The effect of flowering plant density, location, and fitness on pollination rates

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Abstract

Understanding patterns in pollination is important because pollination is vital to the success of many plant species, including cultivated commercial plants and endangered species. Optimal foraging theory predicts that natural selection favors insect pollinators that gather resources in the most efficient method possible. Therefore, we expect that higher densities of plants and flowers will be pollinated more frequently because they provide pollinators with more resources per search and travel time. We conducted observations of (1) a manipulated population of *Hieracium aurantiacum* L. (Asteraceae) to study how pollinator recruitment and pollination rates are affected by open flower density and (2) a naturally distributed population of *Barbarea vulgaris* L. (Brassicaceae) to study how the geographic location, relative density, and fitness of individual plants affect pollination rate. Within one population of *H. aurantiacum,* we observed that increases in the density of open flowers increased the number of pollinators recruited and the pollination rate, especially during times of day with higher light availability. For the *B. vulgaris,* we found that pollination rate was correlated with the number of open flowers on an individual but was independent of a plant's location in the population, nearest neighbor distance, plant height, and whether a studied plot had many or few plants and flowers. This study suggests that flower number within a small area or on an individual is one factor that influences pollinator visitation.

Introduction

Pollination is vital to the reproductive success of many plant species. Over 80% of crop production relies on insect pollinators (Flamini and Cioni 2010). Pollination increases the genetic diversity of plant species by potentially limiting the risks of inbreeding depression and/or extinction (Koelling et al. 2011). Moreover, pollinator limitations control the number of seeds

produced by many species (Bierzychudek 1981), suggesting that access to pollinators may have important consequences for a species' ability to maintain populations. Decreases in pollination rates limit the geographic distribution of plants, as the ranges of the least clonal orchid species are most restricted by access to pollinators (Pauw and Bond 2011). In this study, we examine patterns of pollination to better understand the distribution of individuals in plant populations.

Pollinator behavior is impacted by the plant communities in which they forage. Optimal foraging theory predicts that animals, like pollinators, that maximize resource acquisition per effort spent foraging may be more likely to survive (Pyke et al. 1977). Insects can learn to discriminate between rewarding and non-rewarding flowers based upon flower age, size, sex, symmetry, and sometimes even odors left by previous pollinators (Goulson 1999). Bees and butterflies demonstrate trap-lining behavior where they follow regular routes to avoid revisiting flowers (Goulson 1999). Using floral cues and avoiding previously visited flowers allows pollinators to forage more efficiently by searching only flower patches that are likely to have lots of nectar and bypassing those that may not. Larger, more apparent patches of flowers may recruit pollinators more regularly (Goulson 1999). Various studies suggest that areas with denser distribution of flowers are more frequented by pollinators (Weber and Kolb 2012; Grindeland et. al 2005 and Kunin 1993) potentially because of the higher availability of nectar resources in dense patches.

The behavior of pollinators has implications for the reproductive capacity and abundance of plants in groups of different densities. In a field experiment, Kunin (1993) found pollinator visitation rates to *Brassica kaber* declined with plant density. As a result, populations with greater densities of individuals also had greater reproductive success (Kunin 1997). Metcalfe and Kunin (2005) found that reproductive success of *Cistus ladanifer* was negatively correlated with distance to nearest neighbor. Bartowska and Johnson (2014) observed that on large spatial scales, increasing

the density of *Lobelia cardinalis* led to increased seed production per plant and hypothesized that this increase was due to more frequent pollinator visitation. Given the importance of pollination for the reproduction of plants, we looked at patterns of pollination in two species of wildflower to better understand how plant density, height, and flower number affected pollination rates.

In the first part of this study, we experimentally manipulated the number of open flowers in patches of *Hieracium aurantiacum* L. (Asteraceae) to test whether the number of (a) pollinators and (b) pollination events were higher in patches with higher flower density. *H. aurantiacum* is a vibrant orange flower that can reproduce sexually or asexually (Callahen et al. 1997). It is pollinated by bees and butterflies (Strickler et al. 1996; Voss 1954), which have demonstrated sophisticated foraging strategies such as trap-lining (Goulson 1999). Given that these pollinators demonstrate sophisticated foraging techniques, we expect that they will demonstrate a preference for patches with high floral density where there are potentially more resources.

In the second part of this study, we investigated patterns of pollinator visitation across a single population of *Barbarea vulgaris* L. (Brassicaceae) with natural variation in plant and flower density. *B. vulgaris* can reproduce sexually or asexually (MacDonald and Cavers 1974) and is pollinated by bees and flies (Dailey and Scott 2006). Work done by McQuestion in 2014 at the same location found that plant density and fitness of *B. vulgaris* tended to be higher toward the periphery of the population. As individual abundance and fitness are often correlated with optimal environmental conditions (Van Couwenberghe et al. 2012), peripheral *B. vulgaris* plants may occupy optimal environmental conditions within the population. Pollinators may contribute to these optimal conditions because their behavior may be distinct at the periphery of the population as compared to the center. Furthermore, the higher density of peripheral plants may contribute to

a positive feedback loop in which the higher density of peripheral plants attract pollinators at a higher rate encouraging more reproduction and increasing plant density.

Here, we examined whether pollinator visitation was associated with the density, location, and fitness of *B. vulgaris*. Specifically, we investigated whether pollinator visitation was positively associated with (1) plants in the periphery of the population where individuals are predicted to be taller and produce more flowers, (2) high local plant density, (3) high flower production and (4) taller plants. We expected taller *B. vulgaris* to be visited by pollinators more frequently than shorter *B. vulgaris* because taller plants may be more visible and accessible to flying pollinators.

Methods

Study of artificially manipulated flower density

Study organisms and site

H. aurantiacum is an introduced bright orange flower with a hairy stalk that grows in fields, clearings, and roadsides. (Niering and Olmstead 1979). Plants are 30- 60 cm tall with 2 cm wide bright orange flowers that open between June and August (Niering and Olmstead 1979). *H. aurantiacum* can reproduce sexually or asexually (Callahan et al. 1997) and is pollinated by bees and butterflies (Strickler et al. 1996; Voss 1954). Individuals produce numerous short-lived flowers over their lifetimes that display nyctinasty, the tendency to open in the day and close at night (Oliver 1904).

We conducted observations between June 16th and June 21st, 2015 in an early successional field (46°13'1.39"N 89°31'9.87"W) at the University of Notre Dame Environmental Research Center in the Upper Peninsula of Michigan. The field is bound on all sides by a mixed deciduous-coniferous forest.

Data collection

To test whether different densities of open flowers attract pollinators at different rates, we established four 1 m² plots and randomly assigned the plots to have either 1, 5, 10, or 15 open flowers (Figure 1). We set up three replicates of the four density levels in different portions of the field at approximately equal distances from the forest edge. We monitored the plots to ensure that each plot had the assigned number of open flowers and removed all flowering plants from a 24 m² area around each plot so that only the open flower(s) in the plot could provide a visual signal to pollinators.

We conducted 10 minute observations of each plot in the morning (8- 11:20 AM), midday (11:45-2 PM), and evening (4- 7:05 PM), beginning with a different plot at every time point. During the observation period, we recorded the number 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). We identified all pollinators to order and family when possible.

Statistics

As *H. aurantiacum* open and close with light availability (Oliver 1904), we compared the number of individual pollinators and pollination events across the four flower density levels and the three times of day using 2-way ANOVAs. We used SYSTAT 13 and MYSTAT 12 to analyze the data.

Field study of naturally distributed population

Study organisms and site

B. vulgaris is an introduced mustard that grows in moist fields, meadows, and brook sides. Plants are 30-60 cm tall, with 8 mm wide yellow flowers that open between April and

August (Niering and Olmstead 1979). *B. vulgaris* can reproduce asexually or sexually (MacDonald and Cavers 1974), and is pollinated by flies and bees (Dailey and Scott 2006).

We observed a naturally occurring *B. vulgaris* population between May 30th and June 6th, 2015 in the same early successional field (46°14'42.8"N 89°33'04.8"W) studied by McQuestion (2014). This field is bound on the west side by a swamp and the other three sides by mixed deciduous forest.

Data collection

To determine the extent of the population of *B. vulgaris*, we identified the maximum distance between the eastern and westernmost, and northern and southernmost plants. Based on these distances, we divided the field into a 60 x 140 m grid of 21 400 m^2 zones and identified the central and peripheral regions of the population (Figure 2). We randomly selected four central zones and four peripheral zones and established 1 m^2 observation plots in each to compare pollination rates between the center and periphery (Figure 2). To compare how plant and flower density affect pollination rates, half the selected plots had high plant density ($\geq$ 4 plants/ m^2) and half had a high open flower density ($\geq$109 flowers/m^2; Figure 2). Median values were chosen for categorical cutoffs.

To test whether the data displayed the same patterns observed by McQuestion in 2014, we recorded the plant height and total flower number of all plants in the population. For each of the plants in the observation plots, we recorded the distance to the nearest neighbor, the number of open and about to open flowers at the start of the observation period, and the height of the plant in order to investigate whether pollination visitation was correlated with the plant fitness and/or density.

.   We observed plots three times a day, in the morning (8 -10 AM), midday (12- 4 PM), and evening (6 PM-8PM) for 10 minutes at a time. We rotated the observation schedule to begin with a different plot at each time point and recorded the number of observed pollinator visits to each individual.

Statistics

To see if flower number, density (the average number of plants/ 400 m^2 zone), and plant height were higher in the periphery, as observed by McQuestion (2014) we assessed the whole population using *t*-tests and a Mann-Whitney U test when the data could not be transformed. Because flower number and average number of plants per zone were not normally distributed, we transformed them using $\log_{10}$. Plant height could not be transformed to approximate normality. To test if *B. vulgaris* subsampled in the eight observation plots exhibited the same density patterns the whole population, we used a Mann-Whitney U test to compare the nearest neighbor distance (i.e. density) among peripheral and central individuals because the data could not be normalized. To determine if the same central-peripheral patterns of total flower number and plant height were displayed in the subsampled observation plots as the entire population, we used *t*-tests. We transformed the total flower numbers using $\log_{10}$ to achieve a normal distribution.

To better understand patterns in pollination in *B. vulgaris*, we compared the number of pollinator visits between observation plots in the center and periphery, those with many vs. few plants/ m^2, as well as those with many vs. few open flowers using *t*-tests. As visitation rates were not normally distributed, we transformed them using ln (pollinator visits + 1). To determine if there was a relationship between density (distance to the nearest neighbor) and pollinator visits, we used a linear regression.

Additionally, we looked at the relationship between individual plant fitness (i.e., flower number, plant height) and pollinator visitation rates. To assess whether there was a relationship between the number of open flowers on an individual plant and pollinator visits, we used a linear regression. We designated plants as few-flowered (≤27 flowers) and many- flowered (>27 flowers) using the median flower number and used a t-test to determine if pollinators visited many-flowered plants more often. We used the mean plant height to categorize plants as short (≤37 cm) or tall (>37 cm) and compared whether pollinators visited tall plants more frequently with a t-test.

Results

<u>Study of artificially manipulated flower density</u>

We observed each plot for 130 minutes, for a total of 26 hours of observation of *H. aurantiacum.* Flowers were most often pollinated by bees in the family Apidae and flies in the family Syrphidae (Hoverflies). Bees accounted for 57% of the observed pollination events and hoverflies accounted for 31%. Noctuidae moths and an unidentified species of Diptera also pollinated plants. The number of pollination events to a single observation plot over the total observation period ranged from 1 to 24 (mean=8.33±7.40) and the number of pollinators recruited ranged from 1 to 12 (mean=4.92±3.92).

Pollinator visitation for H. aurantiacum

Consistent with our expectations, flower density impacted pollinator recruitment and the number of pollination events. Individual pollinators visited plots with 15 open flowers significantly more often than plots with 1 open flower (Figure 3; 2-way ANOVA, $F_{3,36}= 3.19$,

$p=0.042$; Tukey's test, $p=0.030$) . Significantly more insects pollinated plants in the morning than in the evening (Figure 3; 2-way ANOVA, $F_{2,36}=5.43$, $p=0.011$; Tukey's test, $p=0.010$). The interaction between time of day and open flower density was not significant for the number of pollinators recruited (Figure 3; 2-way ANOVA, $F_{6,36}=1.55$, $p=0.20$).

For pollination events, there was a significant interaction between open flower density and time of day (2-way ANOVA, $F_{6, 36}=2.75$, $p=0.04$). During the midday when more light was available, plots with 15 open flowers had significantly more pollination events than plots with 15 flowers in the evening, plots with 10 flowers in the midday and evening, plots with 5 flowers in the evening, and plots with 1 flower at all times of day (Figure 4; Tukey's test, all $p<0.03$). Density and time of day also independently affected pollination rates (2-way ANOVA, $F_{3,36}=4.97$, $p=0.008$; and 2-way ANOVA, $F_{2,36}=5.43$, $p=0.01$, respectively). Plots with 15 open flowers were pollinated more often than those 1 open flower and more pollination events occurred in the morning and midday than the evening (Figure 4; Tukey's test, all $p<0.05$).

<u>Field study of naturally distributed population</u>

Of the 395 *B. vulgaris* individuals in the field, 220 were peripheral and 175 were central. Within our subsampled plots, 18 plants were peripheral and 14 were central. We observed each plot for 180 minutes, for a total of 24 observation hours. During the total observation time, plants were visited 0 to 95 times (mean=43.72±53.63) and observation plots were visited between 9 and 155 times (mean =43.88±47.93). Flies in the family Syrphidae accounted for 92% of all pollination of *B. vulgaris* and species of Hymenoptera accounted for the other pollination events.

Patterns of B. vulgaris distribution and fitness

When examining the entire population, there was a significantly higher density of individuals in central zones (mean=0.13±0.07 plants/m^2) than peripheral zones (mean= 0.03±0.08 plants/m^2; t-test, t=5.76, p<0.001). Peripheral plants had more flowers than central plants (mean= 107.66±113.21 and mean=54.06±46.72 flowers, respectively; t-test, t= -6.67, p<0.001). Additionally, peripheral plants were significantly taller than central plants (mean height: 35.57±12.91 vs. 24.67±7.92 cm, respectively; Mann-Whitney U test, U=9,319, p<0.001).

The subsampled observation plots showed some of the same patterns as the whole population of *B. vulgaris.* Peripheral individuals in the observation plots had significantly more total flowers than central individuals (mean=187.06±183.22 vs. 102.93±90.44 individuals, respectively; t-test, t=-2.074, p=0.048). However, when nearest neighbor distance was used to estimate density, peripheral individuals had shorter nearest neighbor distances than central individuals, which was more consistent with the *B. vulgaris* population from last year (mean=18.07±24.95 vs. 44.45±28.62 cm, respectively; Mann-Whitney U test, U=214.000, p<0.001; McQuestion 2014). In contrast to the pattern on the population scale, peripheral plants in the observation plots were not significantly taller than central plants (mean= 39.47±8.46 cm vs. 33.83±10.92 cm, respectively; t-test, t=-1.597, p=0.123).

Pollinator visitation rates for B. vulgaris

There was no significant difference in the number of pollinator visits between central and peripheral observation plots, plots with a high or low number of individuals, or those with many or few open flowers (t-tests, all t ≥-0.77, and p≥0.14). However, when we analyzed individual plants (N=32) in the subsampled observation plots, there was a significant positive relationship between open flower number and pollinator visits (Figure 5; linear regression, r=0.55, p=0.001). Additionally, pollinators visited plants with categorically many open flowers (N=16) more often

than plants with categorically few open flowers (N=16; Figure 6; *t*-test, $t=2.445$, $p=0.021$). There was not a significant relationship between distance to nearest neighbor (i.e. density) and pollinator visits (linear regression, $r=0.29$, $p=0.111$), nor was there a significant difference in pollinator visits between categorically short vs. tall plants (*t*-test, $t=-0.322$, $p=0.750$).

Discussion

<u>Study of artificially manipulated flower density</u>

Overall, the data supported our hypothesis that *H. aurantiacum* plots with more open flowers would be visited by more individual pollinators and that flowers in dense plots would be pollinated more frequently. This finding is consistent with earlier studies that suggest that pollinators frequent denser plots more often (Weber and Kolb 2012; Grindeland et. al 2005 and Kunin 1993). However, the fact that the number of individual pollinators only differed significantly between plots with 15 open flowers and plots with one open flower suggest that differences in density must be dramatic to affect pollinator foraging patterns. In terms of optimal foraging theory, this pattern may imply that the benefits of foraging in one flower patch over another only become substantial given large increases in flower density.

Our study also suggests that the interaction between open flower density and time of day influence whether a plant is visited. More pollinators were recruited in the morning and more pollination events occurred in the morning and midday than in the evening. This pattern is consistent with the fact that the most commonly observed *H. aurantiacum* pollinators, bees, are active during the bright hours of the day (Kelber et al. 2005). Additionally, many bees have vision adapted to a diurnal lifestyle (Kelber et al. 2005). Higher light levels may mean both that there is more pollinator activity and that bees are better able to locate flowers.

Moreover, higher pollination rates during the morning and midday may be related to nyctinasty-the flower-closing behavior that *H. aurantiacum* displays (Oliver 1904). In most floral species, the opening of flowers is caused by cell expansion and may be related to light intensity or humidity (van Doorn and Meeteren 2003). We observed that *H. aurantiacum* flowers were less open when they received less direct sunlight, suggesting that their tendency to close in the evening may be a response to light levels. As a result, when plots were in shadow, the flowers started to close, reducing their signal to pollinators. The combination of lower pollinator activity during evening hours and the reduced floral signal may have coupled to cause a decrease in pollination rates. This finding is important because it suggests that both biotic and abiotic factors, (i.e., pollinators and sunlight), synergistically affect the likelihood a plant will be pollinated. Further study into the effects of light availability on *H. aurantiacum* and how the tendency for the petals to close affects the signal and attractiveness of the flowers to pollinators could examine the implications of this interaction.

Field study of naturally distributed population

Although there was a significantly higher density of *B. vulgaris* in the center than the periphery of the population this year in contrast with McQuestion (2014), this trend was based on the number of individuals per 400 m^2 zone, rather than nearest neighbor distance, so the data did not account for whether individuals were clustered within one part of a zone. The other patterns observed by McQuestion (2014) were consistent with our findings: peripheral individuals had a higher mean flower number and were taller than central individuals. Within the subsampled observations plots, individual plants had more total flowers than central plants suggesting that they were a good representation of the flower-production trend observed within the entire population.

Because peripheral observation plots were denser with respect to distance to the nearest neighbor distance, it was surprising to find that peripheral plots were not visited by pollinators more frequently. Similarly unexpected was that denser observation plots with either more individuals or flowers per square meter were not more frequented by pollinators. However, when individual plants were analyzed, plants with many flowers were pollinated more frequently and there was a relationship between open flower number and pollinator visits. This trend was consistent with previous study that suggests that insects visit plants with many flowers more frequently (Willson and Price 1977).

Patterns of pollinator visitation may have been more apparent on the individual level than when plants were sorted into plots because *B. vulgaris* was observed toward the end of its flowering period and its age may have impacted the nectar resources available to pollinators. Some species of flowers produce less nectar as they age (Southwick and Southwick 1983) and nectar production in two other Brassicaceae species, *Brassica juncea* and *Sinapis alba,* peaks during anther dehiscence (Masierowska 2003). At least one species of Syrphidae shows preference for higher nectar concentrations (Sutherland et al. 1999) and bumblebees travel further between visited flowers in areas where flowers are depleted of nectar (Heinrich 1979). Hence, the nectar quality of individual *B. vulgaris* may have had a larger influence on whether they were pollinated. Schmitt (1983) suggests that in some situations mean nectar intake is more important for efficient foraging than spacing between plants and found that individual *Senecio* plants within a dense plot were not visited more often than plants within a less dense plot. For *B. vulgaris,* a plant with a high flower number may indicate a larger nectar resource, which attracts more pollinators. Additionally, Schmitt (1983) reports that bees foraged more selectively in areas with higher densities of flowers. The optimal foraging strategy for pollinators may be to

visit plants with many flowers more frequently and bypass plants with few flowers even if they are close to other plants. Therefore, we may not have observed increased pollination rates in dense plots because of the variable flower number of individuals within the plots. Similarly, plant height may not have had a significant impact on pollination visits because it may not be as important a signal of nectar production to pollinators as flower number.

The findings for *H. aurantiacum* and *B. vulgaris* contrasted in that the data for *B. vulgaris* suggested that individual flower number make a plant more likely to be pollinated, regardless of the density of the patch surrounding them, while patterns for *H. aurantiacum* suggested plants within dense plots will be pollinated more frequently. However, because *H. aurantiacum* flowers are short-lived, multiple plants were used to maintain the density quota so we do not know the likelihood an individual *H. aurantiacum* plant within a dense plot will be pollinated. One possible important difference between the species is that *H. aurantiacum* only has several flowers at a time and *B. vulgaris* can have upwards of 100 flowers at one time, providing many more flowers for pollinators to visit per individual plant. Even if dense patches of flowers are pollinated at higher rates, the higher flower number in those patches will increase competition between individual flowers for pollination.

Overall, the findings of both studies are consistent with optimal foraging theory. For *H. aurantiacum*, pollinators visited flowers in the densest plots more frequently than the least dense plots suggesting that they were most attracted to plots with the most potential resources. Pollinators favored *B. vulgaris* plants with many flowers. A high flower number may have been the best indicator of nectar resources measured in this study, consistent with the observation that plants with large floral displays are pollinated more frequently than those with fewer flowers (Grindeland et al. 2005) and suggesting that flower number is a cue for pollinators.

Understanding pollinator visitation with respect to plant density is important because of the impact that pollinators have on plant species' ability to maintain their populations (Bierzychudek 1981; Pauw and Bond 2011). This understanding is relevant for the preservation of endangered and fragmented plant species (Metcalfe and Kunin 2005) and for awareness of how plant distribution affects pollination rates in commercial species that rely on insect pollination.

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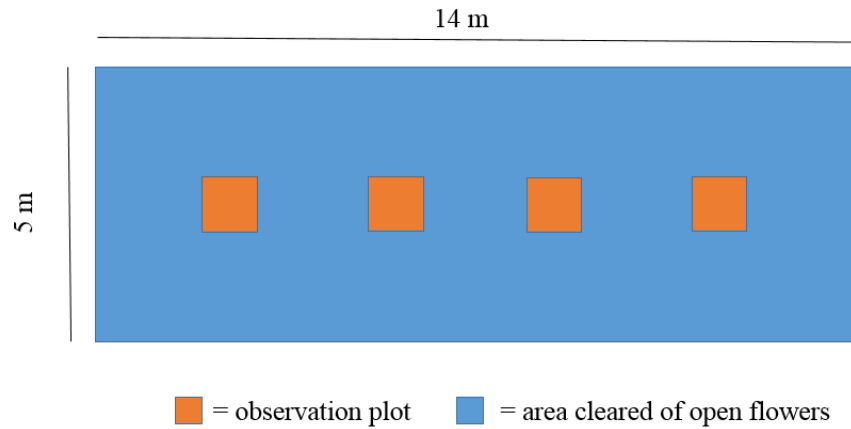(Asclepiadaceae).*Evolution* 31:495-511.

Figures

Figure 1. Plot set-up for pollination observations of *H. aurantiacum.* Observation plots (orange) of 1 m^2 were set-up in the field and randomly assigned to have 1, 5, 10, or 15 open flowers. The 66 m^2 around the observation plots (blue) was cleared of flowering plants. This set-up was replicated at three locations in the field.
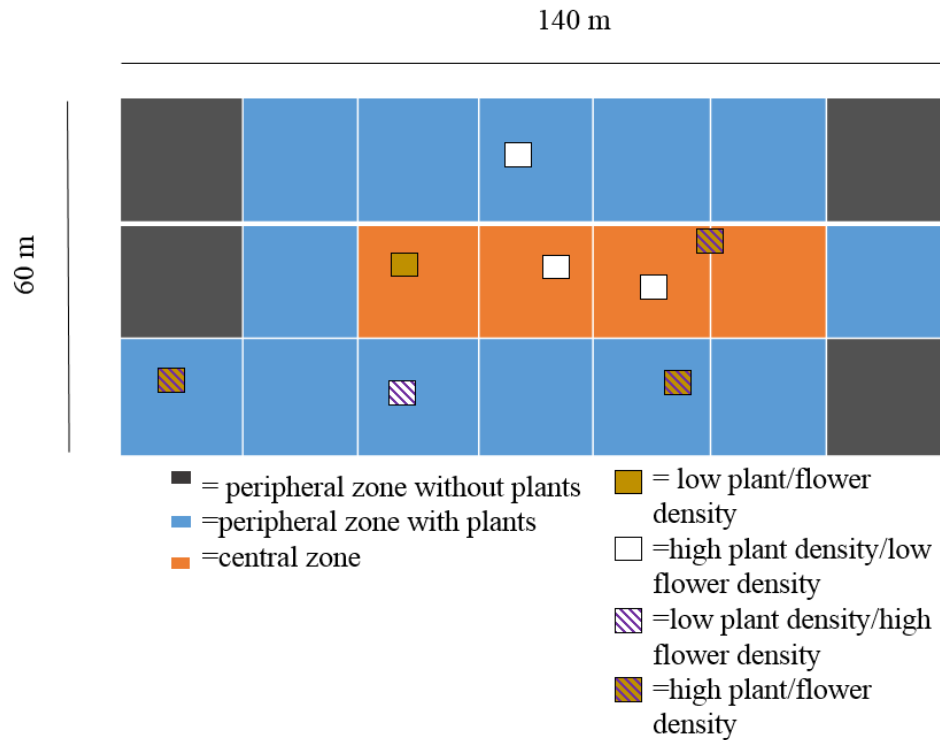
Figure 2. Plot set-up for observations of pollination rates in *B. vulgaris.* The population spanned a 60 x 140 m region of the field and was divided into 21 400 m^2 zones. Zones were separated into central (orange) and peripheral (blue) regions. Four central and four peripheral zones were chosen randomly to establish 1 m^2 observation plots. Half the observation plots had a high plant density/m^2 (gold), and half low plant density (white). Additionally, half the observation plots had a high flower density/m^2 (striped) and half had a low flower density/m^2 (no stripes).

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Figure 3. Mean number of pollinators recruited to *H. aurantiacum* plots of different densities across different times of day. Means were calculated from all 26 hours of observation (2- way ANOVA; flower density, $F_{3,36}= 3.19$, $p=0.042$; time of day, $F_{2,36}=5.43$, $p= 0.011$; flower density x time of day, $F_{6,36}=1.55$, $p=0.20$). Error bars represent standard deviation.
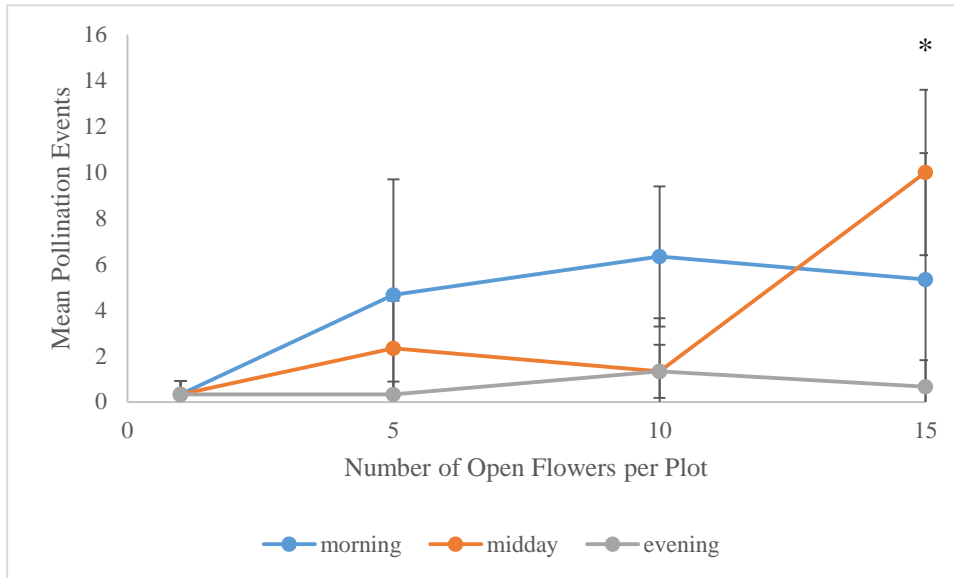
Figure 4. Mean number of pollination events in *H. aurantiacum* plots of different densities across different times of day. We calculated the means from all 26 hours of observation. Error bars represent standard deviation. * denotes significant interaction between flower density and time of day on pollination events (2-way ANOVA, $F_{6, 36} = 2.75$, $p = 0.04$).
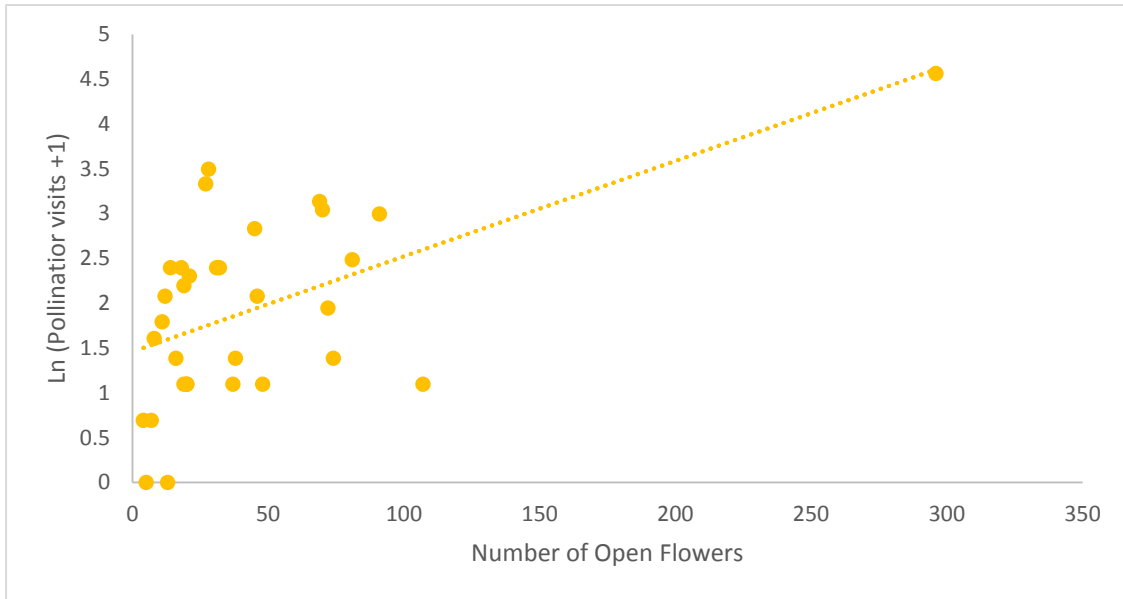
Figure 5. Relationship between number of open flowers on 32 *B. vulgaris* and the ln (pollinator visits+1) based on 24 hours of observation at eight plots in an early successional field (linear regression, $r=0.55$, $p=0.001$).
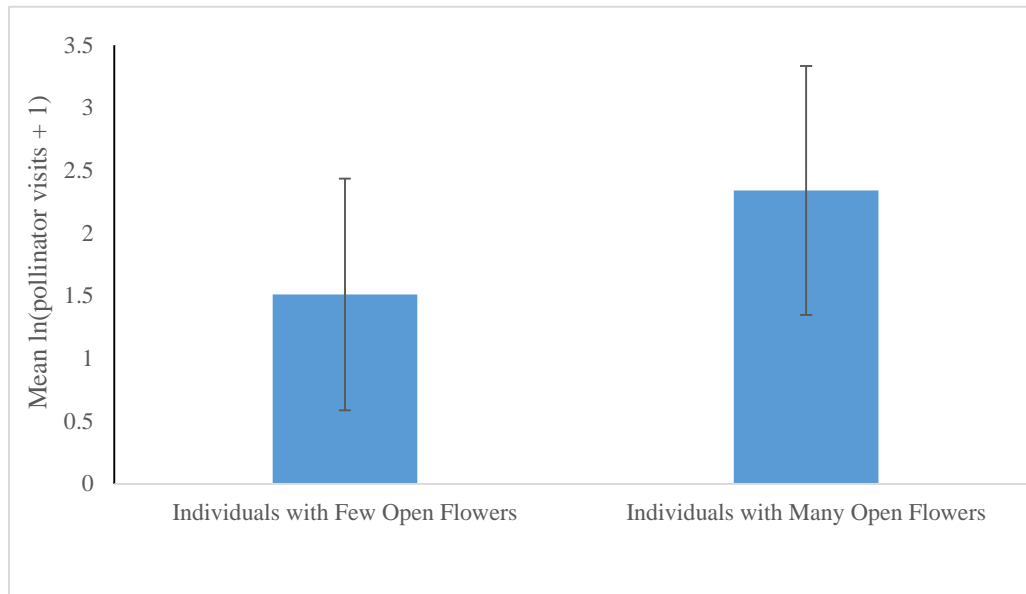
Figure 6. Average number of pollination events between *B. vulgaris* with many (≥28) and few (≤27) flowers based on 24 hours of observation (*t*-test, *t*=2.45, *p*=0.021). Error bars represent standard deviation.