

Measuring the influence of phylogenetic relationships on floral visitation.

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Abstract:

Pollinators are attracted to flowers based on the sensory information they gather concerning the flower they pollinate, and decide to visit the flower, or not. Closely related plants have similar traits because of their similar genetics, and consequently will attract similar pollinators. The abundance of floral visitors a plant gets is dependent on its ability to attract pollinators and the frequency of floral visitation can be able to be predicted based on the phylogenetic isolation of a focal species.

Phylogenetic isolation may be a logistically easier way to predict floral visitation than measuring morphological similarities. There exists a positive relationship between the number of visitors a species gets and the phylogenetic distance (in millions of years) to its nearest neighbor. There also exists a marginal negative relationship between the rate of floral visitation and the mean phylogenetic distance of a focal species in the community.

Introduction:

A new paradigm is emerging in understanding how communities are structured and how community level processes are understood. The phylogenetic composition of a community is a more powerful and more appropriate descriptor of biodiversity than the traditional “species richness”. For instance, insects in forests are shown to have a greater influence on trees through herbivory when their

habitat is composed of trees all in the same class, rather than a mixed stand. (Jactel and Brockerhoff 2007) The expression of similar morphological traits in more closely related trees is the reason why insects can have a greater impact on stands composed of trees in the same class. It is the morphological traits that are stimuli for interspecific interactions like between insects and trees, and the more closely related the tree species, the more similar their morphological traits will be because they will have had less time to diverge evolutionarily and thus develop different genotypes to express. Applying genetic knowledge at the ecosystem level is apply called the “genes to ecosystem concept” (Wymore et al 2011) and is becoming popular because of its greater power to explain certain community level processes like herbivory (Ness et al 2011), frugivory (Rezende et al 2007) pollination, (Evanspeck and Whitney 2012 unpublished) and possibly others like predation or parasitism.

Like with other interspecific interactions, animal pollination of flowering plants is dependent on the expression of plant genes, the ability for pollinators to perceive that expression, and to act on their perception by either visiting a flower and incidentally pollinating it (or not) or by choosing to avoid the flower. The review by Fenster et al 2004 presents an in depth discussion on “pollinator syndromes” which is a concept that explains how the evolutionary trajectory of a flowering plant species is most influenced (with respect to selection) by one or more functional groups of pollinators.

A functional group of pollinators can be defined as multiple species that have similar enough methods of pollination so as to put similar selective pressures on the flowers they frequent (Fenster et al 2004). When added to the fact that closely related species are likely to express similar morphologies because of their similarities in genetic composition (Wymore et al 2011), a group of closely related plants will likely attract the same or similar functional groups of pollinators. This is an extension of the results found by Schuett and Vamosi 2010, who’s work showed that a focal plant species received less conspecific pollen and was consequently less fit when it was in a community of more closely

related species. The floral visitors received by a plant are not necessarily the same as the functional group that are most influential in the reproductive success of the plant, and the overall influence on the reproduction of plant by these no specialized visitors is relatively unimportant (Fenster et al 2004).

We have shown that the phylogenetic isolation of a plant species within its community can predict floral visitation (Evans Peck and Whitney 2012 unpublished), however the data collected in 2012 is somewhat questionable because of the abiotic abnormalities of the ecosystem at the time. The snow fall during the winter preceding the 2012 summer was abnormally low (Barr unpublished) and greatly influenced the abundance of flowering plants across the landscape that was surveyed. Because of this abnormality, it is unclear how general our predictions are. Here we ask what influence phylogenetic isolation has on floral visitation during a more “normal” year with normal refereeing to the overall moisture levels and the timing of the snowmelt in the spring. We focus on floral visitation by any animal rather than the specific functional group so that a greater number of species could be observed. It was logistically impractical to research the functional groups of the 24 species chosen for observation given the restraints of time; and so floral visitation by any “potential animal pollinator” was recorded. This accounts for any visitor that may just be seeking rest or is a nectar robber etc. In alignment with the findings of last year’s analysis, it is expected that a plant species with few close relatives in the community; that is to say a species with a high mean phylogenetic distance (MPD) will experience fewer visits in the same amount of time than will a species with a greater number of close relatives (a lower MPD). Also in line with last year’s findings, a species that has a very close relative (a low nearest neighbor distance [NND]) will experience fewer visits (because of the competition provided by the close relative) than a species that has a distantly related nearest phylogenetic neighbor (a high NND).

Methods:

Site selection:

All floral visitation data were collected in the area surrounding Gothic, Colorado, 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*). Observation sites were restricted to the subalpine to montane habitats of the valley including the Gothic Town site, Virginia Basin, the Maroon Bells Wilderness, Emerald Lake, and Washington Gulch Trail. The summer of 2013 was preceded by a relatively low snowpack, but in comparison to the 2012 winter, the timing of the spring snow melt was much closer to average. (Figure 1).

The data collected in the summer of 2013 was intended to be entirely compatible with the 2012 data set, and so the same species observed in the early and late summer observation periods were again separated into two periods of observation (Table 3). Three of the species observed in 2012 (*Gentiana parii*, *Linaria vulgaris* *Trifolium repens*) were left out of the 2013 survey because they lacked a sufficient amount of data to make significant comparisons. Species were identified with the help of *Wild About Wildflowers* (Darrow 2006) and *Colorado Flora: Western Slope* (Weber and Whitman 201). Family names were updated to the APG III angiosperm taxonomy (APG 2009). The species lists in each time period (for both years) were intended to be wholly distinct. Target species were initially selected in the summer of 2012 based on their abundance (and therefore on their ability to provide a sufficient amount of data) but were limited to two species per family in order to create phylogenetic spread in the dataset. All plants were selected from *Vascular Plants of the Gothic Area* (Frase and Buck 2007). A voucher specimen of each species was collected and processed in 2012.

Each species was observed at three separate sites, with no more than two observations at the same site in the same year. Except in the case of *Polemonium foliosissimum* and *Hackelia floribunda*, sites of the same species were not less than a 5-10 minute walk apart from each other. The three sites

of observation for each species were a 10-15 minute walk in distance from each other except in the case of *Polemonium foliosissimum* and *Hackelia floribunda*, which had sites within closer proximity to each other. Individual plants were not marked for repeated observations; however the GPS coordinates were revisited in the second year of observation, and individuals within ~100m of the original sites were used as subjects for observation. When no individuals could be found at the 2012 sites, new sites were observed.

Floral visitation measurements

Each of the three sites a species was observed at had at most two 40 minute periods of observation. A minimum of five observations were made per species in the 2013 survey. Floral visitation was recorded during these 40 minutes sessions. A visit was counted whenever an insect (no birds were observed visiting focal species in either year) landed on the front face of a flower or inflorescence for the first time. When more than one individual was being observed at a time, visits to different individuals under observation were counted as different; however visits to different flowers or inflorescences on the same individual were not counted as different unless the insect visited another individual between the visits to the different flowers on the first individual. The length of time spent on a flower was not counted, simply landing on an individual plant under observation counted as a visit. Because comparisons of visitation rates are complicated by morphological differences between plant species, three 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), rates to inflorescences (no. visitors/inflorescence/min), and rates to individual flowers (no. visitors/flower/min).

Measurements of plant traits and neighborhood characteristics:

Each observation included a list of measurements other than floral visitation. These measurements included: the number of flowers and inflorescences and individuals watched, local density of flowers or inflorescences (flowers or inflorescences/m²), floral volume (mm³), height of the tallest flower or inflorescence off of the ground (cm), time of day, date, length of observation, the site observed, habitat type, neighboring flowering plants in bloom (categorical), cloud cover, the name of the observer, and if the individual being observed was in the shade or direct sunlight (without regards to cloud cover). Local density was measured by counting the number of flowers or inflorescences within a square plot with the focal individual at the center of the square. Floral volume is essentially the smallest cube that could fit around the flower observed. Inflorescence volume was not measured. To measure floral volume, the length and width of the front face of the flower were measured as well as the distance between the center of the front of the flower to the center of the back of the flower. Habitat type was recorded as well (aspen or conifer understory or meadow).

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) 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 were not 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 for the 2012 and 2013 analyses; 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) was 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 in 2012. In the 2013 analysis; NND, local density, floral volume and the height of the tallest flower were used as predictor variables and the same regression was used. A model selection process was then conducted and AIC values were used to determine which combination of the six predictor variables remained in each final model. Partial regression plots were then generated for 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:

Visits per individual minute

The best fit model for visitation per individual minute included the height of the tallest flower in conjunction with floral volume. The variation in these two traits accounts for 25.34% of the variation seen in visits to the individual for the 2013 survey ($R^2=0.2534$) (Table 1). The relationship between Flower height and visitation to the individual was positive (Table 1), while the relationship between floral volume and visits to an individual was negative (Table 1).

Visits per flower minute

The best fit model for visitation per flower minute included NND, local density of flowering conspecifics and floral volume. Together, these traits accounted for 35.45% of the variation seen in floral visitation rates ($R^2=0.3545$) (Table 2). Floral volume and NND were positively correlated with floral visitation (Table 2), local density was negatively correlated (Table 2) with visits per flower minute.

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 be associated with the highest rates of visitation. After a single summer's observation, this was found to be inaccurate; however MPD and NND were shown to be correlated with floral visitation. A second summer's survey confirmed this initial finding for NND. These two phylogenetic metrics predict floral visitation in different ways. Neither has any significant predictive power concerning visits to an individual (plant), however NND significantly predicts the number of visits a flower will get over time, and the predictive power of

MPD is marginally significant at the scale of the flower. The negative correlation between NND and the rate of floral visitation indicates that a focal species with a very close relative will receive fewer visits than a species with a more distantly related nearest relative. The positive correlation between MPD and the rate of floral visitation indicates that a focal species with many closely related neighbors will receive more visits than a focal species with a group of more distantly related neighbors.

Extreme cases of isolation were not analyzed here because of the lack of observation on invasive animal pollinated flowering plants; however the influence of phylogenetic isolation on invasives with respect to floral visitation may very well be akin to other community processes (like herbivory) where invasives follow similar trends as natives (Ness et al. 2011)

Other morphological traits expressed by flowering plants may have a significant influence on visitation rates, traits like flower color, nectar production and nectar sugar concentration etc. The influence that morphological characteristics have on pollinator attraction is not entirely known, and so the difference between phylogenetic isolation and trait expression similarity needs to be made clear.

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Tables:

VISITS per INDIVIDUAL MINUTE				
	Standardized Parameter Estimate (slope) 2012	Standardized Parameter Estimate (slope) 2013	Significance in 2012	Significance in 2013
Plant Height	0.101	0.08542	p=0.0342	p=0.0465
Floral Volume	n/a	-0.05682	n/a	p=0.0465

Table 1. The variables included in the best fit model for visitation per individual minute, and the significance they had in different years.

VISITS per FLORAL MINUTE				
	Standardized Parameter Estimate (slope) 2012	Standardized Parameter Estimate (slope) 2013	Significance in 2012	Significance in 2013
NND	0.025	0.00263	p=0.0548	p=0.0298
MPD	0.019	n/a	0.0548	n/a
Floral Volume	n/a	0.00316	n/a	p=0.0298
Local density	n/a	0.00349	n/a	p=0.0298

Table 2. The variables in the best fit model for visitation per flower minute, and the significance they had in different years.

Observation period	Species name	Family	Observation minuets
6/28/2013-7/20/2013	Aquilegia coerulea	Ranunculaceae (Darrow 2006, APG III 2009), Helleboraceae (Weber 1996)	239.000
	Castilleja miniata	Scrophulariaceae (Darrow 1996, Weber 2006) Orobanchaceae (APG III 2009)	200.000
	Cymopterus lemmonii	Apiaceae	200.000
	Erigeron elatior	Asteraceae	200.000
	Eriogonum umbellatum	Polygonaceae	235.000
	Erysimum capitatum	Brassicaceae	240.000
	Geranium richardsonii	Geraniaceae	240.000
	Helianthella quinquenervis	Asteraceae	240.000
	Ligusticum porteri	Apiaceae	201.000
	Linum lewisii	Linaceae	200.000
	Polemonium foliosissimum	Polemoniaceae	200.000
	Potentilla gracilis	Rocaceae	240.000

Zigadenus elegans	Liliaceae (Darrow 2006), Melanthiaceae (Weber 1996, APG III 2009)	200.000
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Arenaria congesta	Caryophyllaceae
Campanula rotundifolia	Campanulaceae
Chamerion angustifolium	Onagraceae
Delphinium barbeyi	Ranunculaceae (Darrow 2006, APG III 2009) Helleboraceae (Weber 1996)
Galium boreale	Rubiaceae
Gentianopsis thermalis	Gentianaceae
Hackelia floribunda	Boraginaceae
Lupinus argenteus	Fabaceae
Mertensia ciliata	Boraginaceae
Pedicularis racemosa	Scrophulariaceae (Darrow 2006, Weber 1996) Plantaginaceae (APG III 2009)
Saxifraga odontoloma	Saxifragaceae

Table 3: All of the species observed and their respective families. Where no reference is given, there was agreement between Darrow, Weber, and APG III on the family name. The total minutes of observation per species is also indicated.

Figures:

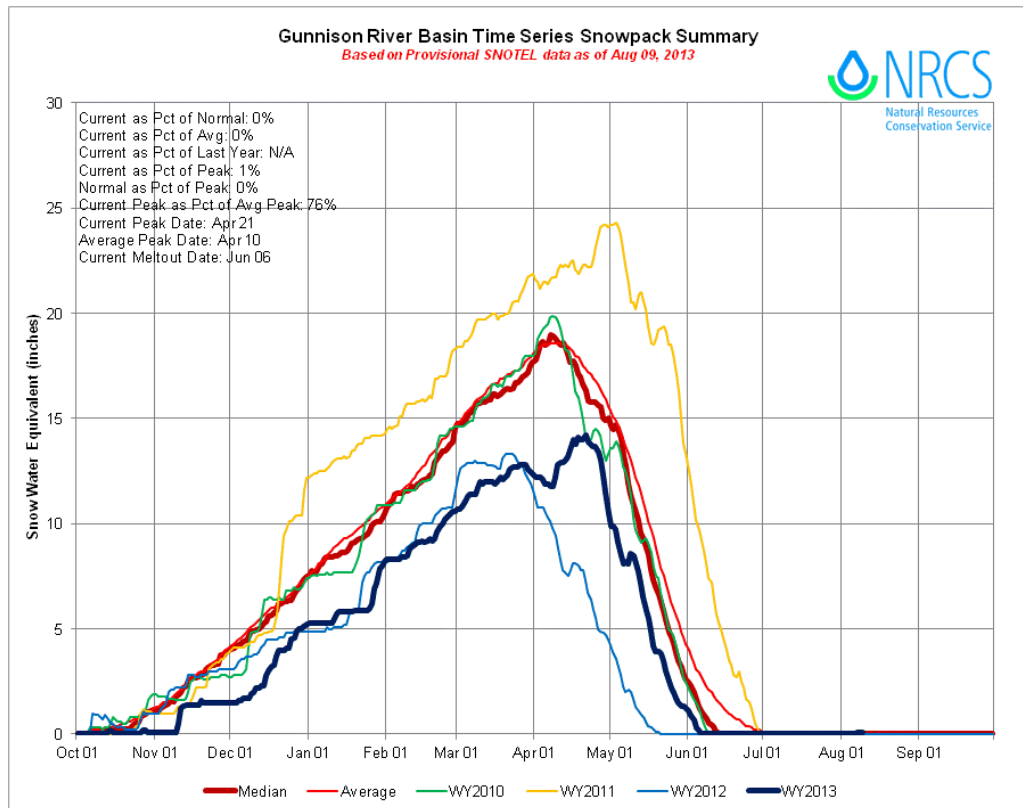
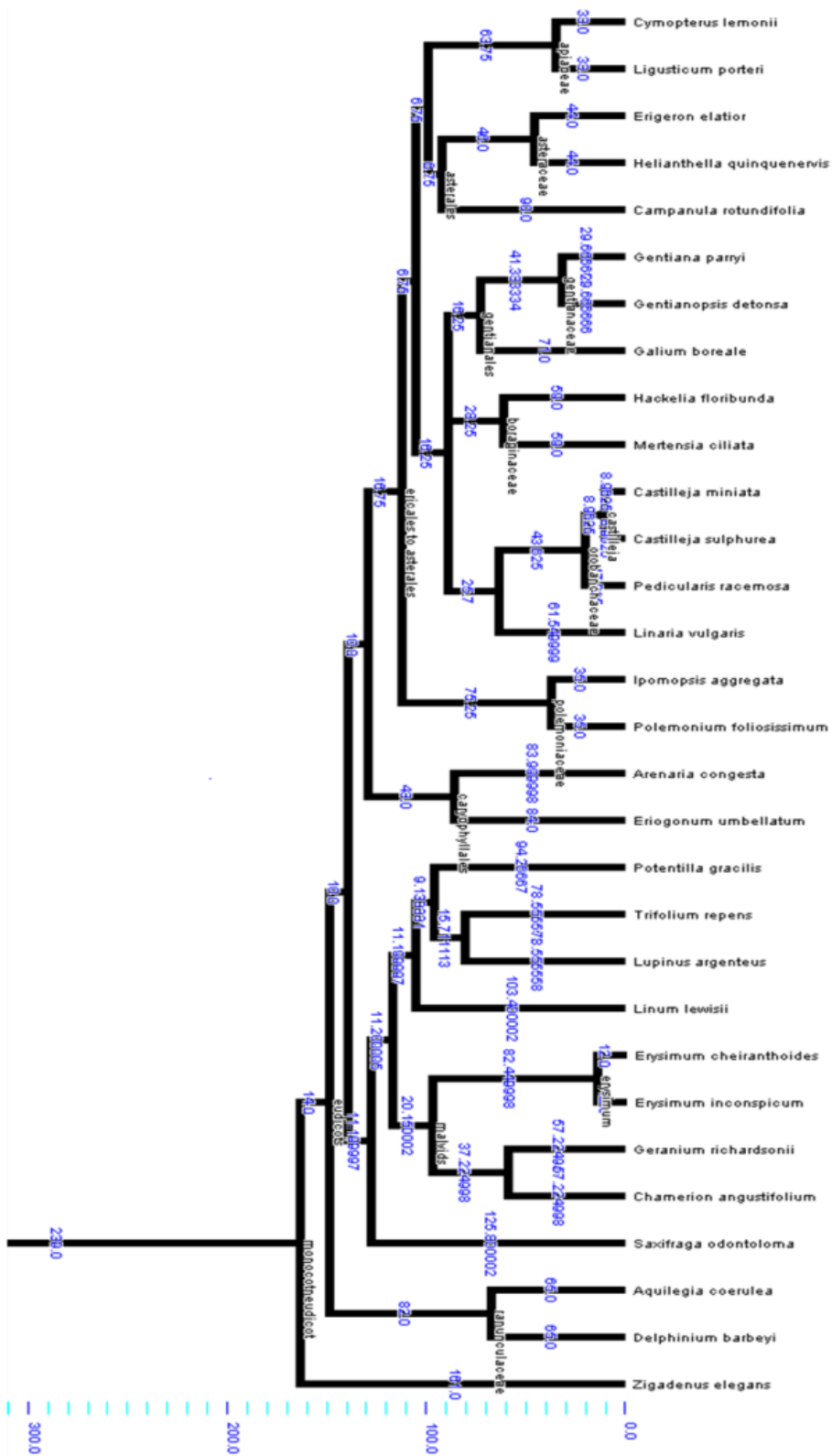
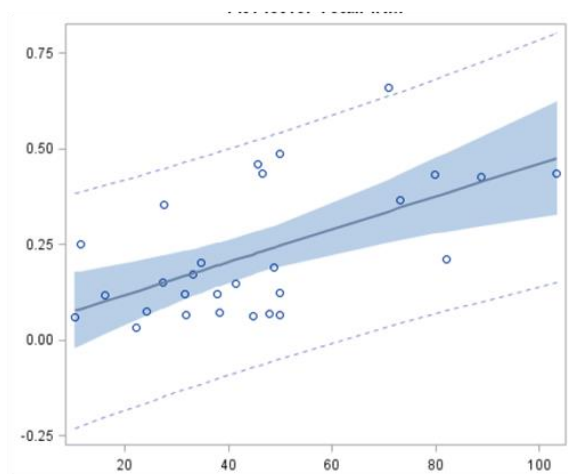


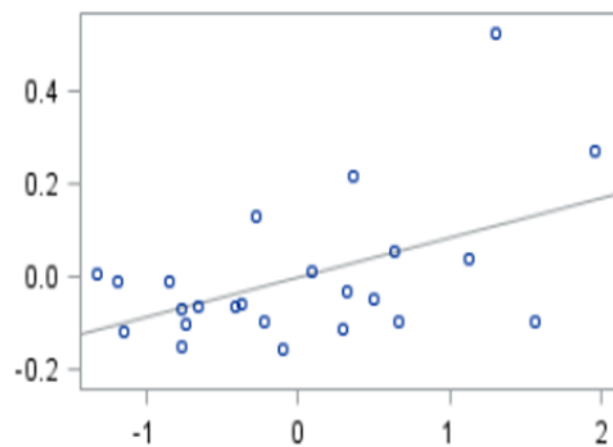
Figure 1. The recorded snowfall during spring over the four years preceding this study.



Visits/individual min



Plant Height



Plant Height

Figure 2. The relationship between plant height and the rate of visitation to individuals in 2012 (left) and 2013 (right).

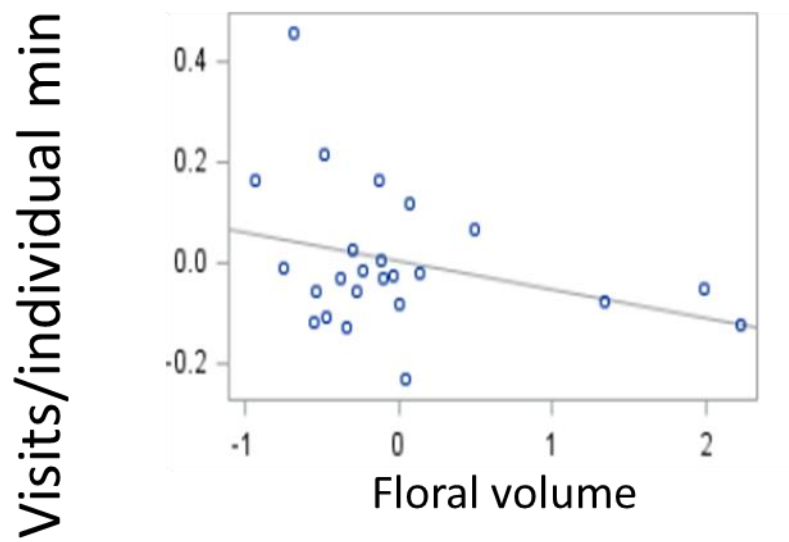


Figure 4. The relationship between floral volume and the rate of visitation to individuals in 2013

Visits/ flower min

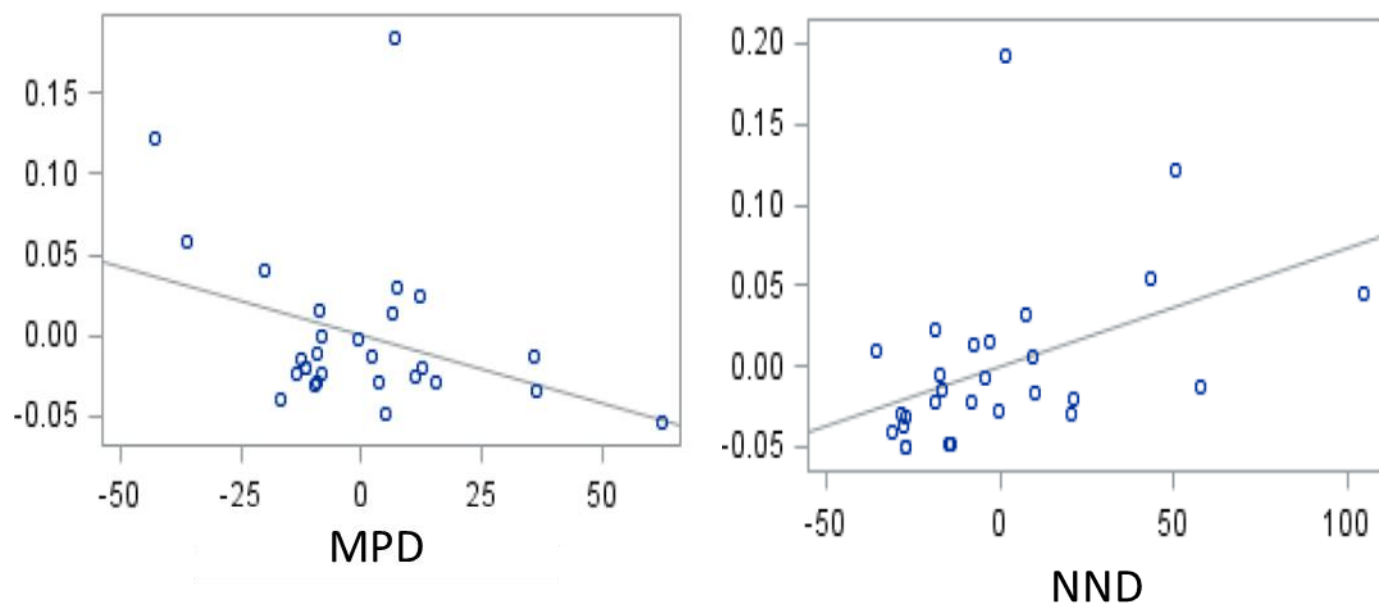


Figure 5. The relationship between either phylogenetic isolation metric and floral visitation to flowers. (2012)

Visits/ flower min

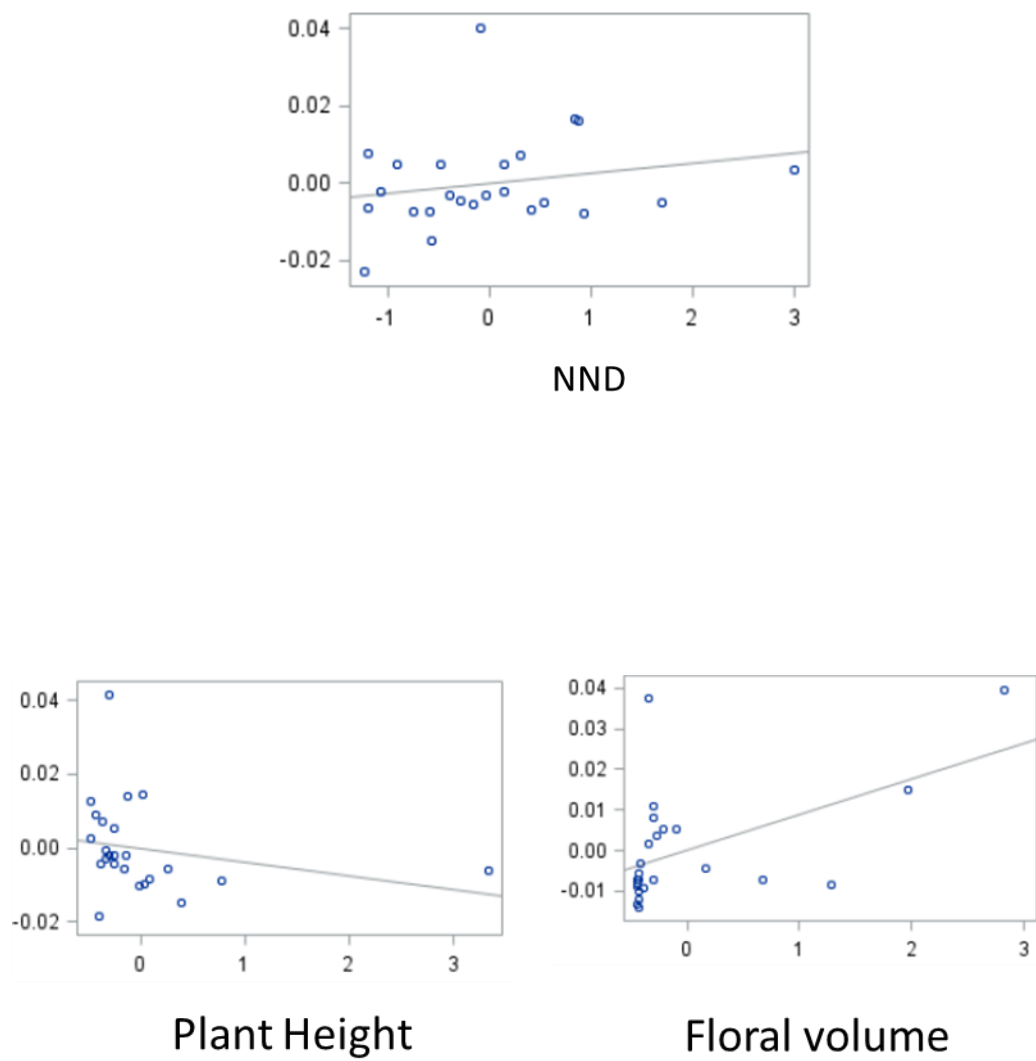


Figure6. The relationship between phylogenetic (top) or morphological traits and rates of visitation to flowers. (2013)

2013 Research Sites

