

Does Floral Nectar Depth Correlate With Pollinator Proboscis Length?

Sophia Bograd | Campbell Lab

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ABSTRACT

Pollination is essential for maintaining global biodiversity and our world's food supply. Climate change is causing inefficiencies in plant-pollinator networks, so understanding the factors that determine which pollinators visit which plants will be exceedingly important for a sustainable future. Our study examined the effects of floral nectar depth on visitor proboscis length in a dry subalpine meadow at the Rocky Mountain Biological Laboratory. We conducted 46.5 hours of pollinator observations in addition to using historic observations in the same plant-pollinator community and historic measurements on the floral nectar depths and pollinator proboscis length, to test if pollinator visitation was affected by size thresholds or size matching. We found that flowers with greater nectar depths were visited by pollinators with longer proboscis, and that the meadow's pollinators tend to visit flowers which have similar nectar depths to their proboscis length. Our results suggest that size-matching influences plant-pollinator networks. As a result, short-tongued pollinators may be particularly vulnerable to the loss of flowers with shallow nectar depths and long-tubed flowers to pollinators with long proboscis.

INTRODUCTION:

Plant-pollinator interactions are essential for maintaining global biodiversity. Approximately three quarters of crops are animal pollinated, meaning our global food supply is highly reliant on pollination (Bailes et al., 2015; Bascompte et al., 2006). Approximately 3-5% of global food production is currently lost due to pollination deficits and inefficiencies, equivalent to an estimated 500,000 people dying of starvation and malnutrition annually (Smith et al., 2022). Many taxa of animals, such as bees, flies, bats, birds, beetles and butterflies, can pollinate flowers. However, pollination efficiency can vary greatly between the many species of pollinators (Eeraerts et al., 2020; Page et al., 2021), and the species which pollinates a plant can even alter

offspring fitness (Travis & Kohn, 2023). As a result, understanding the factors that determine which pollinator species visit which plants will be important to understanding how anthropogenic change is altering pollination services.

Pollinator visitation patterns are highly complex and can be impacted by the distribution, availability, and abundance of flowers (Baracchi, 2019). Pollinators make decisions on which flowers to forage on to maximize the quantity and quality of reward, relying on a combination of learned behavior and environmental observations to determine their foraging patterns (Baracchi, 2019). In addition to behavioral factors, morphology and the physical compatibility of plants and pollinators can affect pollination. Because nectar is only accessible to species with mouthparts that are as long or longer than the distance to a flower's nectar, flower nectar depth is likely to affect which pollinators visit which flowers. Indeed, several studies have found that flowers with deeper nectaries are visited by pollinators with greater proboscis (tongue) length (Gilbert, 1981; Peat et al., 2005). However, most past research on size matching between plants and pollinators has examined only a single family or related group of pollinators rather than studying the full network (Stang et al., 2009). Because plants are often pollinated by multiple families of pollinators, a network-wide analysis would provide clearer results on how nectar depth affects pollination (Stang et al., 2009).

Our research examined how nectar depth affects pollinator visitations in a dry subalpine meadow, by asking two main questions: (1) Is there a minimum proboscis length for each nectar depth? (2) Do pollinators tend to "size-match" by visiting flowers whose nectar depths match their proboscis length? Our null-hypothesis is that nectary depth does not affect visitor proboscis length. Our first alternative hypothesis is that there is a minimum proboscis length for each nectary depth. Our second alternative hypothesis is that pollinators tend to size-match by visiting flowers whose nectary depth is the same size as their proboscis length.

In order to test these hypotheses, we observed the frequency of pollinator visitation to 10 species of flowering plants in the meadow. We then leveraged historic data from CaraDonna et

al., (2017), a previous RMBL study of the meadow's pollination network. Cardona et al., (2017) observed links in the network, proboscis length of the network's pollinators, and nectar depth of the network's plants. Adding size-thresholds did not substantially help to explain interaction turnover and rewiring of links across the season in the meadow's plant-pollinator network. However, Cardona et al., 2017 looked only at links between plants and pollinators, and did not analyze the frequency of interactions between various plants and pollinators, nor did they focus on the distinction between a size-threshold and size-matching. By studying the frequency of interactions between plants and pollinators in the network, our research aims to answer these gaps in knowledge by testing how size matching affects visitation patterns in the meadow.

METHODS

Data Collection:

We observed plant-pollinator interactions in a subalpine dry meadow community. We established five pairs of transects, with each transect made up of six 2x2 meter plots. Within each transect pair, one transect was assigned as a "rainout" and the other was assigned as a control. The "rainout" transects were covered with 2x2 m rainout shelters made out of corrugated polycarbonate roof paneling and 1 inch PVC pipe. On July 15 and July 22, soil moisture was measured in two opposing corners of each of the 60 study plots, using a Campbell Scientific Hydrosense ii 12 cm probe, with prongs inserted 5.5 cm into the ground. If the probes could not be inserted into a plot without the soil cracking around them (impeding electrical current of the probe), soil moisture for the plot was recorded as "na". Mean summer soil moisture did not differ between the two treatments (rainout and control) during the study period ($p = 0.66$).

Pollinator visitation censuses were conducted between 9 AM and 4 PM for 10 days during the month of July. We conducted 186 15 minute plot-level observations, sampling each plot approximately 3 times. Transect pairs (rainout and control) were sampled at the same time if possible, and one after the other if they could not be sampled at the same time due to observer

availability. The order of sampling the transect pairs was randomized, and within each transect plots were sampled in random order. In total, we collected 46.5 hours of observation on plant-pollinator interactions. During each observation period, the observer sat still next to the study plot and recorded all observed interactions. Pollinators were identified into five groups: bumblebees (*Bombus* spp.), other bees, bee-flies (*Bombylius* spp.), other flies, and butterflies. Plants were identified to the species level. Interactions were recorded when the pollinator landed on the reproductive organs of the flower. We observed visits to 10 species of flowering plants during the study period.

Data Analysis

We used data on pollinator proboscis length from CaraDonna et al., (2017) to calculate the average proboscis length for each group of pollinators (bumblebees (*Bombus* spp.), other bees, bee-flies (*Bombylius* spp.), other flies, and butterflies) For the bumble bee group, we calculated the average proboscis length for worker bees. We then used the number of visits of each group of pollinators to species of flower to calculate the weighted mean visitor proboscis length for each species of flowering plant in the meadow. Because mean summer soil moisture did not differ between rainout and control transects, data from all 60 plots were analyzed together. A linear regression (function *lm* in R 4.2.2) was used to test if pollinators with longer proboscis visited plant species with greater flower nectar depth, based on nectar depth measurements from CaraDonna et al., (2017). We created scatter-plots where each point represents an observed link between a plant of certain nectar depth and a pollinator of a certain proboscis length, and point size represents the number of observed interactions. For each species of flowering plant in the meadow, the variance in visitor proboscis length was also calculated, and a linear regression was used to test if plants with smaller flower nectar depths were visited by pollinators with a greater variance in proboscis length. This test allowed us to see if the meadow's plant-pollinator network involved precise size matching or only a size

threshold. A size threshold without size matching would lead to increased variances in visitor proboscis for smaller nectar depths.

In addition to the analyses on the data we collected, we also used plant-pollinator interaction data from CaraDonna et al., (2017) to further investigate the relationship between proboscis length and nectar depth. CaraDonna et al., (2017) observed the entire subalpine meadow for 336 hours (as opposed to individual plots like our experiment), and identified pollinators to the species level. In total, CaraDonna et al., 2017 observed visits to 40 species of flowering plants. Data from CaraDonna et al (2017) were analyzed at both the pollinator species and pollinator group level. The number of observed interactions between each plant-pollinator pair was used to find the weighted mean proboscis length (based on both pollinator species proboscis length and pollinator group proboscis length). A linear regression was used to test if greater flower nectar depth was associated with pollinators with longer proboscis for both data-sets, and to test if there was a relationship between nectar depth and variance in visitor pollinator proboscis length. We created scatter plots based on plant-pollinator observations from CaraDonna et al., (2017) as described above.

RESULTS

Average proboscis length for the five groups of pollinators ranged from 1.6 mm (flies) to 8.0 mm (bumble-bees) (see Table 1).

Analysis of both data from CaraDonna et al., (2017), and our own observations, yielded a positive relationship between floral nectar depth and proboscis length. Although the regression based on data from CaraDonna et al., (2017) was statistically significant both when analyzed by pollinator species proboscis length ($p = .00023$, see Figure 1), and when analyzed by pollinator group proboscis length ($p = .018$, see Figure 2), analysis of our own data led to an insignificant regression ($p = .20$, see Figure 3).

For our observations, there was no significant relationship between variance in visitor proboscis length and floral nectar depth ($p=.20$, see Figure 4). However, when data from

CaraDonna *et al.*, (2017), was analyzed at the species level, variance in visitor proboscis length increased with floral nectar depth ($p = .018$, see Figure 5).

Figures

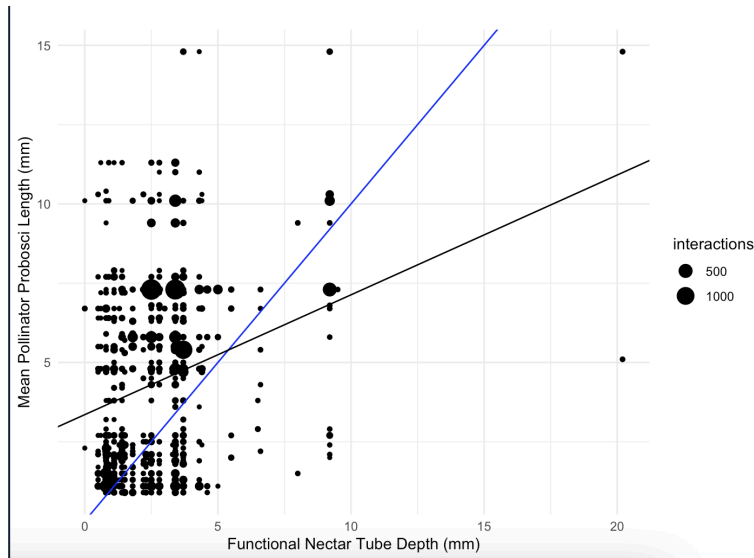


Figure 1: Relationship Between Nectar Depth (mm) and Visitor proboscis Length (mm) for CaraDonna *et al.*, 2017.

Points represent visits by pollinators with a certain proboscis length to flowers of a certain nectar depth, and point size represents the number of observed interactions. Blue line: size-matching line where proboscis length = nectar depth. Black line: regression line based on weighted mean average proboscis length for each nectar depth

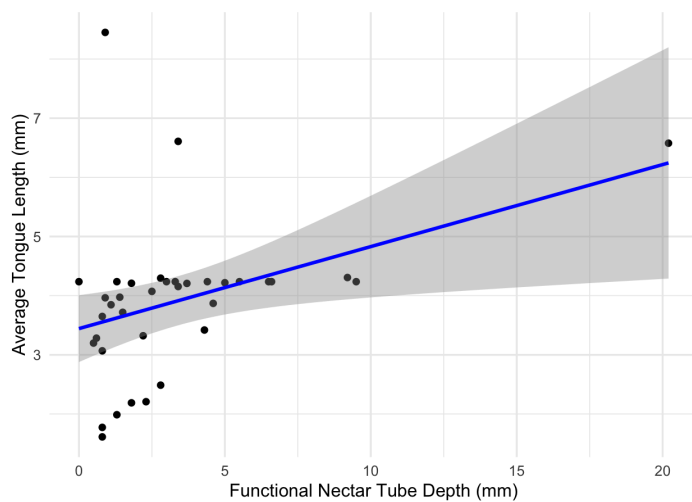


Figure 2: Relationship Between Nectar Depth (mm) and Weighted Mean proboscis Length (mm) analyzed based on Pollinator Groups, for CaraDonna et al., (2017)

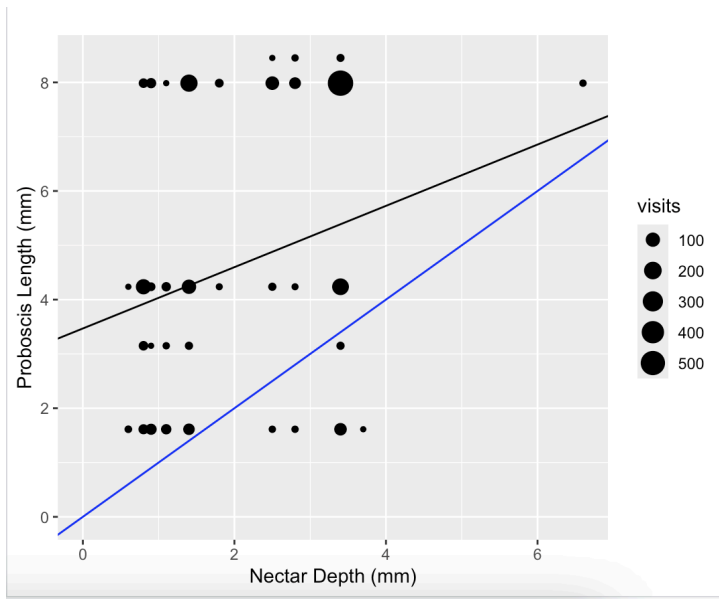


Figure 3: Relationship Between Nectar Depth (mm) and Proboscis Length (mm) in the 2024 data. Points represent visits by pollinators with a certain proboscis length to flowers of a certain nectar depth, and point size represents the number of observed interactions. Blue line: theoretical size-matching line where proboscis length = nectar depth. Black line: regression of weighted mean proboscis length on nectar depth

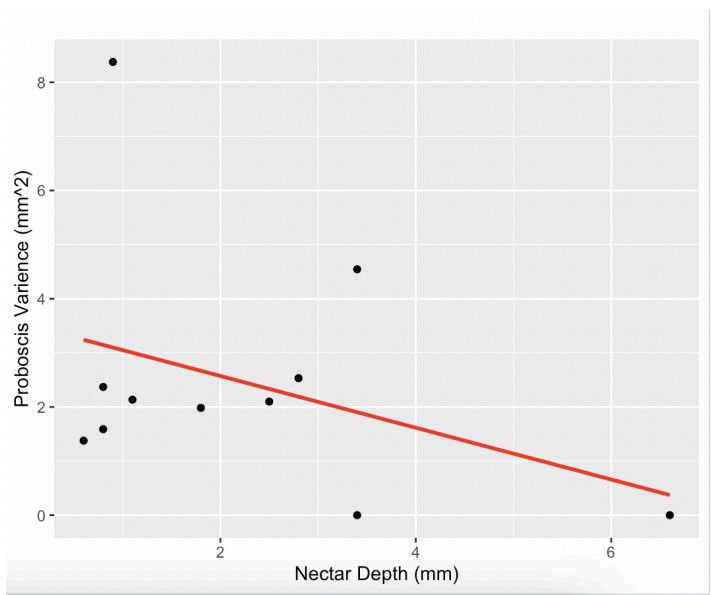


Figure 4: Relationship Between Functional Nectar Depth (mm) and Variance in Proboscis Length (mm^2) in the 2024 data

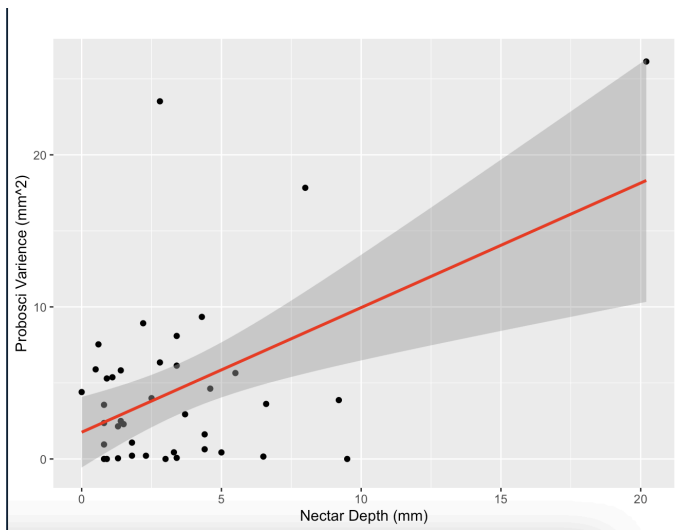


Figure 5: Relationship between Functional Nectar Depth (mm) and Variance in proboscis Length (mm) for CaraDonna. et al., (2017)

Tables

Pollinator Group	Mean proboscis Length (mm)
Bee	4.2
Bee-fly	3.15
Bumble Bee	8.0
Butterfly	8.5
Fly	1.6

Table 1: Mean Proboscis Length by Pollinator Group

DISCUSSION

Results from our experiment suggest that flowers with deeper nectar are visited by pollinators with longer probosces. Comparing floral nectar depth and mean visitor proboscis length for both our experiment and CaraDonna *et al.* (2017), yielded relationships with slopes

less than 1 (0.5819 and 0.3803, respectively). This suggests that the meadow's pollinators sometimes visit flowers which have longer nectaries than their proboscis. A caveat for the 2024 data, however, is that we used average proboscis length for groups of pollinators, so within any group there will likely be some species with longer probosces. However, we found a slope much less than 1 when looking at data from CaraDonna *et al.*, (2017), which identified pollinators to the species level. Several past studies found that hymenoptera and diptera are most likely to visit flowers with nectar depths that are slightly shorter than their proboscis (Klumpers *et al.*, 2019; Stang *et al.*, 2009). For both CaraDonna *et al.*, 2017 and our own observations, some pollinators interacted with flowers whose nectaries were longer than their proboscis. It is possible that some of the pollinators in both our experiment, and CaraDonna *et al.*, (2017) visited flowers with deep nectaries to collect pollen and not nectar.

Our research suggests pollinators may be “size-matching” with plants based on proboscis length and nectar depth. If pollinators were limited by a size-threshold but did not size match with flowers, flowers with small nectar depths would be visited by pollinators with a large variance in proboscis length. However, our research found no correlation between nectar depth and variance in proboscis length for our experiment, and a positive correlation between nectar depth and variance in proboscis length for CaraDonna *et al.*, (2017). This means that size matching likely occurs in this plant-pollinator network. By size matching with plants, pollinators may be able to maximize foraging efficiency and floral rewards. When pollinator proboscis are shorter than flower nectaries, foraging efficiency decreases. However, flowers with deep nectaries tend to have higher quantities of nectar and floral rewards (Klumpers *et al.*, 2019). As a result, pollinators are incentivized to visit flowers with nectar depths similar to their proboscis length (Klumpers *et al.*, 2019).

There are several possible reasons why our experiment did not result in a significant correlation between nectar depth and pollinator proboscis length, while the data from CaraDonna *et al.*, (2017) resulted in a significant correlation, even when analyzed by the 5

pollinator groups. First, CaraDonna et al., (2017) conducted pollinator observations across the entire meadow, while our study only observed several transects within the meadow. It is likely that by studying the entire meadow, CaraDonna et al., (2017) observed more species diversity than our experiment. Throughout our study period, we only observed pollinator visits to 10 species of flowering plants, while CaraDonna et al., (2017) observed interactions with 40 species of flowering plants, with a larger spread in nectar depth. Because data from CaraDonna et al., (2017) led to a model with nearly three times the data points of our study, the precision of his model was likely increased. Additionally, CaraDonna et al., (2017) conducted observations of the meadow's plant-pollinator network for approximately 14 weeks a year during the 2014, 2015, and 2016 flowering seasons. This means that not only did CaraDonna et al., (2017) observe the network for more hours than our study, (336 hours vs 46 hours), they also were likely able to observe a larger proportion of the meadow's plant-pollinator network. Throughout a season, plant-pollinator networks experience both species turnover, and interaction rewiring due to phenology of the plants and pollinators and changes in pollinator behavior (CaraDonna et al., 2017). CaraDonna et al., (2017) found that the meadow where we collected our data experiences substantial interaction rewiring and species turnover throughout each summer. Because our experiment only included pollinator observation data for the month of July, it is likely we missed many of the interactions that make up the meadow's plant-pollinator network.

By providing evidence that plant-pollinator networks in dry-subalpine meadows are influenced by size-matching, our study provides insight into how plant-pollinator networks could respond to climate change. Our study suggests that if ecological changes lead to less abundance of flowers with shallow nectar depths, pollinators with short proboscis may have difficulty finding flowers to forage on. Likewise, if the abundance of pollinators with long proboscis were to decrease, deeper flowers may be left unpollinated. Understanding the effects of size on plant-pollinator networks may also aid in modeling how plant-pollinator networks will respond to climate change. Previous research has found that the structure of plant-pollinator

networks can be largely predicted by considering size-matching, size-thresholds, and the frequency of size-distributions of plants and pollinators (Stang et al., 2007) in mediterranean plant-pollinator networks. By providing evidence that size-matching also influences subalpine meadows such as the ones near RMBL, our research may aid in modeling of plant-pollinator networks.

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REFERENCES

- Bailes, E. J., Ollerton, J., Pattrick, J. G., & Glover, B. J. (2015). How can an understanding of plant-pollinator interactions contribute to global food security? *Current Opinion in Plant Biology*, 26, 72–79. <https://doi.org/10.1016/j.pbi.2015.06.002>
- Baracchi, D. (2019). Cognitive ecology of pollinators and the main determinants of foraging plasticity. *Current Zoology*, 65(4), 421–424. <https://doi.org/10.1093/cz/zoz036>
- Bascompte, J., Jordano, P., & Olesen, J. M. (2006). Asymmetric Coevolutionary Networks Facilitate Biodiversity Maintenance. *Science*, 312(5772), 431–433. <https://doi.org/10.1126/science.1123412>
- CaraDonna, P., Petry, W., & Brennan, R. (2017). *Interaction rewiring and the rapid turnover of plant–pollinator networks—CaraDonna—2017—Ecology Letters—Wiley Online Library*. <https://onlinelibrary.wiley.com/doi/abs/10.1111/ele.12740>
- Eeraerts, M., Vanderhaegen, R., Smaghe, G., & Meeus, I. (2020). Pollination efficiency and

- foraging behaviour of honey bees and non-*Apis* bees to sweet cherry. *Agricultural and Forest Entomology*, 22(1), 75–82. <https://doi.org/10.1111/afe.12363>
- Gilbert, F. (1981). *Foraging ecology of hoverflies: Morphology of the mouthparts in relation to feeding on nectar and pollen in some common urban species—NASA/ADS*.
<https://ui.adsabs.harvard.edu/abs/1981EcoEn...6..245G/abstract>
- Klumpers, S. G. T., Stang, M., & Klinkhamer, P. G. L. (2019). Foraging efficiency and size matching in a plant–pollinator community: The importance of sugar content and tongue length. *Ecology Letters*, 22(3), 469–479. <https://doi.org/10.1111/ele.13204>
- Page, M. L., Nicholson, C. C., Brennan, R. M., Britzman, A. T., Greer, J., Hemberger, J., Kahl, H., Müller, U., Peng, Y., Rosenberger, N. M., Stuligross, C., Wang, L., Yang, L. H., & Williams, N. M. (2021). A meta-analysis of single visit pollination effectiveness comparing honeybees and other floral visitors. *American Journal of Botany*, 108(11), 2196–2207. <https://doi.org/10.1002/ajb2.1764>
- Peat, J., Tucker, J., & Goulson, D. (2005). Does intraspecific size variation in bumblebees allow colonies to efficiently exploit different flowers? *Ecological Entomology*, 30(2), 176–181. <https://doi.org/10.1111/j.0307-6946.2005.00676.x>
- Smith, M. R., Mueller, N. D., Springmann, M., Sulser, T. B., Garibaldi, L. A., Gerber, J., Wiebe, K., & Myers, S. S. (2022). Pollinator Deficits, Food Consumption, and Consequences for Human Health: A Modeling Study. *Environmental Health Perspectives*, 130(12), 127003. <https://doi.org/10.1289/EHP10947>
- Stang, M., Klinkhamer, P. G. L., & Waser, N. M. (2009). *Size-specific interaction patterns and size matching in a plant–pollinator interaction web | Annals of Botany | Oxford Academic*. <https://academic.oup.com/aob/article/103/9/1459/145573>
- Travis, D. J., & Kohn, J. R. (2023). Honeybees (*Apis mellifera*) decrease the fitness of plants they pollinate. *Proceedings of the Royal Society B: Biological Sciences*, 290(2001), 20230967. <https://doi.org/10.1098/rspb.2023.0967>

