Pollinator Response to Patch Distribution and Reproductive Consequences in Lasthenia californica

Elizabeth C. Hendrickson
Portland State University

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Pollinator response to patch distribution and reproductive consequences in Lasthenia californica

by Elizabeth Hendrickson

An undergraduate honors thesis submitted in partial fulfillment of the requirements for the degree of Bachelor of Science in University Honors and Biology

Thesis Advisor
Mitch Cruzan, PhD

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ABSTRACT

Premise: The response of animal pollinators to the spatial distribution of plants is crucial to understanding how plant-pollinator interactions contribute to plant reproductive processes. For plant species that aggregate in patches, population attributes—such as the size and distribution of patches and the density of plants—may affect pollinator visitation to a greater degree than characteristics of individual flowers. We examine how patch characteristics of the self-incompatible, vernal pool species *Lasthenia californica* (California goldfields) impact pollinator visitation using pollen load size and pollen germination rates to infer visitation patterns.

Methods: Plant density and the size and distribution of patches were estimated from aerial images captured during drone surveys of an upland prairie ecosystem in Southern Oregon. Pollen load sizes and number of compatible grains were quantified after staining with Alexander's Stain.

Key Results: We found that denser patches receive the highest deposition of compatible pollen on stigmas. Large patches have an accumulation of low quality pollen and reduced pollen germination success, most likely due to pollinators moving genetically-related pollen within patches.

Conclusions: Patterns of pollinator behavior inferred from the quantity and quality of pollen deposited on stigmas are consistent with optimal foraging theory. Pollinators optimize foraging by preferentially moving within patches that are larger or are in close proximity to larger patches. Our results suggest that individual patch characteristics do not significantly affect overall pollinator visitation rates, but the distribution of patches contributes to pollinator behavior.
INTRODUCTION

Plant-pollinator interactions are crucial for the reproductive success of many plants and mediate gene flow via pollen dispersal (Baker, 1963; Barrett, 2003; Loiselle et al., 1995). Pollination by animal vectors can increase outcrossing opportunities and decrease levels of selfing and inbreeding within populations (Ellstrand and Elam, 1993; Schmitt, 2009). As animal pollinators forage for floral resources, their behavior is influenced by the spatial and temporal structure of plant populations (Loveless and Hamrick, 1984; Karron et al., 1995; Ivey and Carr, 2005). Spatial factors such as the distribution of flowering patches can indirectly influence pollinator movement, and consequently pollen-mediated gene flow, within and among patches in a local population (Jennersten & Nilsson, 1993; Loiselle et al., 1995; Mitchell et al., 2004). Linking the spatial characteristics of plant populations to the plant-pollinator interactions within the larger metapopulation is becoming an increasingly important goal (Steffan-Dewenter et al., 2002).

It is commonly accepted that differences in floral morphology can influence pollinator foraging behavior (Engel & Irwin, 2003, Mitchell et al., 2004), but in plant species that aggregate in patches, the size and distribution of patches may affect pollinator visitation more so than individual floral traits (Mayer et al., 2012). Patch structural characteristics, such as size and density, indicate the resource return of the patch for the pollinator, which is a key factor when considering optimal foraging behavior (Charnov, 1976; Mustajärvi et al., 2001). In a classic patchy system, larger and denser flower patches are typically more attractive and solicit increased pollinator visitation (Emlen, 1966; Kirchner et al., 2005). More attractive patches are predicted to receive a greater quantity of pollen by optimal foraging theory, and as a result, more
pollen deposition yields a positive correlation between individual patch size with fecundity and seed viability (Jennersten and Nilsson, 1993). Similar principles can also be applied at a population scale as well; optimal foraging theory predicts that groups of large patches will be favored and that small neighboring patches may also prosper from local pollinator activity (Delmas et al., 2016).

Plant reproductive success often depends on both the quality and quantity of pollen delivered to stigmas (Brown and Kephart, 1999), so it is critical to consider that patches may tend to consist of closely related individuals because of limited seed dispersal. In sporophytic self-incompatible species, mating is limited to individuals with differing S-alleles, and genetic exchange of plants sharing an S-locus can result in aborted pollen germination or pollen-tube growth (Sih and Baltus, 1987; Byers and Meagher, 1992). The effect of S-alleles is influenced by the spatial and demographic characteristics of the population (Levin et al., 2009) as genetic similarity is higher among neighboring patches (Jankowska-Wroblewska et al., 2016). Patterns of germination success can be influenced by the movement of pollen throughout the population on account of pollinator preference for patch size, density, and distribution characteristics. Further understanding of how variation in pollinator response to local population structure and characteristics affects pollen movement and deposition within and among patches may provide insight into patterns of plant reproductive success (Mitchell et al., 2009).

In this study we examine pollen deposition and germination in the annual plant species, *Lasthenia californica* (Asteraceae) DC. Ex Lindl. *sensu* Ornduff (1966, 1993). Most *Lasthenia* species are known to possess sporophytic self-incompatibility (Ornduff, 1968) and grows in dense patches of varying size across our study area at the Whetstone Savanna Preserve in
southern Oregon. Because this species has limited seed dispersal (P. Thompson et al. unpub. data), and the patch characteristics are relatively consistent across growing seasons (personal observation), pollen dispersal appears to be the primary source of gene flow. Here we examined 1) how population, individual patch, and individual floral factors affect pollinator behavior and visitation, and 2) how the observed pollinator response to these characteristics influenced pollen germination rates.

MATERIALS & METHODS

Study species and study site: Lasthenia californica (Asteraceae) is a small, yellow, composite annual that grows in a wide variety of habitats (Rajakaruna and Bohm, 1999). L. californica was chosen for this study for its self-incompatibility and strong reliance on animals for pollination (Chan et al., 2011). Its range stretches from southern Oregon to southern California and is frequently found in wetland and prairie habitats. Field surveys and flower sample collections were conducted at The Nature Conservancy site of Whetstone Savanna Preserve in Central Point, Oregon, which covers 16 hectares. This population is primarily pollinated by native Oregon bees and flies endemic to the region (Thorp and Leong, 1998). The vegetation in the study area is classified as an oak savanna and upland prairie ecosystem, and annually features patches of L. californica associated with vernal pools.

Micro UAV aerial distribution surveys: Micro UAV aerial surveys were conducted to quantify the density and spatial distribution of L. californica patches across the study site. We used a DJI Phantom 2 Vision+ quadcopter to capture a high density of overlapping images of the entire prairie and construct an orthomosaic (Fig. 1; for further details see Cruzan et al., 2016). In
addition, raw (DNG format) images of individual *L. californica* patches (n=20) of varying
diameters were taken at an altitude of approximately 4.6 meters directly above each patch. All
images included a clipboard as a pixel to meter conversion reference. Two to four patches
located within a 25-meter radius from one another were photographed during each flight, with
the study range spanning the entire 16 hectares of the preserve. We flagged and collected GPS
coordinates on the location of each patch and clipboard as references for spatial analysis.

**Fig. 1**: An aerial orthomosaic (See Cruzan et al., 2016 for further information) of the Whetstone
Prairie Preserve in southern Oregon with GPS sample patch locations of *Lasthenia californica*
shown in green dots.
**Patch image analysis:** The density and spatial distribution of individual flower patches were quantified from aerial images. Images were converted from raw DNG to TIF format using Adobe Photoshop (Adobe Systems Incorporated, San Jose, CA). Each image was cropped to the patch's distinct border using the free selection tool in GNU Image Manipulation Program (version 2.8; GIMP, 2008) and cropped pixels were set to a value of zero within the image's matrix. Using the clipboard's pixel area in each image as a standard, the patch pixels within the cropped selection were totaled and scaled to yield a measurement of patch size. A custom Python pipeline was written to analyze the batch of images (the source code can be found at: https://github.com/cruzan-lab/other-programs/tree/master/Patch_Image_Analysis). Each cropped patch image was converted from blue-green-red (BGR) color space to hue-saturation-value (HSV) color space to account for light fluctuations in the original images. *L. californica*’s unique color range in HSV color space (denoted as lower bound for BGR: 20, 220, 200, upper bound for BGR: 25, 255, 255) was identified in the image and designated with a pixel value of 1. All other pixels were set to a value of 0 (Fig. 2). The number of *L. californica* pixels in each patch was used to determine patch density.

To consider the geographic space of each patch in relation to neighboring patches, the flower color detection script was applied to the orthomosaic projected within the WGS 1984 geographic coordinate system in ArcMap 10.3 (ESRI, Redlands, CA). Changing binary to three-channel color space (BGR) to import into ArcMap was accomplished by applying an Otsu threshold to yield an image with flower pixels in white (BGR: 255, 255, 255) and all other pixels in black (BGR: 0, 0, 0). The 'buffer' tool in ArcMap was applied to the patch location at radius distances of 5, 10, 20, and 40 meters. Finally, the 'zonal statistics as table' tool determined the
sum of pixels within the buffer, to represent the density of patches within the entire prairie. The pixel total within each radius was subtracted from the following distance to eliminate compounding totals. The distance and size of the two closest patches to the sample patch was calculated in the orthomosaic by using the 'line measure' tool in ArcMap to find the distance in meters from the center of the sample patch to the center of the nearby patches. The size of the patch was determined with the 'polygon measure' tool applied around the patch border.

**Individual floral traits**: Flower heads (n=6) were collected from each *L. californica* patch and preserved in 70% ethanol for the analysis of floral traits, pollen load, and the proportion of germinated grains. We included a range of petal and flower sizes by arbitrarily selecting three larger flowers and three smaller flowers within each patch. In the lab, we measured disk width and the total diameter of ray floret to opposing ray floret of each flower head (Fig. 3A) with digital calipers to the nearest hundredth of a millimeter. The difference divided by two of these measurements yielded flower petal length.

![Fig. 2](image)

**Fig. 2**: The original image (2A) taken by the drone at approximately 4.6 meters above the patch. The image on the right (2B) is a visual representation of the size and density image analysis calculations. Pixels within the color range specified were reassigned a white matrix, and pixels outside the specified color range were assigned a black matrix with a value of 0.
**Pollen grain deposition and germination:** The number of germinated and ungerminated pollen grains on stigmas was quantified to estimate pollination success. An average pollen grain deposition per individual was counted by dissecting away disk flower stigmas (n=3) from the preserved inflorescence. Each dissected stigma was approximately 0.5 to 1.0 millimeters in length. On a microscope slide, we submerged the stigmas in Alexander's Stain (Kearns and Inouye, 1993) for at least one minute to allow the stain to adhere. The properties of Alexander's Stain distinguish germinated and ungerminated pollen grains. Cytoplasm in non-germinated pollen grains appeared red, and in germinated pollen grains the empty grain wall appeared blue. Under 40X power, we counted the total number of pollen grains deposited and the total number of germinating grains on each stigma (Fig. 3B).

![Fig 3](image_url): An individual specimen of the species, *Lasthenia californica* (3A). Individual floral traits such as inflorescence diameter (1), disk width (2), and petal length (3) were considered for each individual. Fig. 3B is an image of a *L. californica* stigma taken through a microscope lens at 40X magnification power. The properties of Alexander's Stain allow for pollen grains that germinate upon the stigma to appear blue, while non-germinating pollen grains appear red.
**Statistical analysis**: We tested for the effects of individual floral traits, patch size and density, and distance to and size of closest patches using the stats package in R (version 3.3.1, R core team, 2013). Individual stigma pollen counts were averaged for each individual flower. In addition, stigmas that exhibited clumps of pollen were noted as irregular. Because clumping occurred infrequently and resulted in inaccurate counting as well as reduced contact with the receptive tissue of the stigma, clumped stigmas were excluded from the data analysis (n=6). The average pollen load and germinating pollen grains per flower were assessed as response variables in separate models. We applied a linear regression model function (lm) to determine the best-fit model, including two-way interactions. Finally, residual influence and normality of individual variables was assessed for both models. A path analysis diagram was created with the SemPlots package (Epskamp, 2017) as a visual depiction of the model interactions.

**RESULTS**

**Pollen grain deposition**: The best fit linear model (lm, df=101 R²=0.16, p<0.001), to explain the average number of grains included the factors: pixel totals within 5, 10, 20, and 40 meter buffers, the distance to the first closest patch, and the size of the first and second closest patches (Table 1). All factors had an individual p-value of less than 0.05. Pixel density within buffers at 5, 10, and 20 were negatively correlated with pollen grain deposition, and pixel density within a 40-meter buffer was positively correlated. The distance to the closest patch was negatively correlated with pollen deposition, and the size of the first and second closest patches was positively correlated (Fig. 4). Residuals were normally distributed.
Germinating pollen rate: The best fit linear model (lm, df=96, $R^2=0.29$, $p<0.001$) that explained the number of germinating pollen grains for each flower at each patch consisted of the total number of pollen grains, a two-way interaction between the total number of patch pixels and the number of flower pixels within the patch, pixel totals within 10 meter buffers, and the distance to the second closest patch (Table 1). All factors had a p-value of less than 0.05. The number of germinating pollen grains decreases with the total number of pollen grains deposited. The numbers of patch and flower pixels was negatively correlated with germinating pollen counts. However, the two-way interaction of patch and flower pixels, which represented patch density, was positively correlated (Fig. 4). The number of pixels within a 10-meter buffer and the distance to the second closest patch had a positive and negative correlation with germination rates respectively. Residuals were normally distributed.

**Table 1:** Summary of the effects of population, individual patch, and individual floral factors on the deposition and germination of *Lasthenia californica* pollen grains upon the stigma as a linear regression. The slope and p-value are provided.

<table>
<thead>
<tr>
<th>Total Pollen Grains</th>
<th>Germinating Pollen Grains</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Factor</strong></td>
<td><strong>Slope</strong></td>
</tr>
<tr>
<td>5 Meter Radius</td>
<td>-2.60E+00</td>
</tr>
<tr>
<td>10 Meter Radius</td>
<td>-6.79E-01</td>
</tr>
<tr>
<td>20 Meter Radius</td>
<td>-2.24E-01</td>
</tr>
<tr>
<td>40 Meter Radius</td>
<td>-1.53E-01</td>
</tr>
<tr>
<td>Distance to 1st Closet Distance</td>
<td>-1.38E+01</td>
</tr>
<tr>
<td>1st Closest Patch Size</td>
<td>3.10E+01</td>
</tr>
<tr>
<td>2nd Closest Patch Size</td>
<td>9.69E+00</td>
</tr>
</tbody>
</table>
Fig. 4: These path analyses compare factors influencing total pollen grains and germinating pollen grains. Strong and weak interactions are represented by the thickness of the line. Dashed and solid lines visualize positive and negative interactions respectively. Note, density is expressed as an interaction between total pixels and pixels belonging to flowers in *L. californica* patches.
DISCUSSION

We examined the influence of individual floral, patch, and neighboring patch characteristics on pollinator visitation, as demonstrated through pollen loads and pollen germination on stigmas. Individual floral traits consisted of inflorescence characteristics; individual patches were described by size and density measurements per image analysis; and neighborhood population characteristics were defined by the size and distance to the first and second closest patches, as well as flower density within an interval radius from the sample patch. We found that pollen load and pollen germination success variables responded to different elements of the plant landscape; pollen load responded to patch population characteristics, with patches in close proximity to larger neighboring patches receiving greater pollen loads. Pollen germination was dependent upon individual patch characteristics, with denser patches exhibiting the greatest rate of pollen germination success and larger patches experiencing reduced germination. Individual floral characteristics did not significantly contribute to any model.

In this study system, *L. californica* pollinators are diverse and include: Hymenoptera, Diptera, Hemiptera, and Lepidoptera (Thorp and Leong, 1998). We found these pollinators to move erratically and to be typically small in body size, which reduced the accuracy of visibly monitoring pollinator behavior. Due to these constraints, we used pollen load as a proxy for pollinator behavior, as pollinator visitation is positively correlated to pollen load (Engel and Irwin, 2003; Alarcón, 2010). Because *L. californica* is known to be self-incompatible and exhibits limited seed dispersal, we acknowledge that patches will consist of genetically similar plants. Assuming patches consist of highly related individuals and mating among them reduces germination rates, we inferred pollinator response by using pollen load to indicate the frequency
of pollinator visitation. Rates of germinating pollen represented pollinator movement within and among flower patch populations.

While floral morphology can influence pollinator behavior and the associated pollen load deposited on stigmas (Mitchell et al., 2004; Schmitt, 2009), individual floral traits assessed in this study, specifically petal length, disk width, and inflorescence diameter, did not significantly impact pollen deposition or germination rates. Once within the patch pollinators did not appear to discriminate among variation in individual floral traits. Additionally, many studies demonstrate an increase in pollinator visitation with patch density (Bosch and Waser, 1999; Mustajärvi et al., 2001; Grindeland et al., 2005), however our model does not suggest that individual patch size and density did not significantly affect the rate of pollinator visitation at a local patch scale. In this system, pollen load sizes did not increase in denser or larger patches, which indicate that pollinators were visiting flowers at similar rates regardless of specific individual patch characteristics.

Pollen load was significantly correlated with population-level characteristics, such as the distance and size of the nearest patches, and the total distribution of flowers within a certain distance from the sample patch. Total distribution of flowers within the neighborhood is represented by the number of pixels found within each interval radius from the sample patch. However, the negative relationship observed at 5, 10, and 20-meter radii most likely reflects the absence of accounting for individual patch size in this method, and is not indicative of pollinator response. Upon including measurements of the size and distance of the closest patches, plants in larger and denser groups of patches accumulated more pollen on their stigmas. This indicates that pollinators prefer to forage in patches that are located geographically nearby other patches.
Likewise, greater size of neighboring patches increased the chance of pollen deposition, without regard to the size of the patch itself. The response of pollen load to neighboring patch characteristics implies insect pollinators respond to the broader population scale rather than to the traits of an individual patch.

Our observations are indicative of insect pollinator foraging behavior expected under the optimal foraging theory (Emlen, 1966; Macarthur and Pianka, 1966; Charnov, 1976). The optimal foraging theory assumes animal behavior is dependent upon four principal considerations: which patches to visit, how long to remain in the patch, which food types to eat in each patch, and which foraging path to move among patches (Pyke et al., 1977). Under these conditions and assuming resources are abundant (Emlen, 1966), the classic model suggests that pollinator will trend towards food preferences that minimize energy expenditure and maximum resource gain. In a patchy system, larger patches indicate high resource return for the pollinator and are visited the most frequently. The pollinator response toward *L. californica* supports the hypothesis that patches located in close proximity to large patches will receive more pollen deposited in consequence of more frequent visitation. Although pollen load did not indicate strong pollinator preference for individual patch size and density, the neighborhood distribution of patches was significant in our model. The observed pollen load on stigmas demonstrates that pollinators prefer to forage and remain in large groups of patches with minimal distances to nearby patches.

Because the *Lasthenia* genus is known to be self incompatible, reproductive success is influenced by not only the amount of pollen, but also by the proportion of compatible pollen deposited. In species with sporophytic incompatibility, pollen compatibility is expressed as
germination success; closely related individuals will tend to share S-alleles and consequently are more likely to exhibit self-incompatibility. Assuming limited seed dispersal, we expected *L. californica* patches to consist of related individuals that are more likely to share S-alleles. If pollinators respond to resource availability according to optimal foraging theory, they will tend to remain within dense patches for longer periods of time, and consequently will increase the frequency of mating among closely-related individuals. While pollinator activity may be high within a patch, persistent foraging reduces deposition of compatible pollen and the germination rates of pollen on stigmas will be lower. Pollinator behavior and genetic incompatibility due to relatedness among individuals within patches may reduce pollen germination and fecundity among genetically similar patch populations as an Allee effect. The observed Allee effect in pollen germination success due to the reduction of compatible pollen persists despite substantial patch population size (Ramsey and Vaughton, 2000; Leducq et al., 2010).

Rates of pollen germination in *L. californica* indicate that there may be an ideal individual patch size and density to receive the most compatible pollen. We found a negative correlation between patch size and pollen germination, which contrasted the positive effect of patch density upon pollen germination. A likely explanation for large patches to experience decreased pollen germination rates is the presence of sporophytic self-incompatibility in *L. californica*. In that event, the movement of genetically-similar pollen and S alleles within the same patch reduced pollen germination among related individuals (Levin et al., 2009). While smaller patches accommodated reduced rates of pollinator activity, pollinators visiting small patches were less likely to forage within the patch for an extended time period and more likely to move among patches, consequently transferring greater amounts of compatible pollen to plants.
in small patches. However, higher density patches were more attractive to pollinators than sparse patches and received more compatible pollen (Dauber et al., 2010). Thus the ideal patch size and density for pollen germination success was small enough to decrease the movement of genetically related pollen, yet dense enough to attract pollinators. Several other studies have documented an increase and eventual plateau (Ågren, 1996) or parabolic effect (Elliott and Irwin, 2009) in fecundity with patch density and size, indicating ideal patch traits for optimal pollination.

In conclusion, this study examines how characteristics of aggregated patchy populations, i.e. patch size and distribution of patches in the habitat, influenced pollinator visitation behavior, and how the resulting pollinator response might affect the germination success of the species, *L. californica*. We found that dense patches receive the highest deposition of compatible pollen on stigmas, while larger patches have reduced pollen germination. The distribution of nearby patches also influences pollen load via pollinator behavior—flowers in patches located in close proximity to larger patches received greater pollen loads. Our results indicate that characteristics of the individual patches impact pollinator behavior in accordance to optimal foraging theory, with potential consequences for gene flow in this system. Due to the self-incompatibility attributes and genetic structure of this species, an ideal patch that maximizes germination success is dense enough to attract pollinators, yet small enough to encourage movement among unrelated plant patches.
LITERATURE CITED


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