

Quantifying within-season floral trait distributions of flowers in
Colorado Rocky Mountain sub-alpine dry meadows

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Abstract: This study explored the way in which floral traits, within a community and across (rather than within species), vary in a growing season. It is apparent that the floral resource landscape changes within a summer, and no two weeks are identical in the resources they provide to pollinators. Similarly, pollinator phenology dictates that different species are present and using resources at different times. Because plant-pollinator interactions best occur when trait matching is possible and when phenologies overlap, it is essential to combine these factors and understand the phenology of traits – how floral traits change and are distributed within a season. Measurements were made of nine traits for 20-30 (approx.) flowers of each floral species present in two dry sub-alpine meadows in Gothic, Colorado. Averages were taken for each trait in each species, and distributions were plotted against time, using phenology data for the floral species. These distributions were then weighted for species abundance (also from the phenology dataset), in order to develop accurate graphics for how the real-time patterns of floral traits changed within the season. Six continuous variables were measured and plotted (continuous variables have not yet been analyzed): corolla depth, floral display width, nectar opening width, flower count per stalk and per plant, and plant height. Corolla depth demonstrated a non-linear trend with a peak at mid-season (week 6), most likely the result of high *Delphinium nuttallianum* abundance. Floral display width and plant height both showed strong upward trends, with traits increasing in value across the season. Flower count per stalk showed a non-linear upward trend across the season; however, flower count per plant was had no significant trend, as did nectar opening width (though this greater sampling may have made this pattern significant). Overall, these patterns indicate that, while variation and changes in the trait landscape are present, the community as a whole does not change uniformly. Changes in the community are occurring in a more complex and variable manner, which in turn influences how pollinators must structure their interactions with available floral resources.

Introduction:

Plant-pollinator interactions are critical for ecosystem function (Waser and Forrest 2014). A great deal of research has gone into understanding the intricacies of morphology, phenology, and pollinator interactions for specific plants. However, plants and their pollinators exist within a community larger and more complex than can be understood by studying single species or individual interaction types. More recently, research has begun to focus on entire plant-pollinator communities (e.g., Stang et al. 2009, Vázquez et al. 2009).

It is necessary to take into account size and trait distributions between species *and* across the growing season in order to better characterize large-scale interaction networks, and understand how floral characteristics determine community-level pollinator distributions (Stang et al. 2009; Bosch et al. 1997). It has also been determined that the majority of animal species that interact with plants take the role of moderate to extreme generalist (Waser et al. 1996), where each interaction is influenced by abundance, spatio-temporal overlap, and trait matching between mutualistic plant and pollinator species (Vázquez et al. 2009). As such, greater abundances of “matchable” traits at a given time increase the possibility for interactions between those two species as long as their spatio-temporal distributions overlap (Olesen et al. 2010).

The available evidence strongly suggests that plant and pollinator systems are dynamic and change across the season (Alarcón et al. 2008, Olesen et al. 2008, P.J. CaraDonna *unpublished data*). However, most studies do not investigate how interactions may be influenced by changing plant and pollinator trait distributions within a single season. We see a vast variety of wildflowers across any given growing season here at the RMBL, due to the complexity and heterogeneity of floral communities. In order to make use of models concerning distributions of “matchable” traits, we need to develop a picture of how floral traits are distributed across a given season, and how that spread interacts with the presence of pollinator traits.

The primary goal of this study was to examine within-season patterns of floral traits and pollinator traits, and determine whether floral and pollinator traits vary directionally, remain constant, or are indistinguishable from random across the season. We aimed to establish whether there are quantifiable trends in flower traits and pollinator traits by measuring a broad sample of traits across 35-40 floral species and 60-65 pollinator species throughout the 2015 growing season. The answer to this question can lead to explorations of the mechanisms that create this pattern, and perhaps relate to trait phenology in corresponding pollinator species, ultimately generating a deeper understanding of the community composition and how it fluctuates over the course of a season.

Methods:

Study Site: Samples of 32 flower species were taken in two southwest-facing dry sub-alpine meadows in the Rocky Mountains, Colorado, USA (~2900m a.s.l.). Between the two meadows, a total of 20-30 samples were taken of each species present. Species no longer in season within the two meadows necessitated require trips up the valley, where elevation is greater and community phenology is not as far along. However, this was only necessary for four of the species.

Floral Trait Sampling: The primary methodological component consisted of measuring a collection of floral morphological traits. Floral measurements were made for 32 species between mid-June and late July. Traits recorded, as listed in Table 1, are: flower number per stalk

(randomly selecting a single stalk per plant); flower number per plant; plant height; corolla length; nectar opening width; floral display width; flower color; flower symmetry; and pollen access (is the pollen external, or hidden within the plant and therefore harder to access). In the case of bundled flowers, like *Hydrophyllum capitatum* or *Senecio integerrimus*, the whole inflorescence was measured; with individual flowers, like *Delphinium nuttallianum*, the single flower width was measured at its widest diameter. A meter stick was used to measure height ($\pm 0.1\text{cm}$), and digital calipers ($\pm 0.1\text{mm}$) are used to make finer-scale measurements. Each species had 20-30 replicate measurements (excepting a few, due to issues of abundance and availability) and, for each trait in each species, replicates were converted into mean values. Flowers were only cut when necessary for accurate measurement (ie. very small flowers). In such cases, destructive sampling was minimized, as many plants have numerous flowers and only one flower per plant was sampled.

Table 1: Corresponding floral and pollinator traits that will be measured during the 2015 growing season at the Rocky Mountain Biological Laboratory.

Floral Traits	Pollinator Traits
Corolla length / nectar depth	Tongue/proboscis length
Flower/inflorescence width	Body size (width and length)
Nectar opening width	Head diameter
Flowers per stalk and per plant	Pollinator type
Plant height	Sex
Color	
Symmetry	
Pollen exposure (yes/no)	

Pollinator Trait Measurements: Following floral sampling, measurements were made of traits in pollinators that are relevant to their pollination ability, such as proboscis/tongue length, head width, intertegular distance, body width and length. Measurements were made on species already present in existing collections (RMBL, and Paul CaraDonna). In the event that a species was not present in any existing collection, we collected 1–4 individuals for trait measurements. Where collection was necessary, pollinator samples were collected from Paul CaraDonna’s research sites.

Subject Species: We measured subalpine flowering plants in the subject meadows, consisting of about 32 species that flower from the beginning towards the end of the summer growing season, and have known visiting by pollinators. We measured corresponding traits on the 60-65 pollinators that visit these plants throughout the season. Pollinator measurements were taken for all interaction species known, based on Paul CaraDonna’s plant-pollinator interaction data, to regularly interact with the subject floral species.

Statistical Analyses: We examined patterns in traits across the season using linear and non-linear regression. The predictor variable was time in weeks. The response variables were mean floral and pollinator trait values, as well as variance in those trait values. We calculated mean and variance of floral and pollinator traits within each week in two ways: (1) unweighted trait measurements based on the presence or absence of species, and (2) weighted by plant and pollinator abundance. All measurements for a given trait in a given species were converted into means, in order to define what the “average flower” looks like for each species. Regressions demonstrated how traits are distributed across a season, where there may be no pattern (a

straight line, or random variation) or a variety of linear or non-linear trends, demonstrating whether there are trends in trait values *between* species, rather than within them.

Results:

Patterns within the growing season were found for four of the six continuous traits measured. Corolla length followed a non-linear regression, with a statistically significant peak towards mid-summer (fig. 1a). Flower display width showed a significant linear increase across the season, with peak mean flower display width falling at the end of the summer (fig. 2a). Flower counts per stalk demonstrated a strong non-linear pattern across the season, with an upswing towards late summer (fig. 4a). Plant height, originally measured as a covariate, ended up showing the strongest of our trends, with a strong linear pattern indicating that present plant species were taller as the season progressed, with the maximum heights found in the late summer (fig. 6a). While the results for both nectar opening width and net flower count - per plant, rather than per stalk - were non-significant, non-linear regressions were fit to both these data sets, with opening width showing a mid-summer decline (fig. 3a) and net flower count showing a mid-summer peak (fig. 5a). Corresponding weighted and unweighted box-plots (figures “b” and “c” for each figure 1-6, respectively), created for all variables, demonstrate the above patterns and also show ranges of variance, which will be part of future analyses.

Table 2: Statistical results

Floral Traits	R ²	P-value
Corolla length / nectar depth	0.4875	0.4875
Flower/inflorescence width	0.5157	0.008355
Nectar opening width	0.4147	0.08976*
Flowers per stalk	0.7689	0.001371
Flowers per plant	0.3458	0.1482*
Plant height	0.9577	3.39 x 10 ⁻⁸

* Non-significant p-values

Discussion:

The pattern noted in corolla length is distinct and indicates a strong maximum in community-wide corolla length averages around mid-summer (week 6). This is most likely a result of the presence of *Delphinium nuttallianum*, which has a very long corolla relative to most other species (17.2mm). *D. nuttallianum* is also present in great abundance in our dry meadows of study, with a particularly high abundance this year because of above-average rainfall. It is possible that this trend is particularly exaggerated this year, due to increased abundance of this particular species. To resolve this issue, graphs may be made with floral trait measurement data for 2015 and phenology data for 2014 and 2013, in order to establish, given the average flower trait values available, how abundance affects the nature of these trait distributions from year to year. If the trait persists though multiple years, it is possible that this peak in corolla length values is due to preference or selection by pollinators, who may in turn have longer-than-average proboscises at mid-summer in order to use the most abundant resources – in this case, *D. nuttallianum* (Stang 2009). Nectar opening width, while following a non-significant non-linear pattern with a mid-season minimum, may demonstrate a significant trend given greater sampling. Following a second round of data analysis, with measurements made for corresponding traits in pollinators, we may see patterns in head and body size that appear to

correspond with the current nectar opening width distribution and thus serve to reinforce the possibility that this pattern is indeed present.

Flower (display and/or inflorescence) width demonstrated an overall upward trend across the summer, following a linear model. This is perhaps due to competition, either for pollinators or for resources. In early summer, there are fewer other species to compete with for pollinator attentions. Therefore, having a large and visible display is less profitable, and the plant is benefitted more greatly by having smaller displays and thus conserving resources and regulating energy portioning. Flower count per stalk, another trait that demonstrated an (non-linear) upward trend across the season, may result from similar biotic forces. Fewer flowers in a stalk are necessary early in the season, because there are fewer flowers in general and thus the plant is benefitted more by conserving resources than by splurging on costly, elaborate, and extensive displays (such as seen in sunflowers later in the season). Plant height showed a very strong upward linear trend within the season, stronger than the rest, and this is perhaps due to three factors: (1) the resource-partitioning model discussed above; (2) taller plants may be more successful in direct competition with large numbers of other species as it increases their visibility; and (3), because of time. The later in the season a plant is flowering, the longer it presumably has had to accumulate the necessary resources and grow.

Beyond patterns for any individual trait, this collection of within-season trait distributions indicates clearly that many of these traits vary in clear and distinct manners. Many of the trends vary in different ways – some are negative relationships between the trait value and time, while others are positive or non-linear. This demonstrates that, while variation and changes in the trait landscape are present, the community as a whole does not change uniformly (for instance, all values increasing as the season progresses). Changes in the community are occurring in a far more complex and variable manner, which in turn influences how pollinators must structure their interactions with available floral resources. The fact that the given traits vary differently on the overall community landscape suggests a potential explanation for why many pollinators tend towards moderate to extreme generalism, rather than specializing toward exclusive preferences for individual floral species (Waser et al. 1996). It is most beneficial to be a flexible generalist when your resource landscape is not only constantly changing in terms of the present floral species, but also undergoing constant and non-parallel changes in its trait landscape. A pollinator, under this logic, is more frequently benefitted traits that allow it to undergo trait matching with larger numbers of floral species and to do so flexibly, thus compensating for the variable resource landscape. Overall, these patterns indicate most clearly that the variability present in a given community is far more complex than might be predicted, and this may have a direct impact on the nature of the community's plant-pollinator interaction networks. Future analyses of corresponding pollinator traits will reveal the extent to which trait matching, on a season-wide and multi-species level, may occur, potentially providing further insight into the structuring and plasticity of these interaction networks.

Appendix:

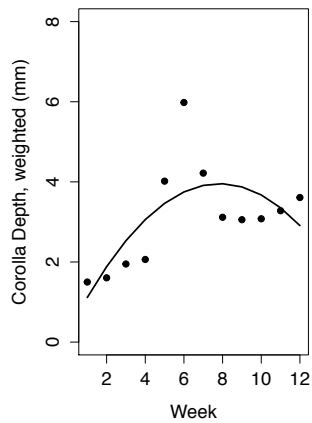


Fig. 1a: Non-linear regression with a mid-season peak in mean corolla depth (mm) across multiple species.

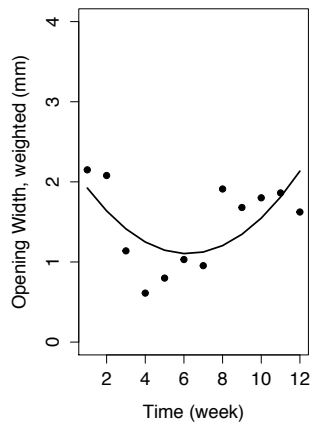


Fig. 2a: Non-linear regression with a non-significant mid-season minimum in mean nectar opening width (mm).

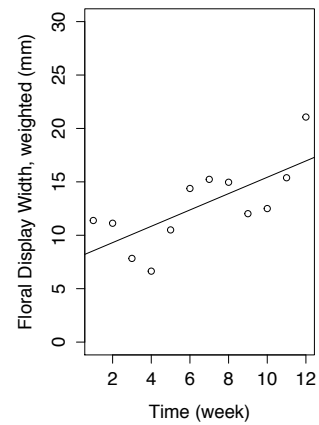


Fig. 3a: Linear regression of an across-season increase in mean floral display width (mm).

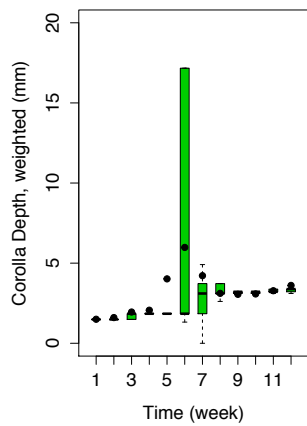


Fig. 1b: Box-plot for mean corolla depth, weighted by species abundance for each week.

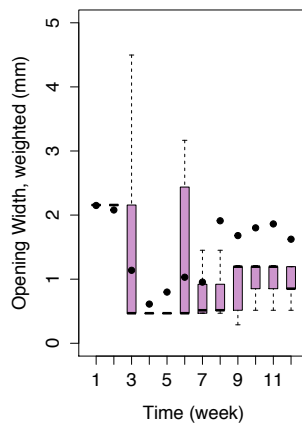


Fig. 2b: Box-plot for mean nectar opening width, weighted by species abundance for each week.

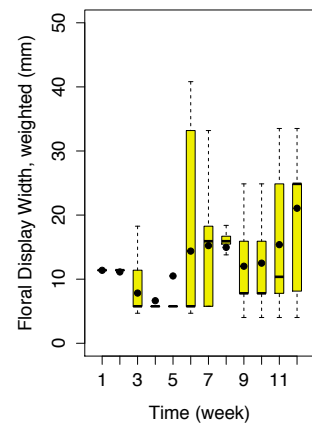


Fig. 3b: Box-plot for mean floral display width (mm), weighted by weekly species abundance.

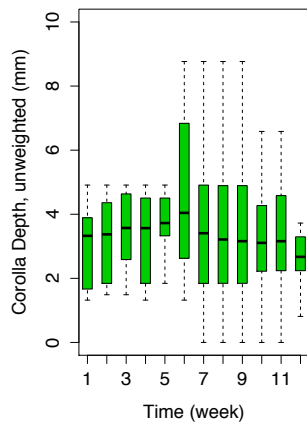


Fig. 1c: Box-plot for mean corolla depth, not weighted by floral abundance.

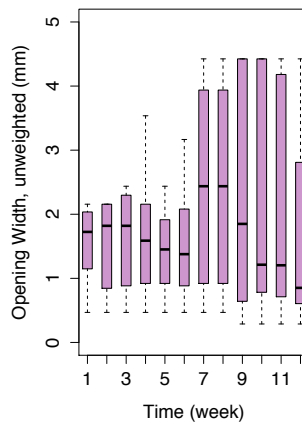


Fig. 2c: Box-plot for mean nectar opening width, not weighted by floral abundance.

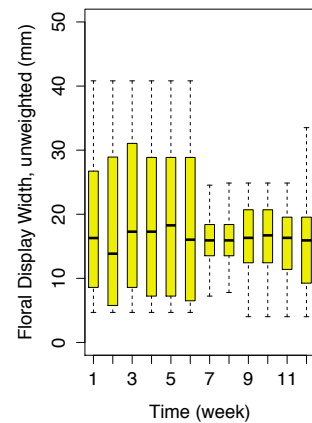


Fig. 3c: Box-plot for mean floral display width (mm), not weighted by floral abundance.

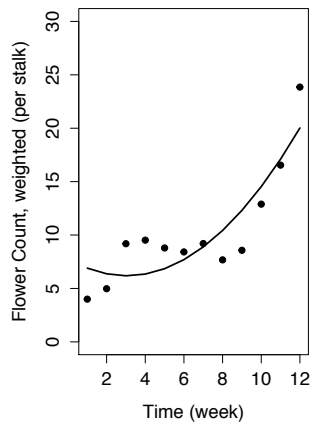


Fig. 4a: Non-linear regression indicating an across-season increase in community-wide mean flower count per stalk.

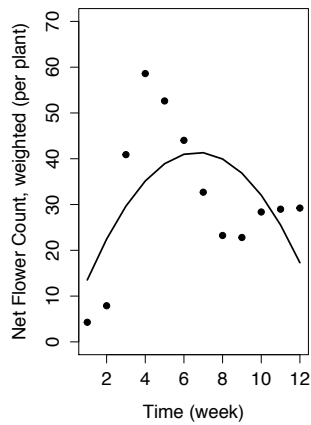


Fig. 5a: Non-linear regression indicating a non-significant mid-season peak in community-wide mean flower count per plant.

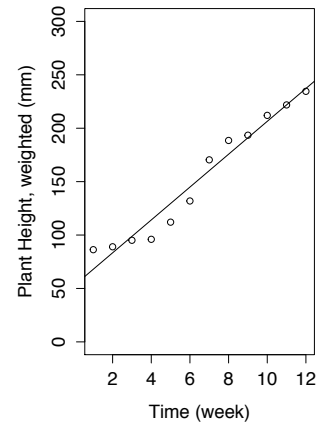


Fig. 6a: Linear regression indicating a strong increase in mean plant height across the growing season.

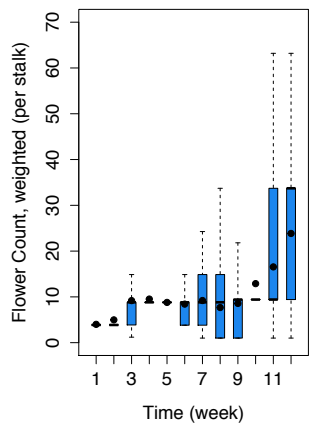


Fig. 4b: Box-plot for mean flower count per stalk, weighted by weekly species abundance.

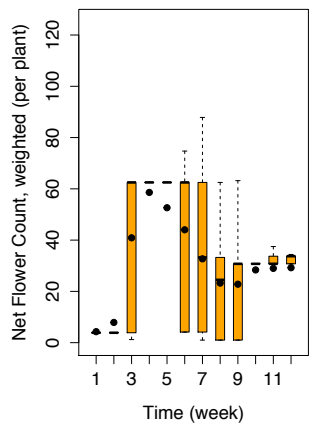


Fig. 5b: Box-plot for mean flower count per plant, weighted by weekly species abundance.

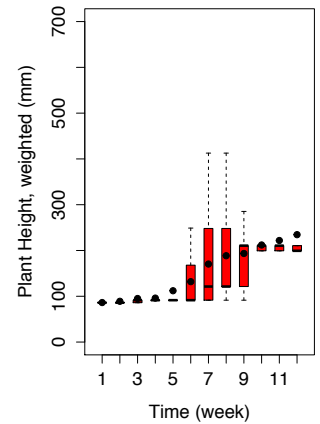


Fig. 6b: Box-plot for mean plant height, weighted by weekly species abundance.

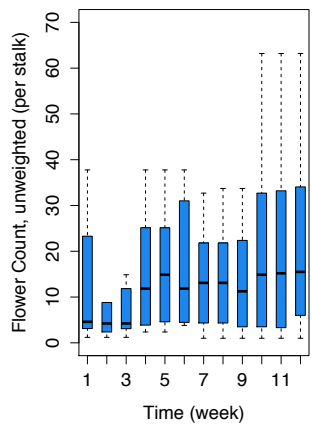


Fig. 4c: Box-plot for mean flower count per stalk, not weighted by floral abundance.

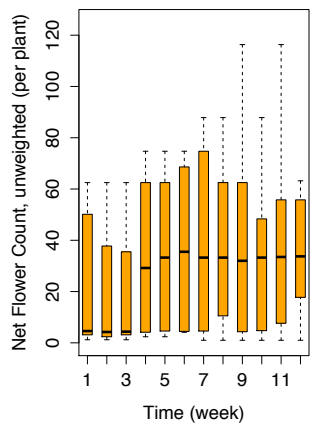


Fig. 5c: Box-plot for mean flower count per plant, not weighted by floral abundance.

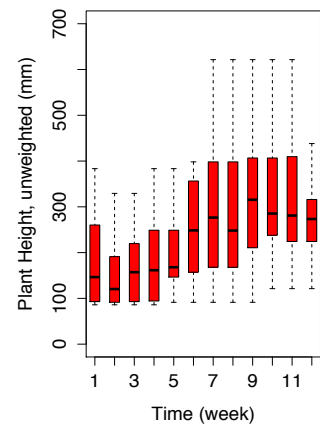


Fig. 6c: Box-plot for mean plant height, not weighted by floral abundance.

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