distribution, then they must be effective at attracting pollinators and therefore must decrease the number of pollination events and pollinator morphotypes for native species. We assessed (1) how many times a pollinator landed on the flowers of the observed plots, and (2) how many different morphotypes of pollinators visited each plot, and determined how the species' native status, average flower number, plant density, and the time of day that observations were recorded affected visitation rates. The study showed that the average number of pollinators and morphotypes that visited the species in the field were not determined by the native status of the species but rather by the plant and flower density of the plot and the time of day that observations were made. This study suggests that introduced species may not affect pollinator behavior as significantly as the floral signal of the species and the time of day.

Introduction

Pollinators are organisms that facilitate the reproduction of flowering plants by spreading pollen between conspecific flowers. There are approximately 200,000 species of pollinators worldwide; roughly 1,000 are vertebrates, such as birds, bats, and small

mammals, and the rest are invertebrates, including flies, beetles, butterflies, moths, and bees (USDA 2013). Pollination is usually the unintended consequence of animals collecting pollen for its protein and other nutritional characteristics or sipping nectar from the flower, when pollen grains attach themselves to the animal's body. When the animal visits another flower for the same reason, pollen can attach to the flower's stigma, resulting in successful fertilization of the flower (Baird et al. 1991). The presence of introduced species in an area can compete with native species through changes in this pollinator behavior as they visit more rewarding introduced species. This change results in either reduced visitation to native plants and less pollen transfer between native flowers or in deposition of heterospecific pollen that reduced pollen quality of the plant (Brown and Mitchell, 2000).

Dietzch et al. (2011) studied the effect that the invasive species *Rhododendron ponticum* had on pollinator visitation, conspecific and heterospecific pollen deposition, and seed set for the native species *Digitalis purpurea*. The study found that flowering invasive plant species that are visited by native insects and overlap with native plant species in their pollinators may facilitate or disrupt native flower visitation and fertilization by forming large, dense populations with high numbers of flowers and copious rewards. Flower visitation by pollinators to *D. purpurea* was significantly lower at higher alien relative plant abundances than at lower abundances or in the absence of the alien. The invasive species nearby did not negatively impact the reproductive success of the native species, although the presence of the invasive plant population disrupted pollinator visitation levels and conspecific pollen disposition (Dietzch et al. 2011). A similar study by Memmott and Waser (2002) found that flowers of native plants were

visited on average by more animal species than flowers of alien plants. This was true even though native plants had a flowering period on average only about half as long as that of alien plants, which would tend to expose natives to a smaller fraction of all potential flower visitor.

Woods et al. (2010) examined how pollinators interacted with the invasive *Lespedeza cuneata* versus native congeners in tall grass prairie. Their research showed that there was variation in which pollinator visited which plant most frequently, and that flower density and visitation rates was correlated for most native species, where greater flower density attracted more pollinators, but not for invasive species. Other studies (Lopezaraiza-Mikel et al. 2007, Molina-Montenegro et al. 2008) have reported that alien plant species attract native generalist pollinators to an area and hence facilitate pollination of native plant species that are in close proximity, but in some alien species, this effect was only observed at low but not high flower densities. This could be due to the fact that low density flowers of invasive plants may be able to attract pollinators to an area that they do not usually visit (Munoz and Cavieres 2008), but higher flower density favors higher pollinator visitation rates to that particular plant as it gives pollinators more pollen or nectar for less effort (Legatzke 2015). Pyšek et al. (2011) stated that the bottleneck effect of species, which is the theory that only the strongest species will be able to survive in a new area by co-opting the services of existing pollinators and successfully integrating into the local pollination web, allows introduced species to increase their visitation rates by pollinators over time.

Given the variation in how pollinators have been found to react to introduced species, this study examines how pollinator visitation to an early successional field in the

Great Lakes region varied between native and non-native wildflower species.

Specifically, we examined whether introduced species have a greater rate of individual pollinator visitation and morphotype rates than native species. We hypothesized that introduced species would average more pollinator visits and pollinator morphotypes than native species would, as introduced species often out-compete native plants for both resources and area to grow (Chrobock et al. 2012) and can attract pollinators to an area that they do not typically go to (Molina- Montenegro 2008).

Methods

Study organisms and site

Four native and four introduced species were chosen for observation based on what was flowering between June 15th, 2016 and July 15th, 2016. The introduced species studied were *Leucanthemum vulgare* (oxeye daisy), *Hieracium aurantiaca* (orange hawkweed), *Potentilla recta* (sulfur cinquefoil), and *Lotus corniculatus* (birdsfoot trefoil). The native species studied were *Erigeron annuus* (daisy fleabane), *Rudbeckia hirta* (blackeyed susan), *Achillea millefolium* (common yarrow), *Asclepias syriaca* (common milkweed). *L. vulgare* Lam. (Asteraceae) is considered a noxious weed, defined in the Noxious Weed Act (1974) as any weeds that can be seen to have adverse effects on man or the environment, that aggressively invades fields where it forms dense populations and decreases plant species diversity. It is typically pollinated by small bees, flies, beetles, wasps, small butterflies, and skippers (www.cals.uidaho.edu). *H. aurantiaca* L. (Asteraceae) is another noxious weed visited by butterflies, bees, and bumblebees, though it can produce its seeds without fertilization and offers minute

quantities of nectar to pollinators (U.S. Forest Service). *P. recta* L. (Rosaceae) is a long-lived plant that is capable of forming a monoculture in fields, and relies heavily on cross-pollination by both long-tongued and short tongued bees, flies, butterflies, and beetles (eol.org). *L. corniculatus* L. (Fabaceae) is a long-lived legume and its flowers grow in clusters low to the ground and are pollinated mainly by bees that can push through the stipules of the flowers (pfaf.org).

E. annuus L. (Asteraceae) have small flowers, usually several per plant, that are pollinated by Lepidoptera, bees, and beetles, but can also form seeds asexually (pfaf.org). *R. hirta* L. (Asteraceae) is a tall, annual, short-lived plant with an average of one flower per plant that attract birds, bees, butterflies, flies, and beetles that are attracted to its nectar (fcps.edu). *A. millefolium* L. (Asteraceae) is a tall plant with bunches of tiny flowers growing at the top of the stem, and it is well known for attracting insects such as flies, short-tongued bees, and bumblebees (pfaf.org). *A. syriaca* L. (Asclepiadaceae) is a tall, fragrant plant with large bunches of flowers growing off the stem of the plant that attract a wide variety of insects including moths, butterflies, beetles, bumblebees, bees, hemiptera and hymenoptera, and wasps (fcps.com).

All plants flowered in the same early successional field, located at the University of Notre Dame Environmental Research Center in the Upper Peninsula of Michigan (Latitude: 46.227685 | Longitude: -89.5237). The field is surrounded on three sides by a mixed deciduous-conifer forest and a wetland on the fourth side and receives full sunlight but little interaction with roads.

Data collection

To test whether native flowering plants attract pollinators at different rates than introduced species, I established five plots of ten plants for all eight species. For *L. vulgare*, which grows evenly over the whole field, plot locations were chosen by randomizing along two perpendicular transects. All of the other species except *A. syriaca* grew in patches sporadically across the field, and their plots were chosen by determining which bunch had at least 10 plants near enough to each other to allow for all plants to be observed simultaneously. Plots for *A. syriaca* had large bunches of flowers that grew along the stem, and so ten of these bunches were chosen as they were easier to observe, carried a large number of flowers, and were spaced well enough that it could easily be determined which bunch the pollinator visited.

Observations occurred between 12 pm and 5:30 pm in order to ensure that the flowers were open and pollinators were active. Excessively cold or rainy days were omitted as pollinators had observably lower activity during those time, and the order plots were observed was varied to control for how pollinator activity varied by time of day. Visitation was defined as the pollinator landing on the flower for any period of time, or an insect walking on the area of the flower where pollen was present.

Each plot was observed for 10 minutes a day for a minimum of 2 times. For each of the ten plants in a plot, the number of flowers was recorded, in addition to the number of pollinator visits and pollinator morphotypes observed during the 10 minute period. This allowed for distinguishing between the numbers of individual pollinators that visited the plot and the number of pollination events that occurred (i.e., one pollinator could be responsible for multiple pollination events). All pollinators were identified to order and

family when possible. Time of observation and weather conditions were also documented.

Statistics

Visitation variation between native and introduced species

For the native and introduced species, we looked at whether the pollinator visitation and morphotype rates were higher for the introduced species than the native species. The observation times varied for each plot, so the visitation numbers for each plant were divided by the total number of minutes that plant was observed to calculate the average pollinator visitation rates for the plant. These values were further averaged to get the visitation rates at the plot and the species level. The morphotype number was calculated by averaging the morphotype that visited observed plants for the species. As none of the data could be transformed to achieve normality, we used a Mann Whitney U Test.

Visitation variation based on flower number

The relationship between a plant's flower number and its average pollinator visitation rate was tested to see if plants with more flowers were more effective at attracting pollinators. The plants were also sorted into high flower density and a low flower density and differences in average pollinator visitation rates between the categories was tested. The flower density was determined to be high if it was greater than 8 flowers, the average flower density of all plants across species. A Spearman Regression was used to see if plants with a higher flower number had a higher average visitation rate,

not including and a Mann Whitney U test was used to be if the plots classified as having a high plant density had more visitors than low density plots. *R. hirta* and *L. vulgare* were excluded from these tests as all plots had only one flower per plant.

Visitation variation by plant and flower density

In order to test if species with a higher density of plants in a plot had more pollination events on average, we used a Pearson Correlation test, where the plant density was determined by dividing the number of plants in a plot by the area of the plot. *A. syriaca* had 3 plants for each plot while all other species had 10 plants per plot. Whether a greater flower density of in a plot, defined as the total flowers in the plot divided by the area of the plot, was related to a greater average pollinator visitation rate was also tested using a Pearson correlation.

Visitation variation by time

How the time of observation affected the total number of pollinator visits to the plot during that time was assessed to see if pollinator activity were varied throughout the day, mainly by decreasing with time. Whether observation time and visitation number were related was determined using a Pearson correlation, and a Mann Whitney U Test was used to compare observations that occurred in early afternoon and late afternoon, before 2:45pm and after 2:45pm, respectively. This time was chosen because it was half way through the average observation time frame.

All test statistics were run using R-Studio.

Results

Overall, we observed *L. vulgare*, *H. aurantiaca*, *P. recta*, and *L. corniculatus* as introduced species; and *E. annuus*, *R. hirta*, *A. millefolium*, and *A. syriaca* for native species. All species had 50 plants that were observed except for *A. syriaca*, where only 15 plants were observed. In total, there were 200 plants and 580 minutes of observation for introduced species, and 165 plants and 750 minutes of observation for native species. The average number of visitations per minute, as well as the average number of morphotypes, for the species is reported in Table 1.

Visitation variation between native and introduced species

No significant differences were found between the values for native and introduced species for average visitation rates (Figure 1, Mann Whitney U test, $U = 12$, $p\text{-value} = 0.3429$) or for average morphotype numbers (Mann Whitney U test, $U = 11$, $p\text{-value} = 0.4857$).

Visitation variation based on flower number

The number of flowers on a plant and the average number of visitors to that plant were positively correlated (Figure 2, Pearson correlation, $t = 9.3097$, $df = 298$, $p\text{-value} < 2.2e-16$, $n=400$). When broken down by species, it is clear that this pattern was driven by *E. annuus* (Pearson correlation, $t = 5.29$, $df = 48$, $p\text{-value} = 2.928e-06$, $n=10$) and *P. recta* (Pearson correlation, $t = 3.3456$, $df = 48$, $p\text{-value} = 0.001601$, $n=10$). There was no significant relationship between flower number, and visitation rates for *H. aurantiaca*, *L. corniculatus*, *A.*, and *A. millefolium* (Pearson correlation; all $t\text{-values} > 0.0726$, all $p\text{-values} > 0.9424$). A positive correlation was also seen between the average flower number of a species and the average visitation number for that species (Figure 4, Pearson correlation, $t = 3.1445$, $df = 6$, $p\text{-value} = 0.01995$). When analyzed categorically, plants

considered to have many flowers attracted more visitors than those considered to have few flowers (Mann Whitney U test, $U = 1202.5$, $p\text{-value} = 0.04259$, $n=400$).

Visitation variation by plant and flower density

The plant density of the plot (i.e. # of plants in plot/ area of plot) had no correlation with the average visitation rate of the plot (Pearson correlation, $t = -1.1428$, $df = 38$, $p\text{-value} = 0.2603$, $n=40$). A comparison of the flower density of the plot to the average visitation rate also found no significant correlation between the two (Pearson correlation, $t = -0.7599$, $df = 39$, $p\text{-value} = 0.4519$, $n=40$).

Effect of time of day on visitation numbers

There was a significant negative correlation between the time of observation for a plot and the total number of pollination events for the plot during that time (Figure 3, Pearson correlation, $t = -2.0203$, $df = 119$, $p\text{-value} = 0.0456$, $n=400$). Visitation rates also showed significant positive difference when observations were done in the late afternoon relative to the early afternoon (Mann Whitney U test, $U = 1202.5$, $p\text{-value} = 0.04259$, $n=400$). Early afternoon observations had an average of 9.67 visits per plot (STDEV= 15.0365, Std. error= 1.6605, $n= 82$) and late afternoon observations had an average of 14 visits per plot (STDEV= 14.4368, Std. error= 2.342, $n=38$).

Discussion

Overall, the data does not support our hypothesis that average pollinator visitation rates of introduced plant species were greater than those of native plant species.

However, the data did show that there were variations in visitation rates among the observed species (Figure 1), as well as the number of morphotypes (Figure 4). The native

A. syriaca attracted the highest number of pollinator visits and morphotypes because of its strong flower signal (fragrant bunches of flowers, tall plant, and high flower density), as well as the fact that butterflies, hemiptera, moths, and some beetles are noted to visit *A. syriaca* but not the other species in this study (Stoepler et al. 2012). *P. recta* is an introduced species and had the second highest number of average pollinator visitations and morphotype numbers, which could be explained by the fact that it is in the same family as *Potentilla simplex*, or common Cinquefoil, which is native to the area of the United States where the study field is located (USDA). A study by Pyšek et al. (2011) found that if an introduced species is closely related to a native species in the area, it is more easily able to co-opt the pollinators of that native plant.

Pollinator visitation and morphotype rates were the lowest for *H. aurantiaca*, which could have been because the species had been flowering for some time before observation, so many flowers were past their peak and had seeded the week after observations (Krushelnycky 2014). *L. vulgare* and *R. hirta* had very similar visitation and morphotype rates (avg. visitation= 0.136666667, avg. morph. =0.48, and avg. visitation= 0.14, avg. morph. = 0.4, respectively). This similarity could be due to the fact that both species had a wide distribution of plants across the field, decreasing the probability that the pollinator would visit a flower in our plot. This could also be explained by the fact that both species only carried one flower per plant which is less attractive for pollinators, as data for this study and from a study conducted by Legatzke (2015) found a positive correlation between the number of flowers on a plant and the of number pollinators that visit it.

Our study did suggest that having a greater number of flowers per plant was related to a higher average pollinator visitation rate when all species were considered, though this relationship was species dependent- only *E. annuus* and *P. recta* had a relationship when species specific tests were run between flower number on a plant and average visitation rate to that plant. The reason for this is unclear, as neither of these species had the highest average flower number (6.52 and 1.64, respectively) and the species were not of the same family nor had similar patterns of flower growth.

The flower density and plant density were also positively correlated to how often a plot was visited, which is consistent with a study by Harmon-Thereatt et al. (2008) that also found that a species with higher plot densities had more pollination events than species with lower plot densities. This could be explained by the optimal foraging theory, which states that animals such as pollinators will maximize resource acquisition per effort spent foraging by choosing food sources that provide more reward with less effort (Pyke et al. 1977). Visiting species that have densely packed plants with many flowers allows the pollinators to collect pollen more efficiently as the pollen density is also greater, so it is more likely that pollinators will choose these highly dense species.

The time of observation also affected the average visitation rate for the species. Observing plots later in the day was correlated with lower rates, possibly because the peak flowering time of species is earlier in the afternoon and the floral signal (i.e. scent) of the plant decreases with time (Rodriguez-Saona et al. 2010). This correlation could also be due to decreases in sunlight and temperature with time, which causes flowers to slowly close and then reopen during the mid-morning, meaning that the flower is more attractive to pollinators earlier in the day (He et al. 2005).

Overall, the results of our study indicated that the average visitation rate to a species was not related to the native status of the species, but rather on the specific characteristics of the species as explained by the optimal foraging theory. High flower numbers on the plants and a high density of the plants and the flowers in an area were positively related to the average rates because they allow the pollinators to visit more flowers without expending much energy, so these species would be the most beneficial to forage on. This understanding is important for determining what effect future introduced species to an area that have long flowering times and large floral displays would have on native species in the area (Krushelnycky 2014).

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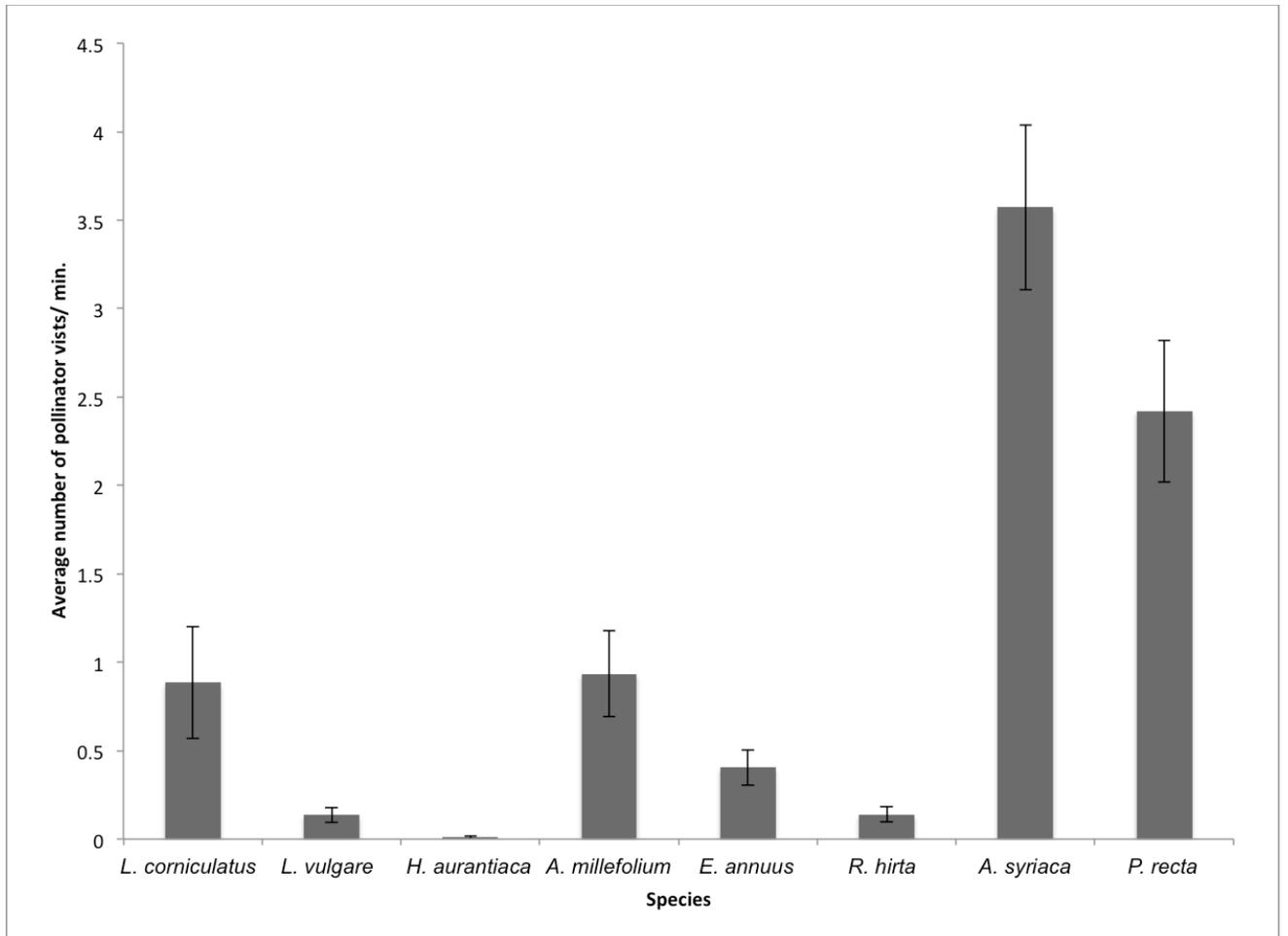


Figure 1: The graph shows the average number of pollinators for each species during the observation period, and the standard error of those averages. All averages are adjusted to account for the actual length of time that the species were observed by dividing the visitation numbers by the observation time. No difference was found between average visitation rates for introduced and native species (Mann Whitney U test, $U = 17$, p -value = 0.4206).

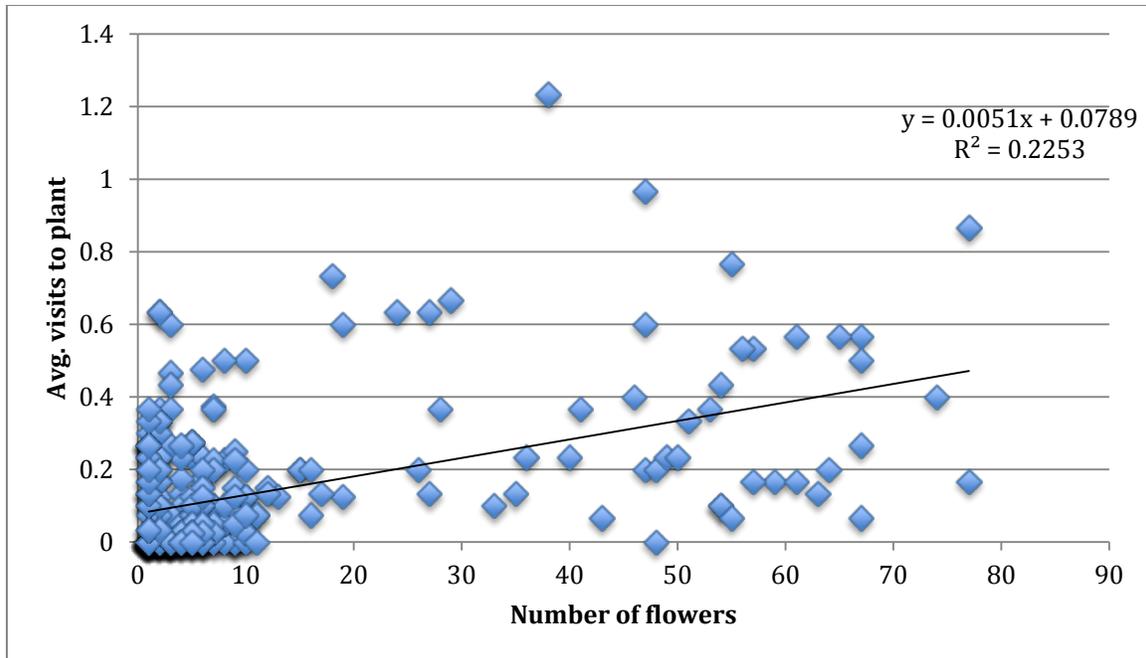


Figure 2: The number of flowers on a plant was graphed against the average pollinator visitation rate to that plant. It was shown that flower number and visitation rates were positively correlated (Pearson correlation, $t = 12.1686$, $df = 398$, $p\text{-value} < 2.2e-16$, $n=400$), though this correlation was not true for all species. Categorical distribution of plants showed that those that had greater than 8 flowers on them had greater visitation rates than those with less than 8 flowers (Mann Whitney U test, $U = 1202.5$, $p\text{-value} = 0.04259$, $n=400$).

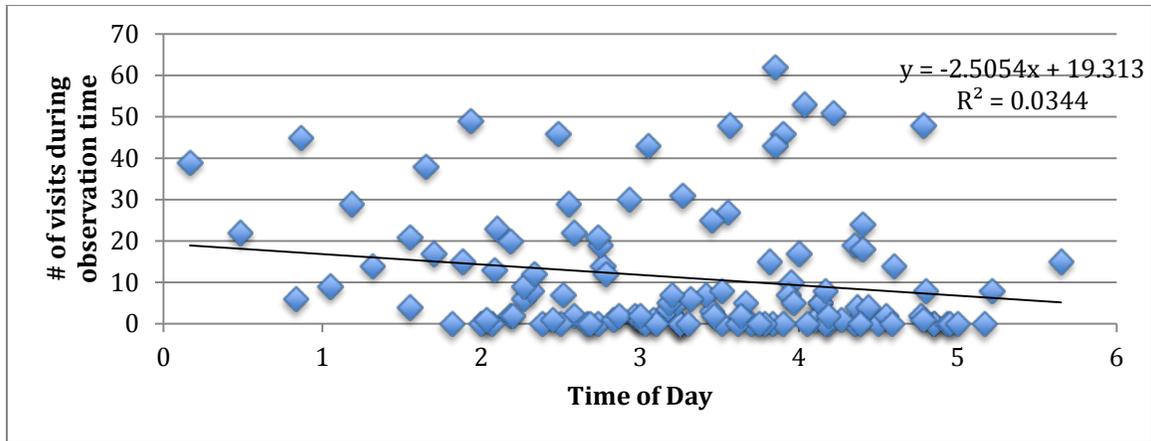


Figure 3: The total number of visits to a plot during an observation time plotted against the time that the observation period had begun. A negative correlation was found between the time of observation and the pollination rates (Pearson correlation, $t = -2.0203$, $df = 119$, $p\text{-value} = 0.0456$, $n=400$), and observations taken in late afternoon had lower average visitation rates than those taken in early afternoon (Mann Whitney U test, $U = 1202.5$, $p\text{-value} = 0.04259$, $n=400$).

Table 1: The average number of visits and morphotypes per minute, as well as the standard deviation and standard error, for all of the species.

Species	Avg. visit./m in	STDEV	Std. error	Avg. Morpho.	STDEV	Std. error
<i>L. corniculatus</i>	0.8850	0.7063	0.3159	0.5800	0.5325	0.0753
<i>L. vulgare</i>	0.1367	0.0941	0.0421	0.4800	0.5741	0.0812
<i>H. aurantiaca</i>	0.0100	0.0200	0.0089	0.0200	0.1400	0.0198
<i>A. millefolium</i>	0.9350	0.5414	0.2421	1.3600	0.8663	0.1225
<i>E. annuus</i>	0.4050	0.2238	0.1001	0.7800	0.7820	0.1106
<i>R. hirta</i>	0.1400	0.0998	0.0446	0.4000	0.6000	0.0849

<i>A. syriaca</i>	3.5733	1.0434	0.4666	3.5800	1.7560	0.2483
<i>P. recta</i>	2.4200	0.8961	0.4007	2.3000	1.0817	0.1530

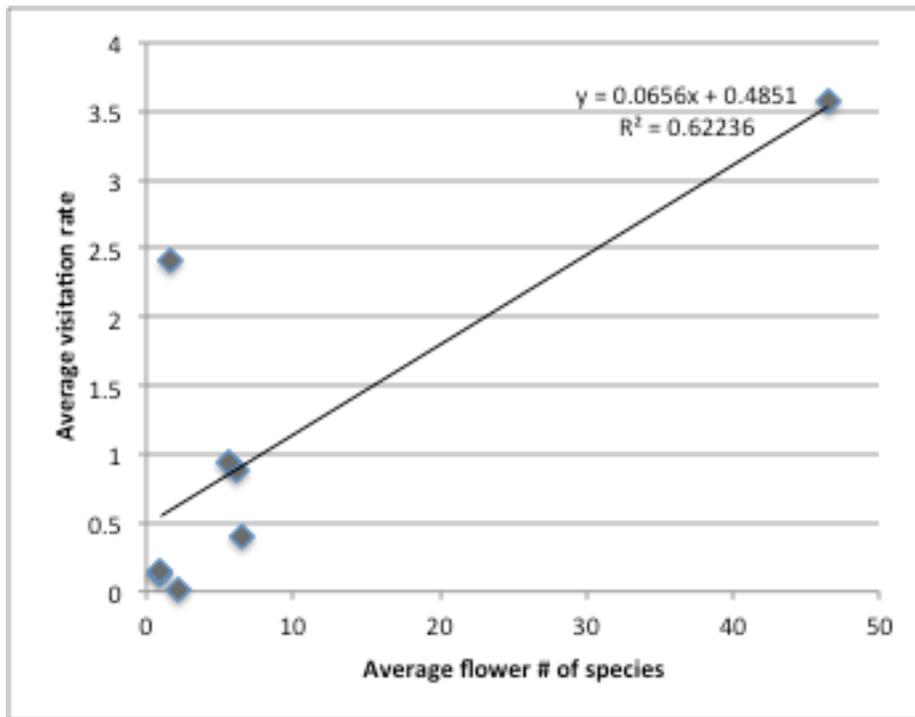


Figure 4: Graph of the average flower number for a species versus the average visitation rate for that species. There was a strong positive correlation between the two variables (Pearson correlation, $t = 3.1445$, $df = 6$, $p\text{-value} = 0.01995$)