

Floral visitation determined by phylogenetic distance in the plant communities of Gothic, CO

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Abstract:

Pollinator's attraction to various species of angiosperms is based upon various sensory outputs. These attractants could be floral/inflorescence size, color, scents or other various types of pollinator attractants. It can be inferred that plants species that are more closely related will have similar morphological traits and pollinator attractants due to the similar genes within them. This could mean that plant species that are closely related could attract the same pollinators to themselves, and thus allow us to predict floral visitation based upon phylogenetic relationships. Using phylogenetic relationships to predict floral visitation has been found to be a useful tool in the past few years that this study was done, and a positive relationship was found between floral visitation and the distance (in millions of years) to its nearest neighbor along with a small correlation between a species and the mean distance (in millions of years) between it and all of its neighbors. But, this year these relationships were found to be stronger and opposite of what was seen in previous years.

Introduction:

Over the past few years, phylogenetic approaches have been spreading throughout the field of community ecology. Such approaches assume that phylogenetic relationships can help predict the outcomes of processes including species interactions and community assembly. This has come about because of the rapid acquisition of phylogenetic data that has been obtained in recent years, the increased computing power, and computation tools that have become available (Cavender-Bares 2009). But, this idea has not been at the forefront of the community ecology even though phylogenies have been recognized to be useful for community ecology work (Brooks & McLennan 1991, Losos 1996, Thompson et al. 2001). Phylogenetic relationships research has already demonstrated its predictive power of community interactions.

In a study done by Ness et al 2011 herbivore damage was found to decrease as a species' phylogenetic isolation increased. The prediction of why this occurs is that species that are more closely related have similar morphological traits than species that are not closely related. This is because they have diverged evolutionary, and if their morphologies are similar then the impact of the herbivores can be greater since the similar morphological traits of the plant species are stimuli for the herbivores (Ness et al 2011). This application of genetic knowledge to ecosystems is called the "genes to ecosystems concept" (Wymore et al 2011) and puts the genetic interactions between species at a community level to explain interspecific interactions like herbivory (Ness et al 2011), pollination, and could possibly be used to explain other interspecific interactions.

When brought into the realm of pollination biology, phylogenetic relationships amongst host plants in a community could predict floral visitation rates. Pollination is one form of interspecific interaction between species that relies on the sensory perception of the pollinator. In a review by Fenster et al 2004, a concept called "pollinator syndrome" is introduced which explains that the evolutionary pathway for angiosperms is influenced by one or more functional groups of pollinators interacting with the host species. These functional groups are defined as species that behave in similar fashions on flowers and exert similar selection pressures (e.g. hummingbirds, flies, long tonged bees, etc.) on the flora they frequently visit (Fenster et al 2004). Closely related species express similar morphologies because of their similar genetic composition (Wymore et al 2011), and it can be said then that plants species that are closely related will attract the same or similar pollinator functional groups. But, when flowering plant species are too closely related the high amount of conspecific pollen can clog pollen tubes or ovule abortion could occur in the pollinated plant (Schuett and Vamosi 2009). Previous studies at

Gothic have shown that phylogenetic isolation of a plant species within its community can predict floral visitation (Evans Peck, Agosti, Whitney unpublished).

Given large variation in abiotic conditions across years, I repeated the study in 2014 in order to examine the consistency of the effect of phylogenetic isolation on floral visitation patterns. The summers of 2012 and 2013 were preceded by a low snowpack compared to the yearly average that is seen in the Gothic, CO area, but the summer of 2014 was preceded by a more “average” snowpack than the previous years for the Gothic, CO area (Fig. 1; SNOTEL data for the Gunnison Basin). This relatively “wet” year could yield different results as the extra moisture could shift the ratio of floral visitors to flowers. In accordance with the last two studies it will be predicted that a plant species that has few close relatives (i.e. a high mean phylogenetic distance [MPD]), will experience a deficit in floral visitors when compared to a species that has close relatives (a lower MPD). We also predict that, a species that has very close relatives (a low nearest neighbor distance [NND]) will experience fewer visits (because of the competition that it experiences by having a close relative) than a species that has a distantly related nearest phylogenetic neighbor (a high NND).

Methods:

Site and Species Selection

All floral visitation data was collected in the area surrounding Gothic, CO in and around the site of Rocky Mountain Biological Laboratory. The East River Valley, where Gothic is situated, is dominated by three subalpine plant communities: open meadows, Aspen (*Populus tremuloides*) forest, and coniferous forest (dominated by *Picea engelmannii* and *Abies bifolia*). The observation sites was restricted to the subalpine to montane habitats of the valley including

the Gothic Townsite, Virginia Basin, the Maroon Bells Wilderness, Emerald Lake, and Washington Gulch Trail.

Data that was collected in the summer of 2014 is intended to be entirely compatible with the 2012 and 2013 data sets, and so the same plant species were observed. Each species was verified by using *Wild About Wildflowers* (Darrow 2006) and *Colorado Flora: Western Slope* (Weber and Whitman 1996). Family names were updated to the APG III angiosperm taxonomy (APG 2009). The target species were selected in 2012 based upon their abundance in the field; only two species were chosen per a family to create a phylogenetic spread in the dataset. A voucher specimen of each species was collected and processed in 2012.

Each species was observed in three separate sites, and there will not be more than two observations at the same site in the same year. Most species observation sites will be within a 10-15 minute walk from each other. The previous GPS locations were visited to see if the same species could be observed used in the summer of 2012 and 2013, and a few sites needed to be replaced due to there being an absence of the species in question.

Floral visitation measurement:

Flora visitation was observed in five 40-minute observation periods per species. Observations were conducted between 10am and 5pm on sunny and partly cloudy days, because this is when potential floral visitors are active. A visit was only counted whenever an insect (no birds were observed visiting focal species in both previous years) landed on the front face of a flower or inflorescence for the first time. Visitors were placed into separate categories: dipterans, hymenopterans, lepidopterans, and “other insects”. A visitation only counted if the insect visit the flowers or inflorescences for the first time, and only counted as a visitor again if it visited another individual before returning to the focal individual(s). Time spent resting on a flower was

not recorded. Because comparisons of visitation rates are complicated by morphological differences between plant species, two different visitation rates were analyzed, with visitation rates being calculated by averaging across all of the above observations of a given focal species: 1) rates to individuals (no. visitors/plant/min), and rates to individual flowers (no. visitors/flower/min).

Measurements of plant traits and neighborhood characteristics:

For each observation made additional measurements were taken. These measurements include: the number of flowers and inflorescences and individuals being watched, local density of flowers or inflorescences (flowers or inflorescences/m²), floral volume (mm³), height of the tallest flower or inflorescences measured from the ground (cm), time of day, date, length of observation, the site observed, habitat type, neighboring flowers plants in bloom (categorical), cloud cover, the name of the observer, and if the individual is observed in the shade or sunlight. Floral volume is the smallest cube (LxWxH) that can fit around a flower. It was recorded by using the length and width of the front of the flower along with distance from the center of the flower to the back center of the flower. Local density was also taken by counting the number of flowers or inflorescences within a square plot with the focal individual at the center of the square. Depending on the species, the area could range from 25 m², 4 m², and 1m². The areas will vary in size depending on the abundance of plants and their inflorescences or flowers.

Estimation of phylogenetic distances:

A list of all the animal pollinated plant species in the Gothic area was created from Fraser and Buck (2007). From the complete angiosperm list, all non-animal pollinated taxa were excluded, such as those in the families: Betulaceae, Cyperaceae, Juncaceae, Poaceae, Typhaceae, Juncaginaceae, Salicaceae, and the genus *Potamogeton*. A phylogenetic tree for this species list was then created using the Phylomatic web service (Webb and Donoghue 2005) from a complete

angiosperm phylogeny in North America created by Davies et al. (2013) and imported into Mesquite ver. 2.75 (Maddison and Maddison 2009). Fossil-based branch lengths (in millions of years) were obtained from Wikstrom et al. (2001). Phylocom (Webb et al. 2008) was used to generate a phylogenetic distance matrix for all species, from which we calculated the average phylogenetic distance between each of our focal species and all of the remaining animal pollinated species in the region, as well as the phylogenetic distance to each focal species' closest relatives. Two phylogenetic metrics were then calculated: the distance to the nearest phylogenetic neighbor (nearest neighbor distance, NND) and the mean phylogenetic distance (MPD) to all species in the community. We also investigated two alternative measures of phylogenetic distance (mean inverse phylogenetic distance and inverse nearest neighbor distance, see Ness et al. 2011), but as these metrics performed similarly to MPD and NND, they will not be used in the final analyses.

Examination of the relationship between phylogenetic isolation and floral visitation:

Because an initial regression found no significant relationship between visitation rate and date in 2012, we opted to include all species in the same analysis rather than splitting species by observation period (late June and early July vs. late July and early August) and doing two sets of analyses. The same analysis was done concerning the two observation periods of 2012. We also made the decision to lump all visitors together; future analyses will examine patterns for individual groups (bees, flies, etc.). For each plant species across all observation dates, visits were totaled and observation effort (minutes) were calculated. Visitation rates to each species were then quantified as visitation per individual (plant) minute (PINM) and visitation per flower minute (PFM). A multiple regression analysis was then run on both of these visitation rates, with two measures of phylogenetic distance (NND, MPD), local density, floral volume, and inflorescence height as predictor variables. A model selection process was then conducted and

AIC (Akaike information criterion) values were calculated using SAS and were used to determine which combination of the six predictor variables remain in each final model. Partial regression plots were then generated from our main predictors of interest (MPD, NND); these plots isolate the effect of the variable in question by accounting for the confounding effects of the other variables in the model (Rawlings et al. 1988).

Results

Visitation per Individual Minute

The best fit model for visitation per Individual minute [TPINM] included: mean phylogenetic distance [MPD], nearest neighbor distance [NND], average height [AvgHeight], and average floral volume [AvgFloralVol] as predictor variables and explained 79.71% of the variation in the visitation witnessed ($p < 0.001$, $R^2 = 0.7971$). A positive relationship was found between MPD and TPINM, AvgHeight and TPINM, and AvgFloralVol (Standardized slope=0.05499; Standardized slope=0.03353; Standardized slope=0.48593 [Respectfully]) (Fig. 2). For NND and TPINM a negative relationship was found (Standardized slope=-0.03612) (Fig. 2).

Visitation per Floral Minute

The best fit model for floral visitations per minute [TPFM] include: average local density of flowers [AvgLocalDensityFlowers] and average floral density [AvgFloralVol] as predictor variables and explained 35.26% of the variation in the visitation witnessed ($p = 0.0248$, $R^2 = 0.3526$) (Fig 2.). A positive relationship was found between AvgFloralVol and TPFM (Standardized slope=0.07229) while a negative relationship was found between AvgLocalDensityFlowers and TPFM (standardized slope=-0.05295) (Fig. 2)

Discussion

The goal of this study was to determine if the phylogenetic isolation of a plant species is correlated with its floral visitation rates. Previous studies have shown that pollination success for a single focal species is influenced by the phylogenetic diversity of a plant community (Schuett and Vamosi 2009, Sargent et al 2011), yet to our knowledge no community-wide research on floral visitation and phylogenetic distance has been conducted. Initially, it was hypothesized that an intermediate level of phylogenetic isolation would associate with higher levels of visitation.

Previous year's data showed that species with a further away relative would receive more visitation (Higher NND) compared to a species that has a closer relative (Low NND) (Agosti 2013, Evans Peck 2012). It also showed that a species with many closely related neighbors (High MPD) would receive more floral visitations compared to a species with fewer closely related neighbors (Low MPD) (Evans Peck 2013). In 2013 only NND was found to have an effect on floral visitation while MPD was not found to have any significant effect on floral visitation (Agosti 2013).

The slope of the partial regression graph for MPD was positive instead of 2013's negative slope and had a much higher R^2 value, 79.71% compared to 2012's R^2 value of 35.23% (Evans Peck 2012), for the relationship between MPD and TPINM (Fig. 2). The relationship between NND and TPINM was found to have a negative slope (Fig. 2), and is opposite to the previous year's results which showed a positive correlation in both years studied (Agosti 2013, Evans Peck 2012). The reason for this may be because this year was an "average" year for snow pack (Fig. 1) and this abiotic factor may have influenced our results. Follow up studies should be conducted using the same methods to see if the results would be similar in another "average" snow pack year, and if similar results would be comparable to the "dry" year's results.

In the results found this year for floral volume [AvgFloralVol] was again seen to be correlated with floral visitation per minute and had a similar R^2 , 35.26% compared to 2013's R^2

value of 35.45%, but this year's results has a steeper standardized slope (standardized slope=0.07229) versus last year's standardized slope (standardized slope=0.00316) (Fig. 3, Agosti 2013, Table 1). Thus, we are still seeing that larger flowers, and more conspicuous flowers, are attracting more floral visitation.

The conspecific density results had a steeper standardized slope (standardized slope=-0.05295) compared to 2012's slope (standardized slope=-0.020) and 2013's slope (standardized slope=0.00349) (Fig.3, Evans Peck 2012, Agosti 2013). This makes sense because the increased species within a plot would create competition among the individuals of the same species, and thus result in lower floral visitation for the host species. The occurrence of larger pollinator visits in high conspecific density environments has been previously conducted by Bosch et al. (1999) where they found similar results. This may be due to a "wetter" year allowing for plants to have higher rates of growth and density within the community allowing the conspecific density of the host species to increase.

With the results found this year it would be important to repeat the study over another three year period to compare and contrast different year's results for phylogenetic distance affecting floral visitation. This experiment has been performed during two "dry" years and one "average" year for snow pack, and in the "average" year we have found results that are the direct opposite of the two previous years. And, they have stronger explanation for the phylogenetic variables in the system. This may be the result of different abiotic conditions or some other mechanism that is at play, and it would be beneficial to the project if a larger data set was collected to analyze the effects.

Along with collecting a larger data set the additional co-variants measured (surrounding species, sun or shaded plants, and weather) could be analyzed to see if they have any significant impact on the visitation levels of the host species studied. If so, the project could be more fine-tuned to account for the variability that may occur due to these co-variants (i.e. observing the host species in only sunny environments if the impact on floral visitation is greatly influenced by shaded or sunny

area) and this would allow the experiment to get more realistic results. The surrounding species that were recorded within the 5m² plots could also be analyzed along with MPD, NND, and floral visitation to see if there is influence on floral visitation within a small plot caused by species phylogenetic distance.

Acknowledgements

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Wymore AS, et al. 2011. Genes to ecosystems: exploring the frontiers of ecology with one of the smallest biological units. *New Phytologist* 191:19-36.

Figures

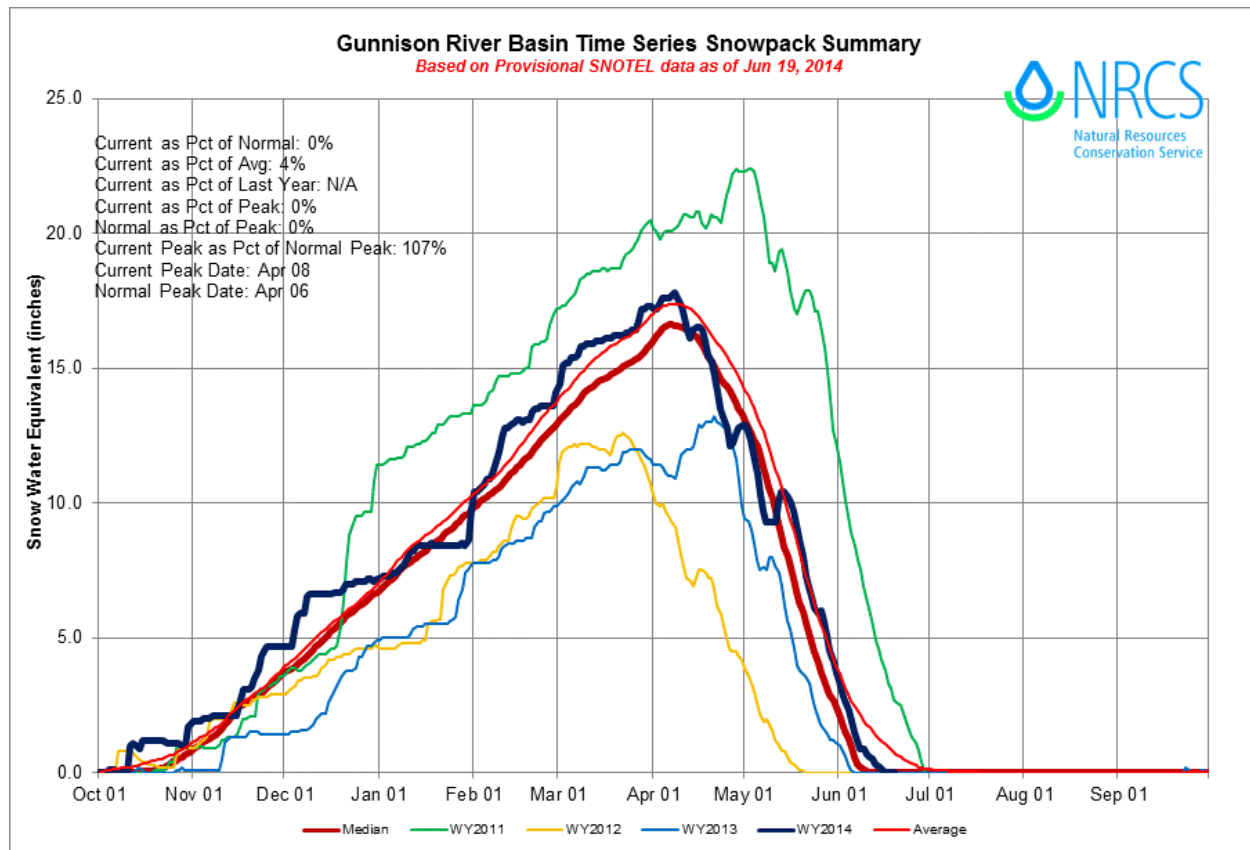


Figure 1. The snowpack levels for the last four years near the research sites.

Visitations per an Individual Minute

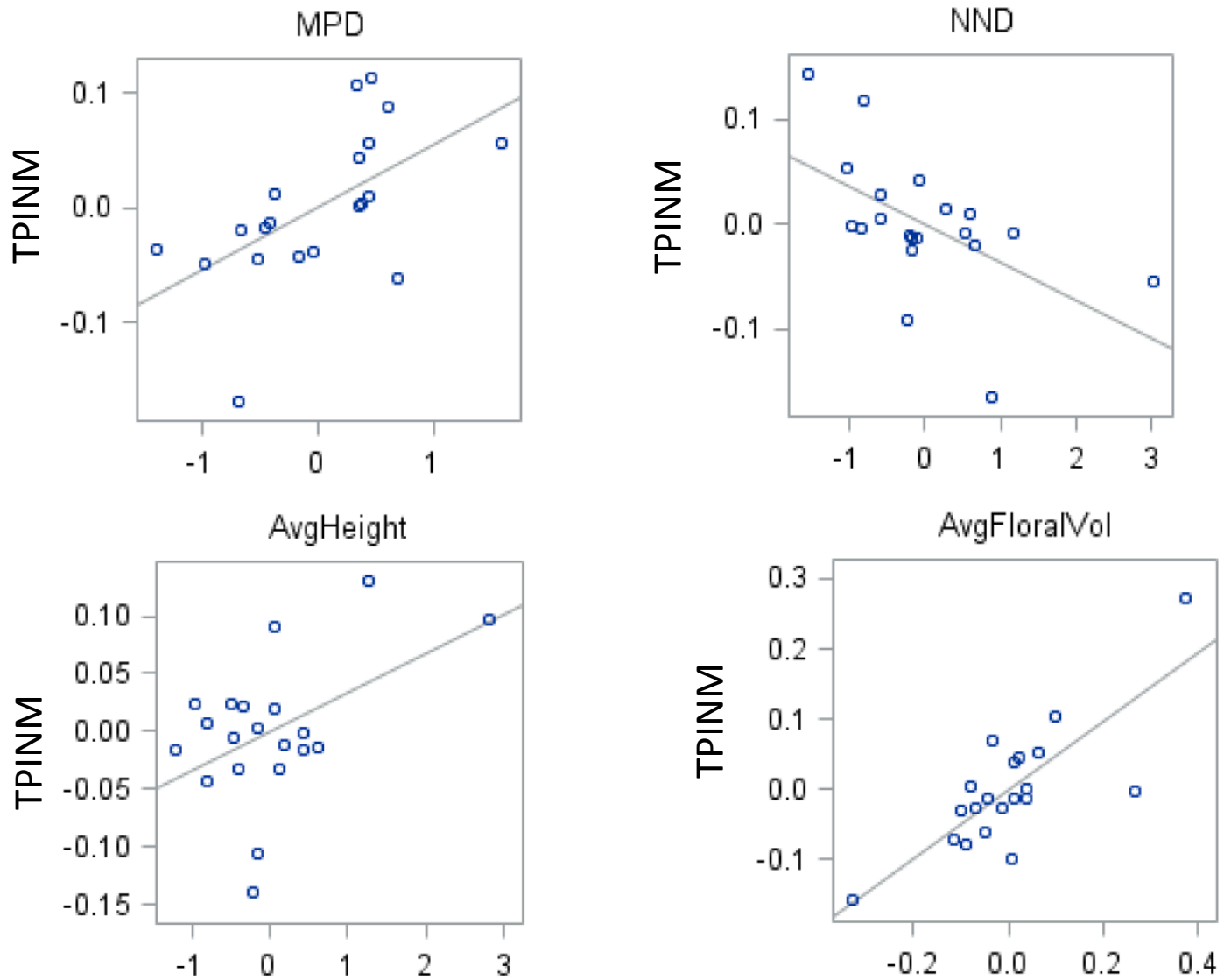


Fig. 2. The best fit model for Individual visitation per minute included 4 variables: NND, MPD, AvgHeight, and AvgFloralVol as predictor variables ($p = <.0001$, $R^2 = 0.7971$). The partial regression shows that NND has a negative correlation with visitation per inflorescence minute (slope = -0.03612). The partial regression for MPD shows a positive correlation with visitation per inflorescence minute (slope = 0.05499). The partial regression for AvgHeight shows a positive correlation with visitations per inflorescences minute (slope = 0.03353). The partial regression for AvgFloralVol shows a positive correlation with visitations per inflorescences minute (slope = 0.48593).

*Both x-axis are standardized

Visitations per a Floral Minute

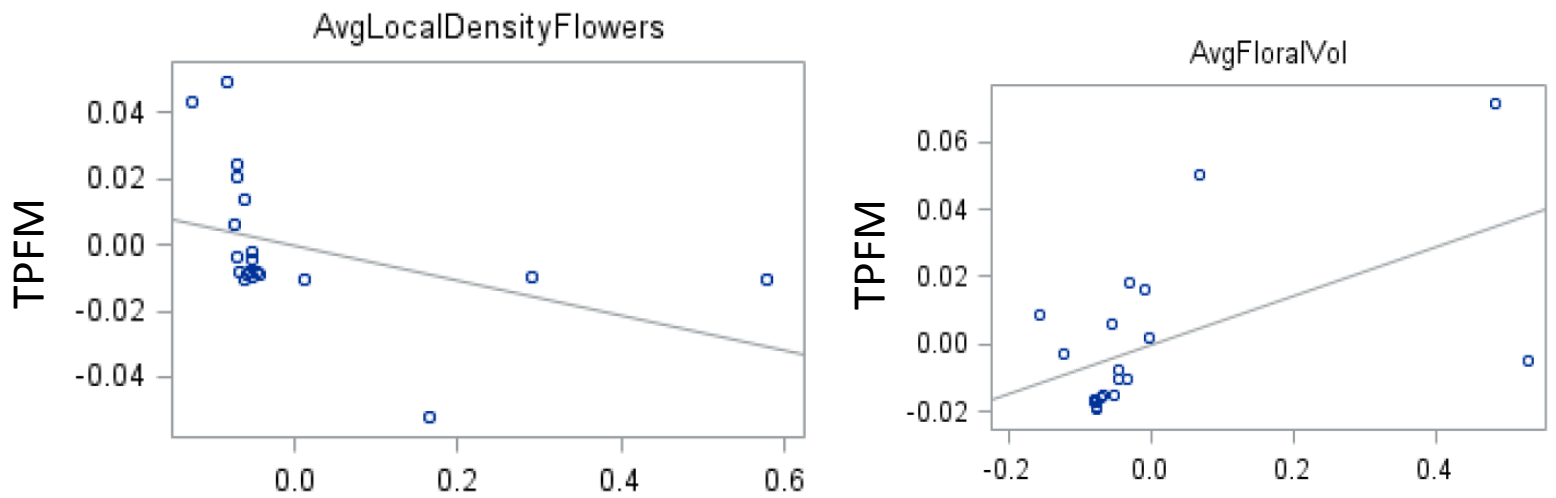


Fig. 3. The best fit model for flower visitation per minute included two variables: AvgLocalDensityFlowers and AvgFloralVol as predictor variables ($p=0.0248$, $R^2=0.3526$). The partial regression for AvgLocalDensityFlowers has a negative slope (slope=-0.05295). The partial regression for AvgFloralVol has a positive slope (slope=0.07229).

*Both x-axis are standardized

Observational Sites 2014

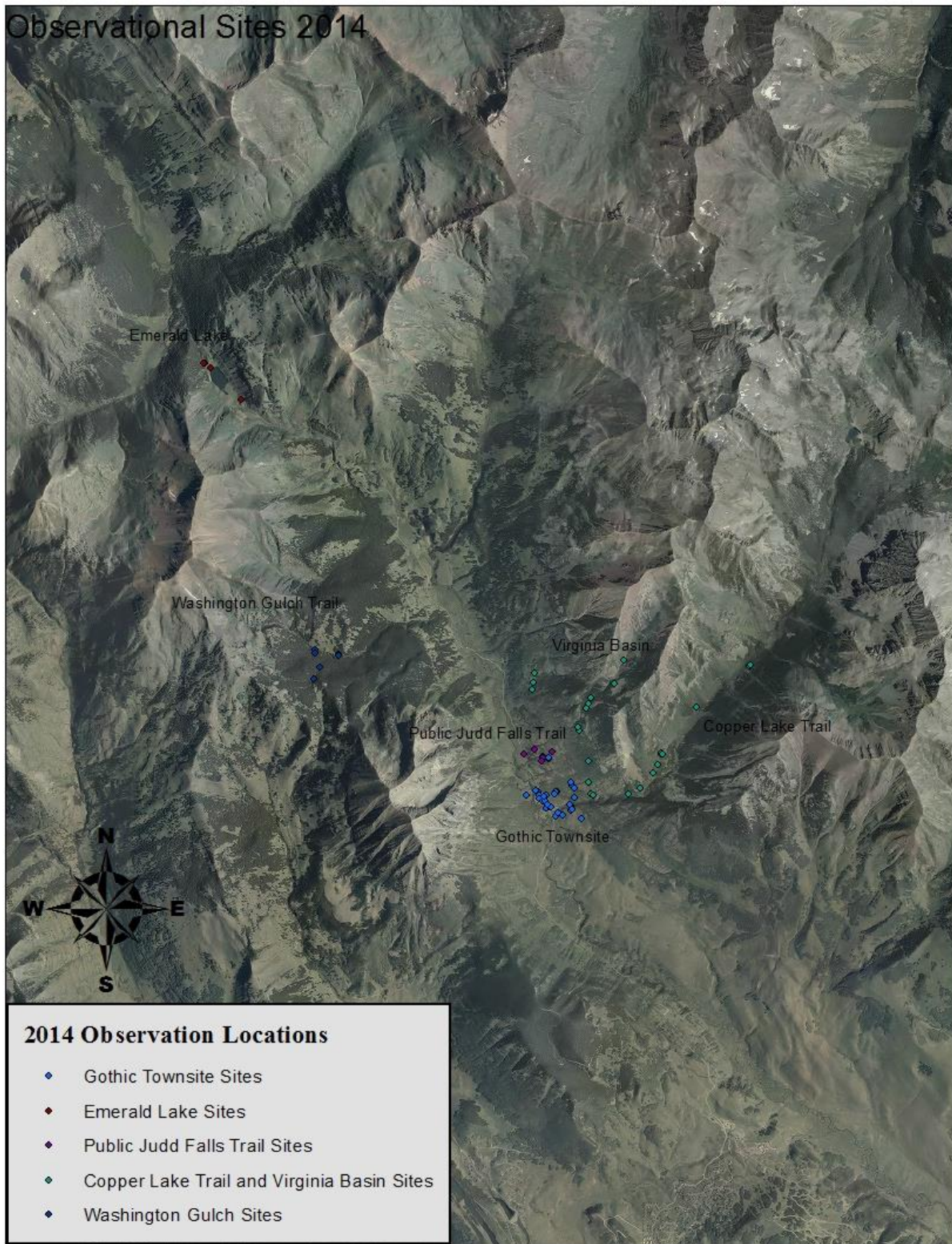


Fig. 4. Map of all observational sites in Gothic, CO

Tables

	Standardized parameter estimate (slope)
Mean phylogenetic distance	0.05499
Nearest neighbor distance	-0.03612
Average Floral Height	0.03353
Average Floral Volume	0.48593

Table 1. The slopes of the individual visitation per minute variables.

	Standardized parameter estimate (slope)
AvgLocalDensityFlowers	-0.05295
AvgFloralVol	0.07229

Table 2. The slope of the flower visitations per minute variables.