

Exploring within season temporal variation in plant-pollinator interactions in sub-alpine meadows using weekly pollination interaction networks.

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Advanced Independent Research
Summer 2013

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James L. Cunningham

Abstract:

It is known that sub-alpine meadows experience significant community level changes throughout the spring/summer season due to phenology. In this study we sought to interpret the within-season community-level changes in the sub-alpine meadows of Gothic, CO, USA through the construction of weekly plant-pollinator interaction networks. Creating a plant-pollinator interaction networks for every week from 24 May to 2 August 2013, allowed us to quantify and visualize the changes occurring in this system across the season. We found a large amount of variation between weeks as shown in the varying structure of the networks. Connectance was highest at the beginning and end of the season, showing a direct relationship with floral richness. This is most likely a result of biotic factors, specifically, the phenology of participating species, although to a lesser degree, abiotic factors may influence variation in weekly network structure. These results advance our understanding of community level trends in Gothic's sub-alpine meadows.

Introduction:

Up to 90% of flowering plants, including many economically important crop species, are animal pollinated (Ollerton et. al 2011). Despite the widespread use of the honey bee, native pollinators are more effective pollinators in many cases (Garibaldi et. al 2013). However, plants and pollinators have shown population declines which has been attributed to decreased temporal overlap and function (Burkle et. al 2013). It has also been shown that there has been a parallel loss of plants and insect-pollinated flowers (Biesmeijer et. al 2006). A greater understanding of plant-pollinator interactions is needed to understand why these changes are taking place. The community level approach to pollination biology allows for a large range of mutualistic interactions to be quantified, often allowing us to make valuable insights regarding ecological and evolutionary processes (Waser et. al 1996). However, plant-pollinator communities are often difficult to study and understand because of their inherent complexity. One way to understand pollination interactions as a community is through the construction of pollination networks. These networks allow plant-pollinator interaction data to be rigorously quantified. In addition to allowing pollination biologists to make inferences regarding community stability, often pointing to nestedness and connectance as the means to do so, networks have been used to allow complex and numerous pollination interactions to be easily understood (Vásquez et. al 2009). Currently, most pollination networks are created by pooling the interactions across an entire season in order to make a single network, thus making inferences on community structure based on the entire season's visitations. Indeed, plant-pollinator networks show considerable variation across years (Alarcón 2007), yet within season variation in patterns of interactions has been investigated much less (Kaiser-Burnbury et al. 2010), (Medan et. al 2006). Although we have learned a lot from season-wide interaction networks, the variation of interactions within time and space is

often overlooked (Bascompte et. al 2007). We believe that utilizing weekly networks is a logical way to capture within season temporal patterns.

In this study we investigated how patterns of plant-pollinator interactions within a season contribute to the overall structure of the season-wide community network. The ecosystems around the RMBL exhibit substantial within season abiotic and biotic variation (D.W. Inouye and b. barr, unpublished data), both of which are liable to have a large impact on the patterns of interactions between plants and pollinators throughout the season. We assembled weekly plant-pollinator networks to better understand how temporal variation within a season influences patterns of plant-pollinator interactions. Using networks as a tool we sought to ask two main questions 1) How do subalpine plant-pollinator interaction patterns change throughout the spring and summer season, specifically by looking at how connectance (the proportion of potential links that are actually realized; Jordano, 1987) varies throughout the season. 2) How does floral abundance and diversity relate to the number of observed interactions? Specifically, we created plant-pollinator interaction networks for every week from 24 May to 2 August. As a result of these networks we were able to answer these questions using the characteristics of the networks and statistical models, allowing us to further understand the internal workings of Gothic's plant-pollinator communities.

Methods:

Two south-western facing meadows north of the RMBL were selected as study sites in the Rocky Mountains of Colorado, USA (38 57N 106 59 W ~2953m). Meadows are separated by a mix of spruce and aspen forest, a linear distance which represents approximately 100m. Within each meadow, four 25 x 1m permanent belt transects were established in order to capture existing ecological variation in plant community composition. All subsequent network and floral abundance sampling occurred between 24 May and 2 August 2013.

Plant-pollinator interactions were consistently sampled at the meadows for approximately 6-10 hours each week between 1000-1500 hours from 24 May 2013 until 2 August 2013. We concentrated our sampling effort during these hours because our preliminary observations reveled that this time frame coincided with higher pollinator activity periods. A network survey typically involved a pair of observers slowly walking belt transects, taking 10-15 min in the process, and recording only those visits in which an animal arrived at a flower and contacted the plant's reproductive structures (following Alarcón et. al 2008). Sampling for pollinator visitation occurred within a 3m buffer on both sides of the transect. All new pollinators were netted, and then identified to lowest taxonomic level and released. In order to obtain even sampling of each entire transect, pollinators are observed for up to 30 seconds or until they leave the transect; this prevents us from observing only a single portion of the transect for excessive periods of time. Transects are walked barefoot as much as possible to reduce disturbance to the plant community.

Flowers were counted on Monday and Friday along the 25m x 1m transects, and an average between the two dates was calculated to estimate flower abundance and diversity for each week. Compact inflorescences in the family Asteraceae were treated as individual flowers, and all other families are treated as individual flowers. Wind pollinated plants were not included in these bi-weekly surveys.

Networks were created using the network package bipartite in R. Interactions between plants and pollinators were shown by lines connecting plants and pollinators, and to show

weighted interactions, line size (or thickness) is indicative of visitation frequency. As stated, these networks made it possible to make inferences and understand themes regarding overall community structure at any one point throughout the season. To summarize the changes in amount of connections over the summer we created a time series graph showing the changes in amount of connectance, the proportion of potential links that are actually realized (Jordano, 1987) over this 11 week period (Fig. 2 A-K). We then created a time series chart indicating total plant-pollinator interactions along weeklong increments, displaying when in the season is a peak and a low point for visitations. Utilizing the visitation information used to create the networks we performed linear regressions relating floral abundance to number of pollinator visitations. This process was repeated using floral diversity relating to number of pollinator visitations.

Results:

Overall, 65 hours of observation were recorded over a period of 11 weeks (24 May 2013 to 2 August 2013). This resulted in 1448 observed interactions, representing 30 species of plants and 62 species of pollinators. As weeks progressed more plant and pollinator species interact as shown by the increased number of interaction types, which then decreased towards the end of the season (Fig. 3 A-K). Similarly to the first week of the season (fig. 2-A), the end of the season had the fewest plants being visited over the season, 4 (fig. 2-K) and (table 1). Number of visits showed a marginally significant positive relationship with week number (fig. 1)

Peaks of connectance were during weeks 1 (0.4444) and 11 (0.3929), lowest connectance is at week 9 (0.1625), weeks 5-9 all share a connectance value $< .25$ (fig. 3) and (table 1). Floral abundance was highest at week 1, lowest at week 2. Varying levels of floral abundance relate to connectedness evenly, ($P = 0.04334$, $R^2 = 0.06769$; fig. 4). Flowering species richness was lowest at weeks 1 and 11, highest at week 2 (fig. 5) and (table 1). There is a strong negative trend between connectance and flowering species richness, ($P = 0.0007931$, $R^2 = 0.731123$; fig. 6).

Number of visitations was variable through the season, reaching a peak at week 10 (fig. 7). Floral abundance was slightly negatively related with number of visits, but not significantly so (fig. 8). No statistically significant relationship was found between flowering species richness and amount of visitations through the season.

Discussion:

1) *How do subalpine plant-pollinator communities in the Rocky Mountains change throughout the spring and summer season.* Our analyses of these eleven weekly pollination networks support the idea that there is much variation among plant-pollinator communities throughout the summer season in Gothic, Colorado. Floral abundance was variable throughout the season, Week 1 being highest, when the meadows were dominated by *Claytonia lanceolata* (table 1) and (fig. 2-A). Visitation frequency was also variable through the season, spiking late in the season at week 10, due to the emergence of *Bombus* workers. The overall number of visits tended to increase slightly as the weeks progressed (fig. 1), however this proved to be an insignificant trend, most likely due to the variability in community structure these networks display. As demonstrated in (fig. 2 A-K) there is a great deal of variation in the weekly structure of the plant pollinator communities as seen by the changing amount of plant species on the top level of the networks and the changing amount of pollinator species on the bottom level of the networks. Week 1 (Fig. 2-A) is of a simple community structure, with only four species of flowering plant being visited,

interacting with four pollinator groups. However this changes quickly as the total number of plant species and overall floral abundance increase in number once out of the colder and harsher spring this system has to offer. A similar pattern is seen at the end of the summer season, where the number of available plant species has returned to four, (fig. 2-K).

At these periods of low species diversity, occurring at the beginning and end of the season, connectance is also the highest (table 1). Connectance relates well to the species richness in a network time period, (fig. 5 and 6). This similarity in connectance values at the beginning and end of the summer season is most likely a result of the lower diversity of plants allowing for more potential interactions to occur, as week 1 and 11 show the lowest richness of visited flowering plants. We found that in the meadows of study, there was a significant negative relationship between connectance and number of species. This makes sense, as connectance is a measure of realized interactions between plants and pollinators. With fewer available floral species available, a higher percentage of flowering plants may have the potential to be visited.

This also gives us insight into the community structure of the shoulder seasons in Gothic, specifically while there are fewer available plant species to visit in the meadows, the pollinators during this time may visit a larger proportion of available plants than they do in the middle weeks. This may suggest that the pollinator species at the beginning and end of the season are generalists, visiting multiple plant species and causing the connectance values to rise. Of the four plants visited in week 11 for example, three are large and showy members of Asteraceae (*Heterothica villosa*, *Heliothus multiflora*, *Erigeron speciosus*). These three plants offer a high reward to visiting pollinators, suggesting that connectance may also rise in with the increase of flowers offering high rewards. The high connectance values later in the season may be best predicted by the emergence of *Bombus* workers. Weeks 10 and 11 (fig. 2-J and 2-K) showed the largest amount of *Bombus* workers (*Bombus flavifrons*, *Bombus sylvicola*, *Bombus bifarius*), such that they dominated all visitations. This emergence in conjunction with a low over all number of species available contributes to the high connectance values at the end of the season.

We did not find a relationship between the number of flowers available and connectance, likely because floral abundance was not associated with floral richness, and thus not impacting the amount of potential types of interactions. Our findings were not unlike Alarcon et. al (2008), who found variation among networks between years. While we found variation within a season, most likely due to the varying phenology of the plants and pollinators involved, these interactions are subject to variation across many different time scales. Another factor that undoubtedly contributes to the within season variation we found is the abiotic variation these meadows experience throughout the season. Intimately tied to phenology, abiotic factors such as snowmelt, precipitation, and temperature may also impact pollinators' decision to forage and thus alter visitor abundance for a certain time period.

2) *How does floral abundance and diversity relate to the amount of species interactions with pollinators?* We found that neither floral abundance nor floral richness predicted the number of species interactions with statistical significance, (fig. 8 and 10). However when discerning between the two, floral abundance may be a better indicator of visitor interactions. Floral abundance seems to be negatively related to number of visits possibly because as more flowers are available, fewer pollinators will be in the transects available for observation. A slightly positive relationship is seen between number of floral species available and number of visitations. A greater diversity of floral resources may cater to a larger group of visiting pollinators, increasing overall visitation amount. When considering weeks 1-9, there is a much stronger relationship between visitor frequency and flowering species available. Weeks 10 and

11 alter this trend as the emergence of *Bombus* workers increase overall numbers of visitations despite the relatively low number of species available during these last two weeks. It may be that for systems not dominated by late season *Bombus* workers, floral diversity may better predict number of visitations. In a future study we may examine if floral diversity and visitor frequency experience similar trends throughout the season. In conclusion, when considering the full season, neither floral abundance or floral richness predict amount of recorded visitations, but of the two floral abundance may be the better choice.

Very few studies have sought to examine within season variation using networks as a tool to do so. We consider this a successful and informative way to interpret and understand a changing community, given the amount of information we were able to extract utilizing this method, and the amount of tests and question that remain to be analyzed. This project exposed the high degree of within-season variation that is rarely addressed in most pollination network studies. For ecologists interested in within-season community level changes this is an effective way to answer otherwise difficult questions. This method can also be used in areas where little is known about interactions in plant pollinator communities. This approach documents many aspects of a plant-pollinator community, potentially helping to structure conservation plans for an area by addressing which interactions are the most rare and involve certain species known to be at risk. These networks would allow managers to know when in a season a certain interaction is going to take place. Further, if this project is continued for more years, it may be possible to identify new and lost interactions under the conditions of climate change induced phenological shifts.

In summary this project allowed us to address the within-season community level changes in the meadows of Gothic, Colorado. A large amount of weekly variation was observed and analyzed throughout the season. We can most likely attribute the variation of these eleven plant pollinator networks to variation in phenology and other influences of abiotic factors. Particularly we found that connectance is negatively related to plant richness, and is highest at the shoulder seasons, where plant richness is lowest. In addition we found that floral abundance may be a better predictor of visitation amount than floral species diversity, although neither showed a significant association with visitor amount.

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Figures and Tables

Table 1. displaying Week # (1-11), Dates refereeing to each week, Plant richness indicating total number of species recorded per week, pollinator richness indicating total umber of visiting species recorded per week, and connectance values.

Week #	Dates	Plant richness	Pollinator richness	Connectance
1	5/20/13-5/24/13	4	7	0.4444
2	5/27/13-5/31/13	10	10	0.25
3	6/3/13-6/7/13	8	8	0.2031
4	6/10/13-6/14/13	5	13	0.2769
5	6/17/13-6/21/13	9	19	0.1696
6	6/24/13-6/28/13	9	23	0.1691
7	7/1/13-7/5/13	7	17	0.2071
8	7/8/13-7/12/13	7	15	0.2286
9	7/15/13-7/19/13	8	20	0.1625
10	7/22/13-7/26/13	6	19	0.2983
11	7/29/13-8/2/13	4	14	0.3929

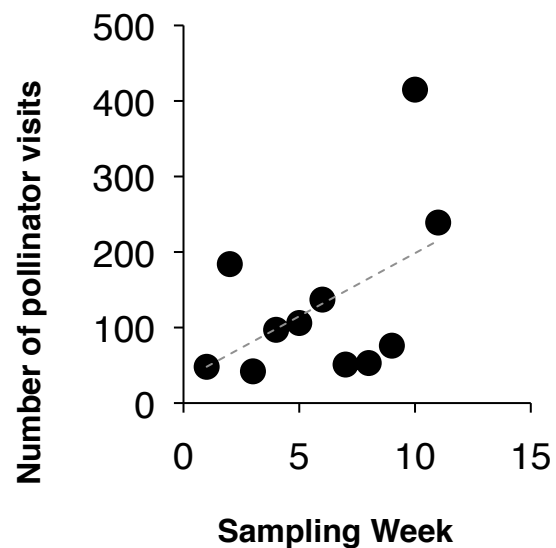


Figure 1. Number of pollinator visits in relation to sampling weeks1-11, ($P= 0.1429$, $R^2 = 0.24133$)

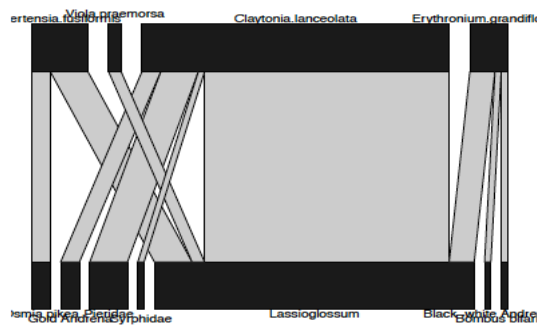


Fig. 2-A: Network displaying interactions from week 1 (5/20/13-5/24/13), connectedness = 0.4444.

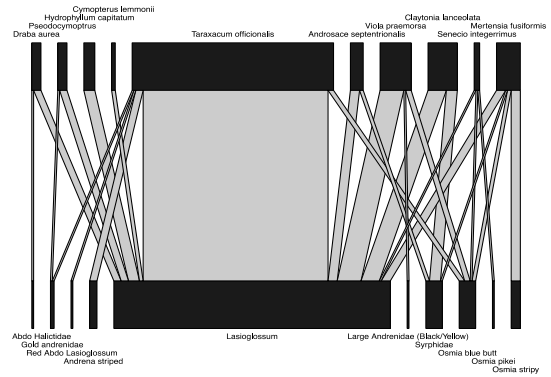


Fig. 2-B: Network displaying interactions from week 2 (5/27/13-5/31/13), connectedness = 0.2500.

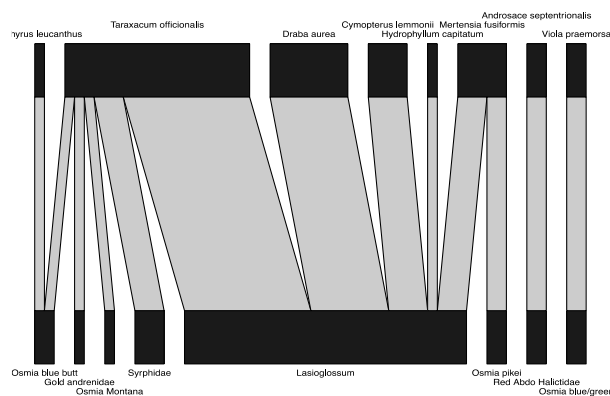


Fig. 2-C: Network displaying interactions from week 3 (6/3/13-6/7/13), connectedness = 0.2031.

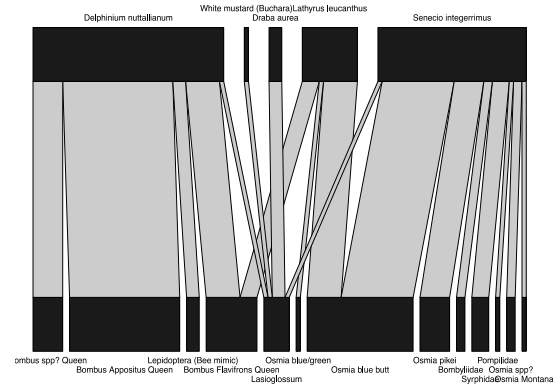


Fig. 2-D: Network displaying interactions from week 4 (6/10/13-6/14/13), connectedness = 0.2769.

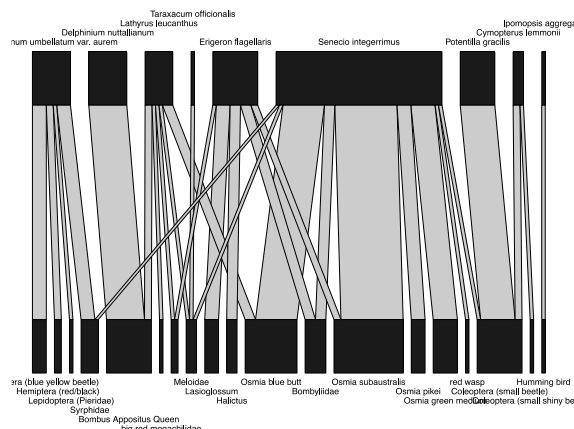


Fig. 2-E: Network displaying interactions from week 5 (6/17/13-6/21/13), connectedness = 0.1696.

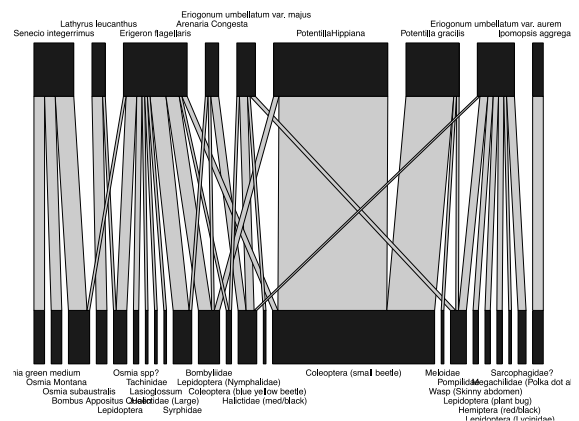


Fig. 2-F: Network displaying interactions from week 6 (6/24/13-6/28/13), connectedness = 0.1691.

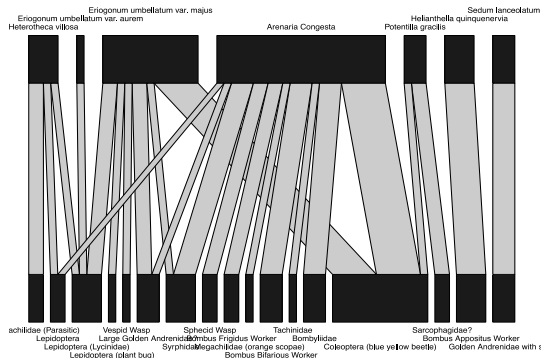


Fig. 2-G: Network displaying interactions from week 7 (7/1/13-7/5/13), connectedness = 0.2071.

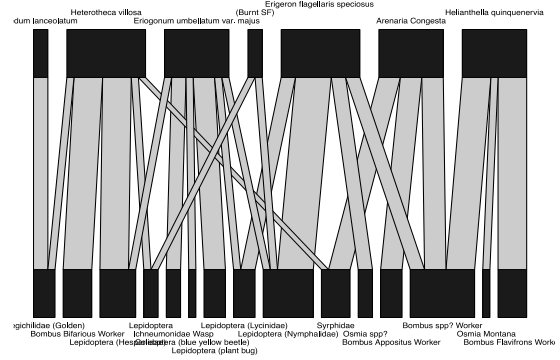


Fig. 2-H: Network displaying interactions from week 8 (7/8/13-7/12/13), connectedness = 0.2286.

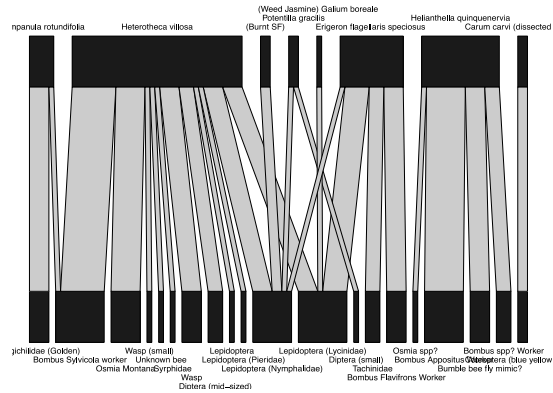


Fig. 2-I: Network displaying interactions from week 7 (7/15/13-7/19/13), connectedness = 0.1625.

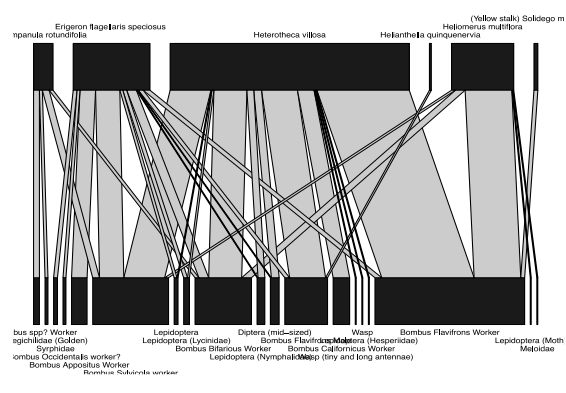


Fig. 2-J: Network displaying interactions from week 8 (7/22/13-7/26/13), connectedness = 0.2983.

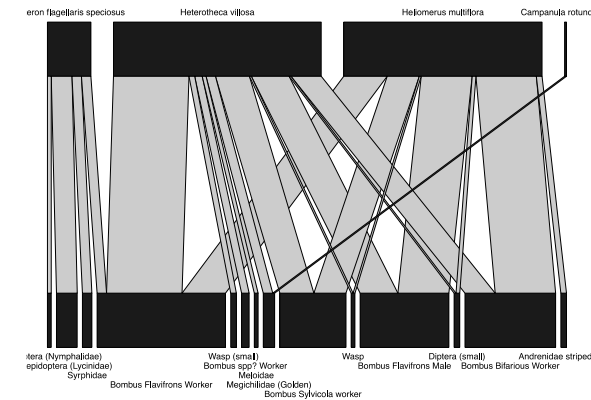


Fig. 2-K: Network displaying interactions from week 11 (7/29/13-8/2/13), connectedness = 0.3929

Figures 2 A-K. These networks display the variation in community structure throughout the 11 weeks that this study was performed.

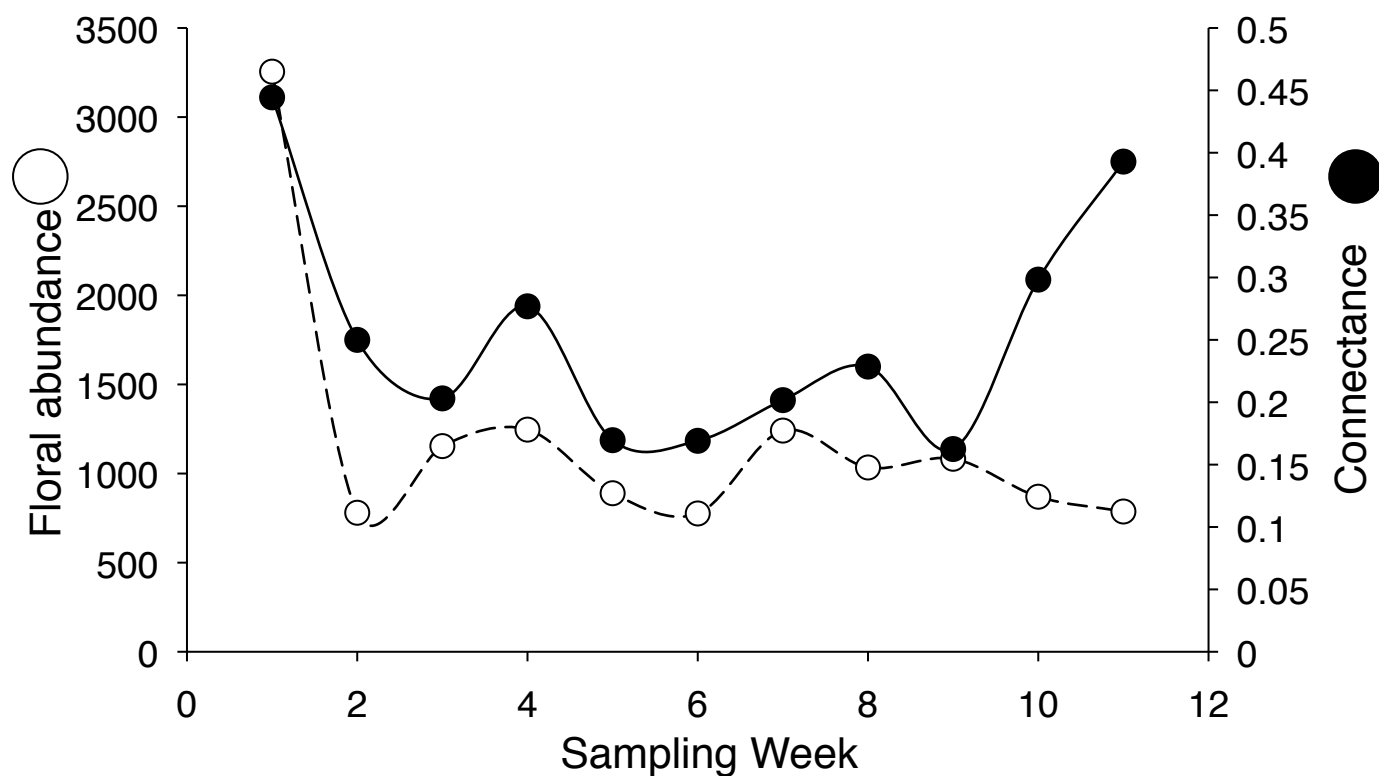


Figure 3. Time series of floral abundance and connectance. Connectance shown over a period of 11 weeks shown in black symbols. Floral abundance over a period of 11 weeks in white symbols.

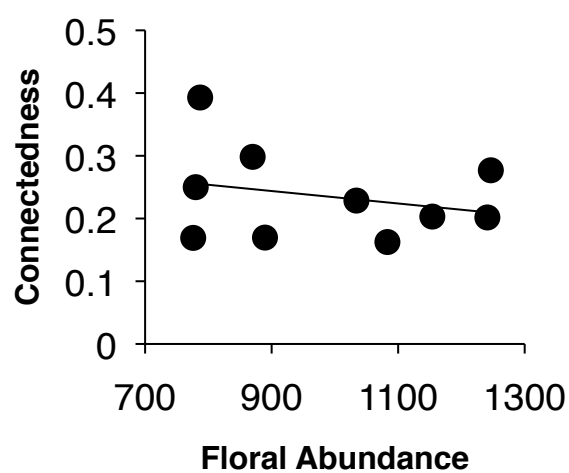


Figure 4. The relationship between connectedness and floral abundance from weeks 1-11. ($R^2=0.06769$ $P=0.04334$)

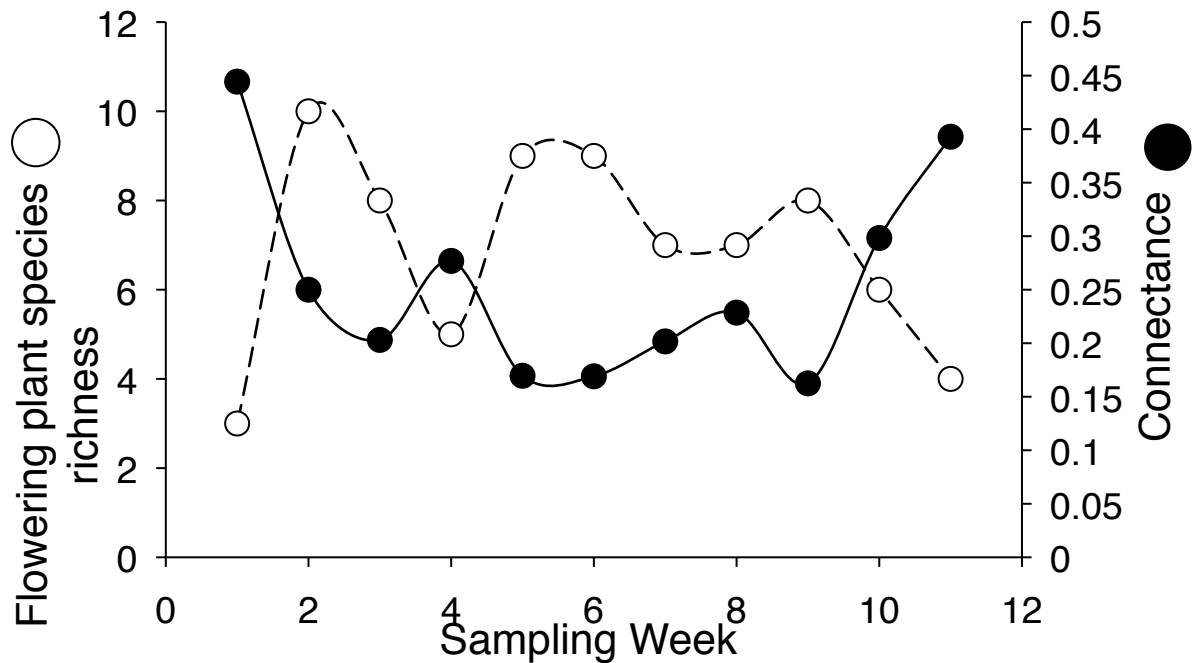


Figure 5. Times series of Flowering plant species richness and connectance. Connectance shown over a period of 11 weeks in black symbols. Flowering plant species richness over a period of 11 weeks in white symbols.



Figure 6. The relationship between connectedness and floral abundance from weeks 1-11. ($R^2=0.731123$ $P=0.0007931$)

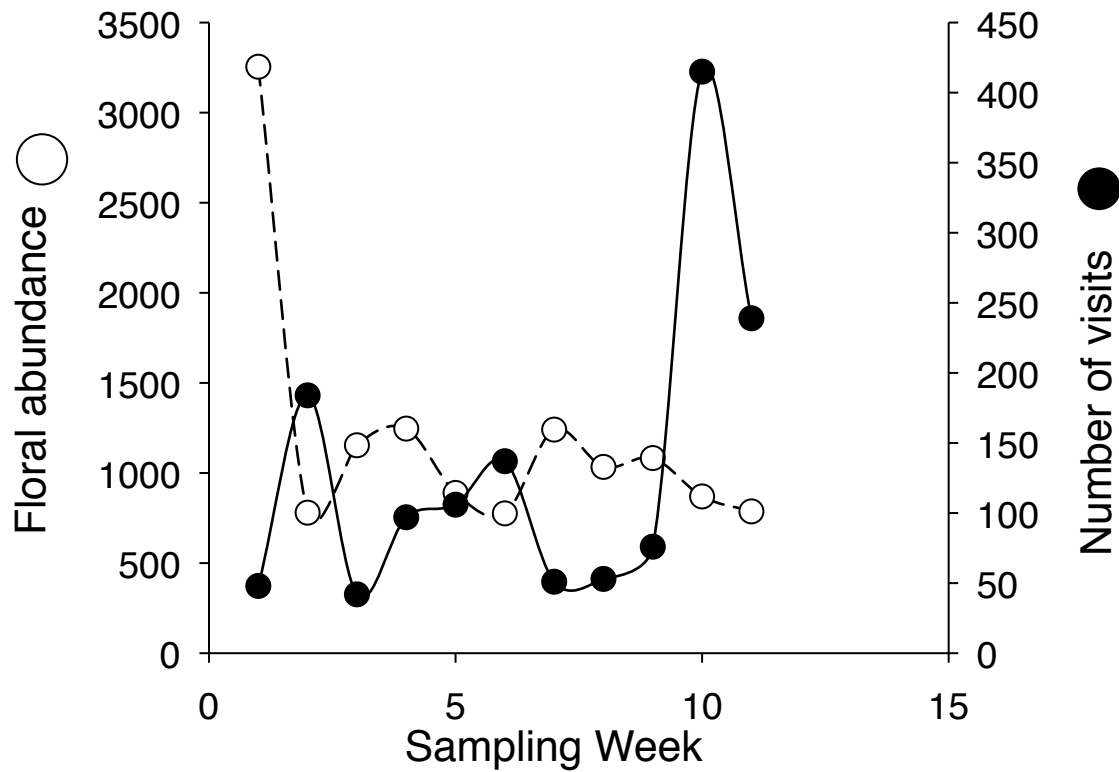


Figure 7 Time series of floral abundance and # visitations. Floral abundance in white symbols, Number of visits in black symbols.

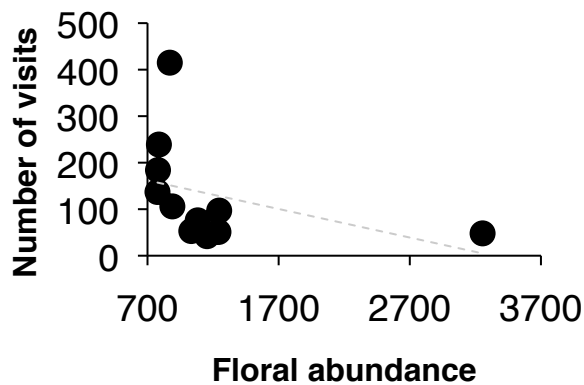


Figure 8. Relationship between number of visits and floral abundance. ($P= 0.2421$ $R^2 = 0.1483$)

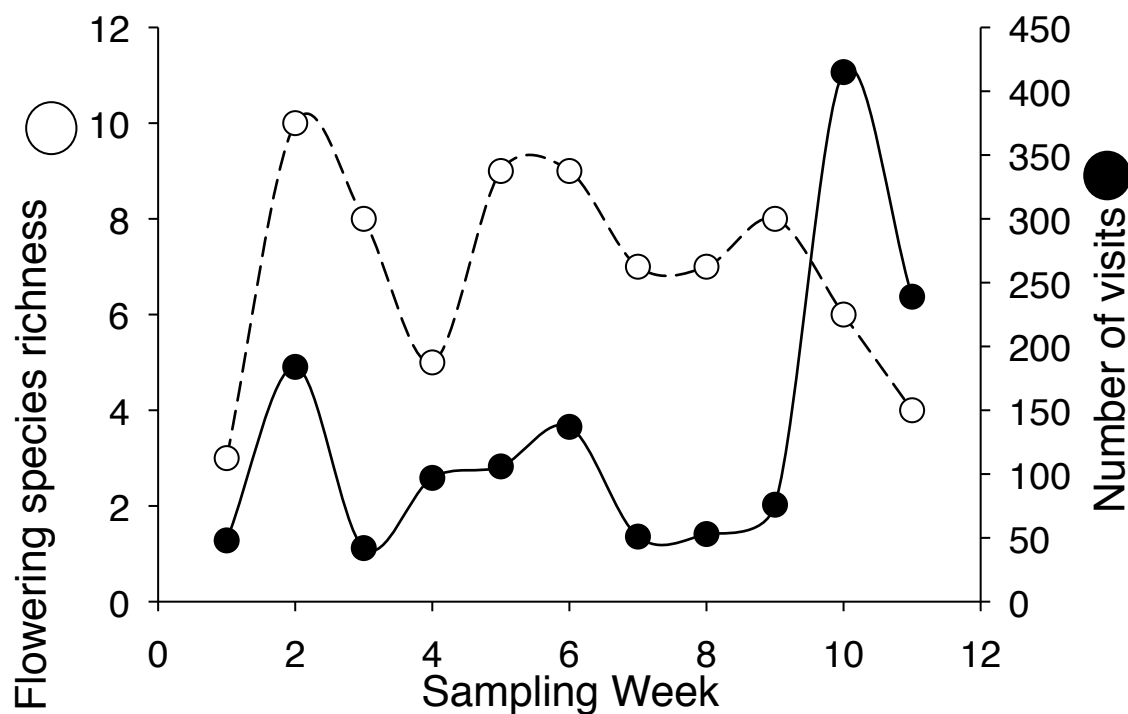


Figure 9. Time series of flowering species richness and amount of visitations. Flowering species richness in white symbols. Number of visits in black symbols.

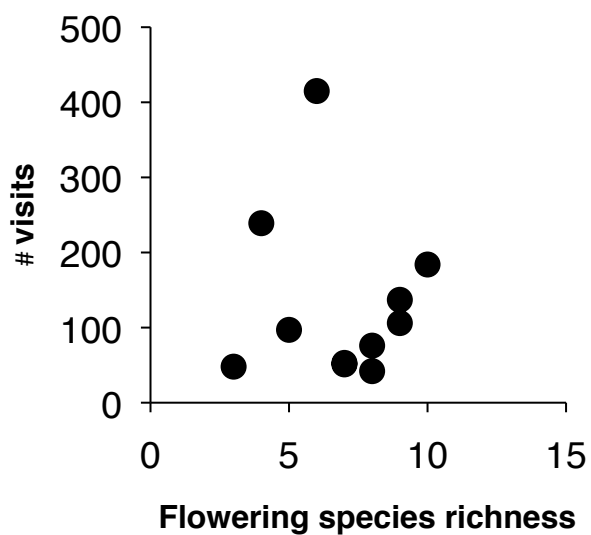
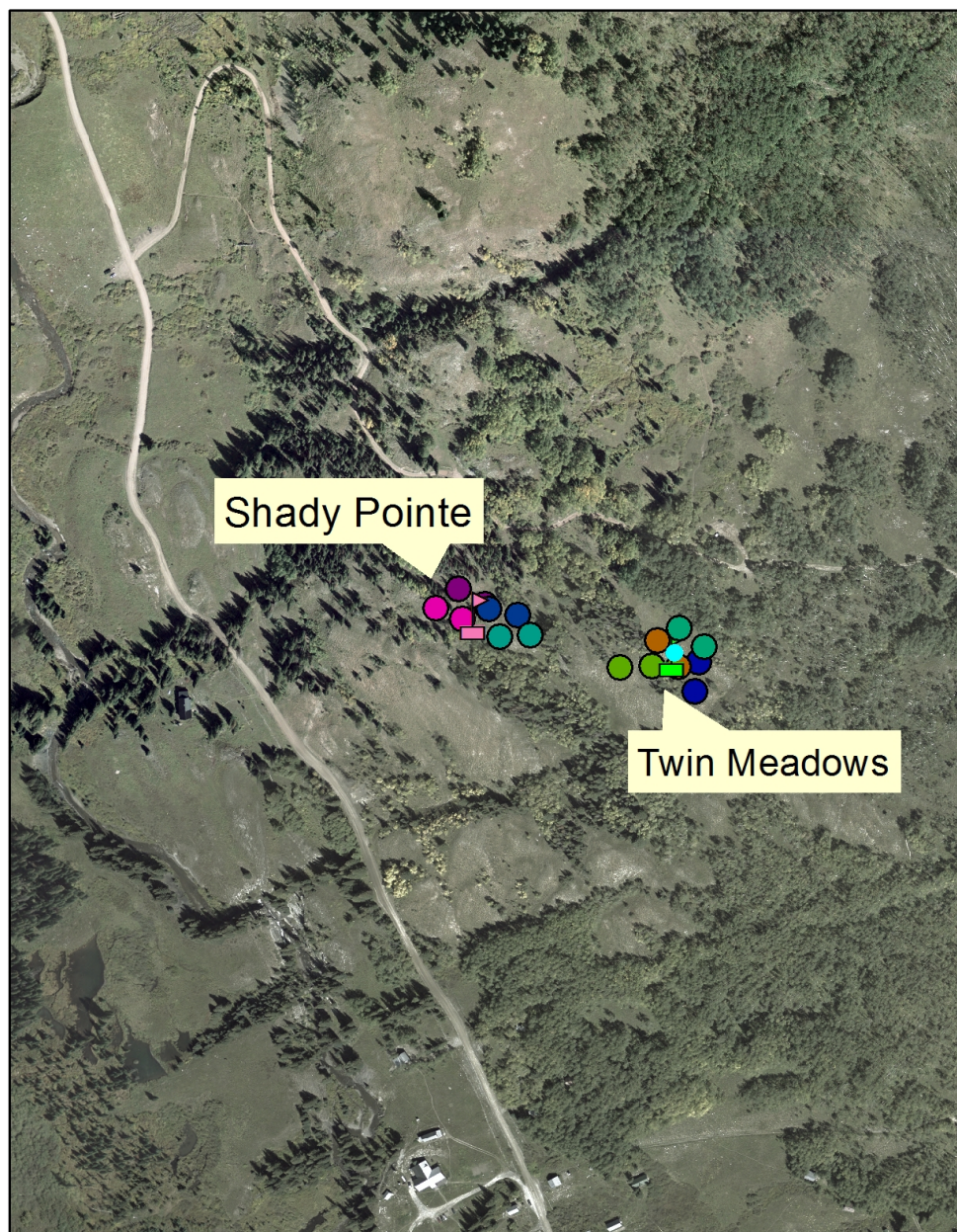


Figure 10. Relationship between number of visits and flowering species richness. ($P = 0.7871$, $R^2 = 0.0085$)

Map

James Cunningham's 2013 Field sites



0 0.020.04 0.08 0.12 0.16 Miles