Biotic and Abiotic Drivers of Plant-Pollinator Interaction Rewiring

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A dissertation
submitted in partial fulfillment of the
requirements for the degree of

Doctor of Philosophy

University of Washington
2023

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Program Authorized to Offer Degree:
Biology

University of Washington

Abstract

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In this dissertation, I examine variation in plant-pollinator interaction patterns. I present two observational studies exploring drivers of interaction variation, followed by an experiment in which I demonstrate the effect of changing plant-pollinator interactions. First, I use a structural equation modeling framework to demonstrate that temperature variation and plant community dissimilarity directly drive variation in plant-pollinator interaction patterns. Both variables also provide pathways for geographic distance to indirectly impact rewiring. Second, I use a multinomial logistic regression to show that pollinators prefer different flowers at different temperatures, even within a constant plant community. Third, I present an experimental manipulation that connects the removal of the most abundant bumble bee in a community to the production of fertilized seeds in a focal plant. In this experiment, I find that the composition of the plant and pollinator communities as well as the distribution of morphological traits both guilds influences the way in which pollinator removal influences pollination function. As a whole, this dissertation demonstrates that interspecific interactions cannot be considered static components of plant-pollinator communities. Rather, ecologists must consider how changes to biotic and abiotic conditions are likely to affect not only the organisms present in a community but also the interactions between them.

Contents

Chapter 1

Introduction

How and why do species interact? These questions, which form the basis of much of community ecology, continue to intrigue researchers. Despite a wide range of models describing community dynamics, the factors regulating interaction patterns remain obscured by nonlinear and multi-dimensional drivers of interaction occurrence (Abrams, 2001). Though co-occurrence can be thought of as a minimum requirement for an interaction to occur, it is not the sole determinant of interaction patterns. Interaction turnover—the variation between interactions under different ecological conditions—can occur as a result of a change in the species present in the community (species turnover) or due to some behavioral change among species that co-occur under multiple contexts (rewiring) (Anderson et al., 2011; Poisot, Canard, Mouillot, Mouquet, & Gravel, 2012). Studies have identified both species turnover and rewiring as significant sources of interaction turnover along spatial and temporal gradients (Burkle, Marlin, & Knight, 2013; CaraDonna et al., 2017; Carstensen, Sabatino, Trøjelsgaard, & Morellato, 2014; Trøjelsgaard, Jordano, Carstensen, & Olesen, 2015). However, while the processes underlying spatial and temporal patterns of species turnover have received substantial attention in ecological literature, the relationships between these gradients and rewiring remain poorly understood.

Rewiring is the term given to variation in interactions between species that co-occur under multiple ecological contexts. Since rewiring occurs despite the continued presence of both interaction partners, it often reflects a driver external to the interaction that alters the attractiveness or availability of resources in that community. In a recent review, Poisot, Stouffer, and Gravel (2015) propose that such drivers of rewiring include environmental context, biological compatibility, and community composition. For instance, a bee may forage on different flowers at high and low temperatures depending on its metabolic needs even if the floral assemblage remains constant, but those metabolic concerns may become less important if trait-matching incentivizes a particular interaction or if strong competition from other pollinators disincentivizes another. While this framework represents a valuable way to conceptualize the drivers of rewiring, more empirical evidence is necessary to understand how interaction turnover relates to specific environmental and biological contexts. Further, research must connect changes in interaction patterns to the broader maintenance of biodiversity and ecological function in order to fully understand the multi-faceted ways in which global change pressures are likely to impact ecological communities.

Plant-pollinator communities are important biological systems with which we can expand scientific understanding of interaction turnover while also identifying specific functional implications associated with these ecological changes. Plant-pollinator interactions play an essential role in the maintenance of biodiversity: plant reproduction relies upon the consistent transfer of pollen between individuals, which is often vectored by mobile organisms like insects as they forage for floral resources. However, despite the ecological importance of plant-pollinator interactions, the mechanisms structuring these interactions and the variation therein remain poorly understood. We have very little insight into how interactions between species will change in a rapidly changing world, which limits our ability to predict how global environmental change will impact important ecological processes like pollination. For instance, what flowers will pollinators visit if temperatures increase? Will altered foraging patterns occur throughout a pollinator's entire range? What plant or pollinator characteristics might make species resistant—or susceptible—to interaction change? How will these changes influence pollination services? Answering such questions will help to determine how global environmental change will influence the persistence and maintenance of biodiversity and ecosystem function via pollination systems.

With this dissertation, I examine the causes and consequences of plant-pollinator interaction turnover in the Rocky Mountains of southern Colorado. I combine observational and experimental studies in an effort to understand how both the abiotic environments and biological contexts within which plants and pollinators interact affect the structure and function of these communities. In Chapter 2, I examine environmental and biological drivers of plant-pollinator interaction rewiring. I utilize a natural environmental gradient to explore the role of temperature in mediating spatial patterns of rewiring. I also discuss the niche and neutral processes that may underlie the effect of community composition on interactions between spatially persistent partners. In Chapter 3, I further explore the effects of temperature on pollinator foraging preferences. Specifically, I isolate the effects of temperature variation from variation in plant community composition in an effort to ascertain how pollinator foraging patterns vary within a consistent resource landscape. Finally in Chapter 4, I present experimental results that demonstrate the functional effects of single-pollinator-species losses. I show that the effect of these species losses on seed production in a focal plant species depends on the composition the remaining pollinator and plant communities as well as the distribution of key morphological traits within these communities.

Chapter 2

Temperature and community composition underlie geographic patterns of rewiring in plant-pollinator networks

2.1 Abstract

Plant-pollinator communities experience variation in the interactions between guilds that are unrelated to turnover in either the plant or pollinator community. This process, which is known as rewiring, often increases with the spatial separation of communities; however, any relationships between this spatial pattern and biologically meaningful drivers have not been identified. In this study, we find that both temperature difference and plant community dissimilarity provide pathways through which geographic distance drives rewiring. Geographic distance between sites does not have a direct effect on rewiring when these proximal drivers of rewiring are included in analyses, which further supports our hypothesis that spatial patterns in interaction variation reflect underlying ecological processes rather than inherent spatial structure. These results illustrate the importance of environmental and biological conditions to interaction variation in plant-pollinator communities and ultimately demonstrate key pathways through which global change dynamics may influence an important ecosystem function.

2.2 Introduction

Interspecific interactions, which are crucial components of biological communities, exhibit substantial variation over time and space (Burkle et al., 2013; CaraDonna et al., 2017; Carstensen et al., 2014; Simanonok & Burkle, 2014; Trøjelsgaard et al., 2015). The plant-animal interactions that facilitate pollination in over 85% of flowering plants (Ollerton, Winfree, & Tarrant, 2011) are susceptible to disruption via temporal (phenological) mismatches if pollinators are not actively foraging at times that flowers are producing pollen, to or spatial mismatches if the geographic ranges of plants and pollinators diverge due to environmental change (Burkle et al., 2013; CaraDonna et al., 2017). Since synchrony—both spatial and temporal—in the life histories of these organisms is essential to their ecological function, substantial work has been done to understand where and when plants and pollinators are likely to co-occur under shifting climatic and anthropogenic pressures (Dalsgaard, 2020; Gérard, Vanderplanck, Wood, & Michez, 2020; Miller-Struttmann et al., 2015). However, even if plants and pollinators achieve both spatial and temporal overlap, plant-pollinator interactions themselves experience turnover independent of species co-occurrence (Carstensen et al., 2014; Trøjelsgaard et al., 2015).

Rewiring—the name given to interaction turnover that is not explicitly driven by the loss of one or both interaction partners—can be partitioned into several distinct drivers (Poisot et al., 2015). Pollinators are likely to partition their interactions among plants in a way that maximizes the rewards obtained from each foraging interaction (Fontaine, Collin, & Dajoz, 2008; Valdovinos, Moisset de Espanés, Flores, & Ramos-Jiliberto, 2013). The type, amount, and quality of that reward may vary with environmental context (McCallum, McDougall, & Seymour, 2013); for instance at lower temperatures heat rewards might be more valuable than nectar (Rands & Whitney, 2008; Sapir, Shmida, & Ne'eman, 2006; Seymour, White, & Gibernau, 2003), while higher temperatures may affect the quality of nectar in terms of volume, sucrose concentration, and viscosity (Descamps, Quinet, Baijot, & Jacquemart, 2018;

McCombs, Debinski, Reinhardt, Germino, & Caragea, 2022; Nicolson, de Veer, Köhler, & Pirk, 2013; Russell & McFrederick, 2022; Tan et al., 2014). Pollinators also likely respond to the composition of the plant (resource) and pollinator (competitor) communities and partition their interactions in a way that minimizes interspecific competition while maximizing the rewards obtained from each foraging interaction (Valdovinos et al., 2016). Thus in order to effectively predict how rewiring may influence plant-pollinator communities experiencing global change, it is crucial to understand how these distinct environmental and biological drivers affect rewiring directly and in tandem with one another.

Spatial patterns of community dynamics are often used as proxies for environmental and biological drivers of ecological processes (McIntire & Fajardo, 2009). Environmental dissimilarity often covaries with geographic distance and predictable negative relationships exist between distance and community similarity (Dray et al., 2012; Nekola & White, 1999; Soininen, McDonald, & Hillebrand, 2007). Plant-pollinator interaction similarity also generally decreases with the distance between two communities, with parallel distance decay curves reported for species-driven and rewiring-driven interaction turnover (Carstensen et al., 2014; Trøjelsgaard et al., 2015). However, research to date has not explored whether these spatial patterns of rewiring can be considered proxies for more biologically meaningful variables like changes in temperature stress or altered competitive landscapes. Examining the biotic and abiotic drivers of rewiring across geographic space is therefore a valuable step in understanding how global change may impact the persistence of interaction-mediated ecosystem functions.

In this study, we explore potential spatial, environmental, and biological drivers of rewiring among plant-pollinator communities. We calculated the turnover in interactions among species that co-occurred between pairs of plant-pollinator networks from three years and 18 sites. We also calculated the difference in temperature (average and at the time of sampling), plant community composition, and pollinator community composition among these network pairs. These variables reflect a range of possible niche and neutral processes that could affect pollinator foraging patterns, such as altered competitive dynamics and encounter probabilities. We then used a structural equation modeling framework to examine the pathways through which these potential drivers directly and indirectly affected rewiring in order to better understand what ecological processes underlie spatial patterns of plant-pollinator interaction turnover. Specifically, we examine whether geographic distance predicts rewiring independently of its effects on other environmental and biological factors as well as how geographic distance indirectly affects rewiring through these variables. We also examine three variables that we expected to have strong effects on the availability of floral resources—air temperature, plant community composition, and pollinator community composition—and determine whether differences in the environmental and biological contexts of plant-pollinator interactions affects their similarity between networks.

2.3 Methods

2.3.1 Study system

We conducted this study over three summers from 2020–2022. Each summer, we surveyed the plant-pollinator interactions at a subset of 18 subalpine wildflower meadows, all of which were within 20-km of the Rocky Mountain Biological Laboratory (Gunnison National Forest, Colorado, United States). During these visitation surveys, we observed plant-pollinator interactions for 45 minutes of active sampling, evenly distributed across three 1m x 10m transects. We caught any insect that touched the reproductive organs of a flower rooted within our transects. We identified all bumble bees to species level on the wing and released them once identified; all other insects were collected and subsequently identified to the genus level in the lab. We also surveyed the plant community on each sampling day, noting the identity and abundance of every plant rooted in our transects. We measured air temperature 10-cm above the ground every 30 minutes using a data logger (HOBO Pendant, UA-001-08) covered by a radiation shield that remained at each site for the duration of each summer.

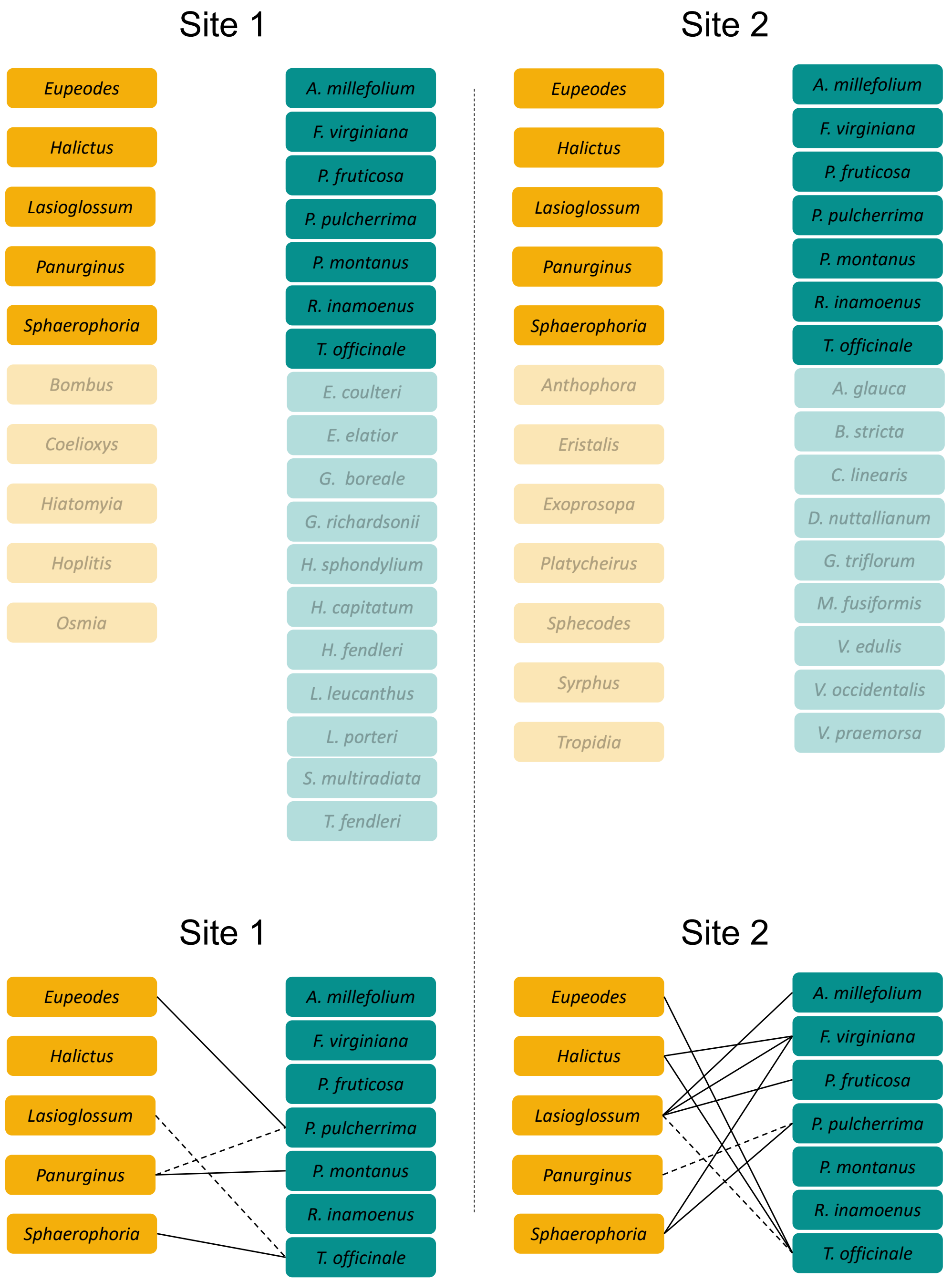


Figure 2.1: Visual depiction of rewiring between plant-pollinator networks at two sites. a) List of all pollinator genera (yellow) and plant species (teal) observed at two sites over the summer of 2020. Taxa shared between sites are indicated with dark colors and brackets and those that vary between sites are shown in light colors. b) Shared taxa with observed interactions indicated. Solid lines represent rewired interactions; dashed lines represent constant interactions.

2.3.2 Calculating rewiring

Since rewiring is a metric of interaction dissimilarity between two communities, all of our analyses involving rewiring comprise pairwise comparisons of every combination of sites. Rewiring is only possible if both partners are present in the two communities being compared. Thus, we began our rewiring calculations for each pair of sites by creating edge lists comprising just the plants and pollinators shared between both sites (Figure 2.1a). In order to ensure that our networks were not biased by uneven sampling effort, we performed this step for just a subset of five sampling rounds for each site/date combination (1SD below the mean) and then aggregated these possible and observed interactions from each site/date into single networks for the entire year. We then filled the edge lists with the number of times that we observed these possible shared interactions at each site (Figure 2.1b). This process yielded two numbers for each site pair—the number of interactions that occurred at one site but not the other (rewiring occurrence) and the number of interactions that occurred at both sites or neither site (no rewiring occurred).

2.3.3 Assessing drivers of rewiring

We used a structural equation modeling (SEM) framework (J. Grace, 2022) to understand how rewiring in our system was affected by geographic, ecological, and environmental differences between sites (Figure 2.2a). This SEM framework allowed us to test both direct and indirect relationships between predictor variables and rewiring that would be impossible to explore using a single regression model (J. B. Grace et al., 2012). Specifically, we examined (1) whether geographic distance and temperature difference had direct relationships with rewiring and (2) whether these two variables indirectly affected rewiring via changes to plant and pollinator community composition. We used the *piecewiseSEM* package (Lefcheck, 2016) to calculate standardized coefficients for the individual causal pathways in Figure 2.2a, with a linear model describing the relationship between geography and temperature and generalized linear models following a binomial distribution for all other relationships.

Since rewiring is a pairwise process, it involves an inherent lack of independence that violates the assumptions of standard linear models. Thus, the p-values estimated by the linear models underlying our SEM were not appropriate for interpretation of the statistical significance of these relationships. Instead, we developed a matrix regression model (MRM) based on the *MRM* function from the *ecodist* package (Goslee & Urban, 2007). MRMs can appropriately assess drivers of similarity or dissimilarity between pairs of sites using permutation tests (Lichstein, 2007). However, since our data included multiple records within the same years, we modified the *ecodist* code with the *permute* package (Simpson, 2022) to include a block-level control for year.

We modified our MRM to accept a response in the form of a binomial variable with two matrices—one containing the shared interactions that experienced rewiring and one containing the shared interactions that did not rewire. We formatted our predictor variables as matrices of pairwise differences in temperature (mean across all sampling events), plant and pollinator community composition, and geographic distance. We calculated community dissimilarity using the Horn-Morisita method in the *vegan* package (Oksanen et al., 2022) and geographic distance using *geosphere* (Hijmans, 2021). Mirroring the paths included in our SEM, we used a linear regression to test the relationship between geographic distance and temperature difference and logistic regression for all other relationships. Because these MRMs differed from the (general) linear models in our SEMs only by the use of permutation tests of significance, we are confident that the standardized coefficients from our SEMs and significance values from our MRMs can be assessed in tandem.

2.4 Results

We observed 1,816 plant-pollinator interactions across the three years and 18 sites included in this study. Of those interactions, 69.4% involved bees, 13.8% involved syrphid flies, and

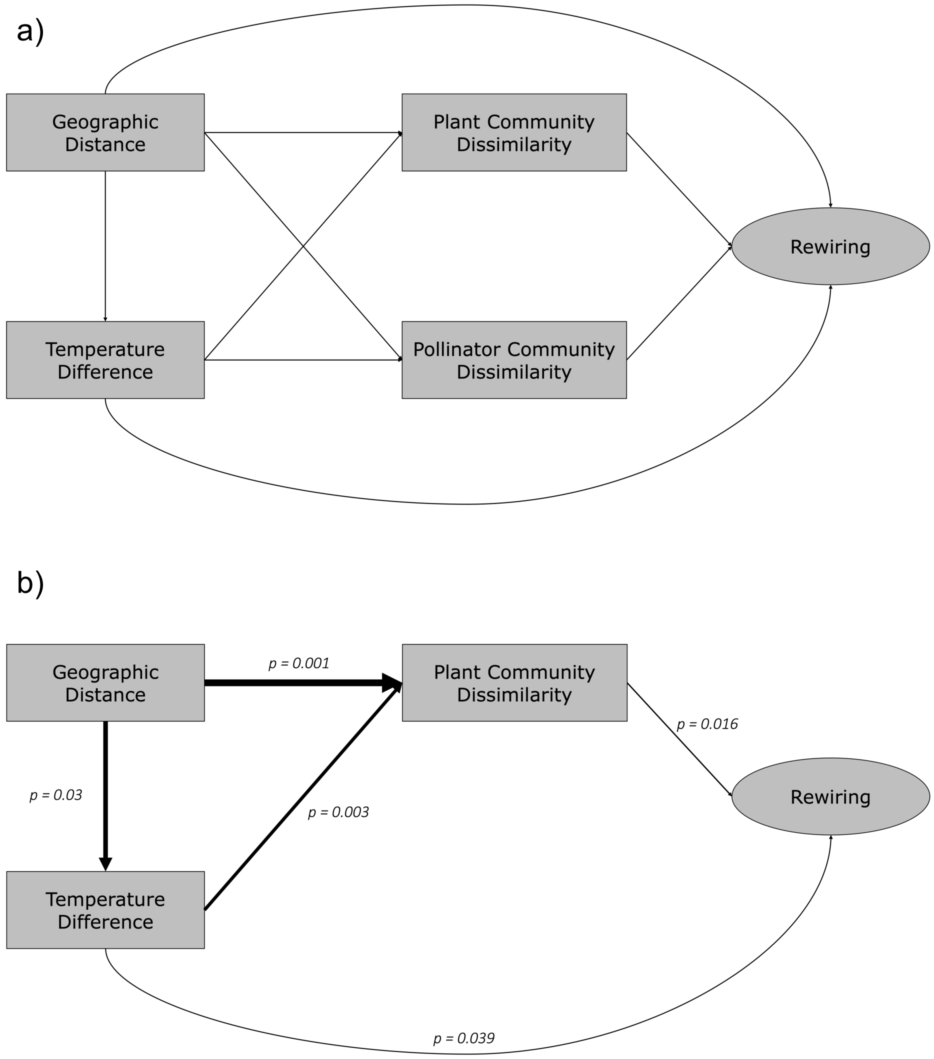


Figure 2.2: a) Conceptual diagram of the potential drivers of rewiring that we examined in this study. b) Results of our structural equation model, with non-statistically significant ($p > 0.05$) pathways removed. Width of the arrows indicate the strength of the relationship. All relationships are positive.

16.8% included non-bee hymenopterans, non-syrphid flies, and other insect orders. Rewiring occurred in an average of 23% of shared interactions between networks. Mean pollinator community dissimilarity was 0.56 ± 0.28 and mean plant community dissimilarity was 0.76 ± 0.23. Our year-specific site-level temperatures ranged from 20.2 °C to 28.7 °C, with a mean ± SD temperature difference between sites of 3.6 ± 1.9 °C. After performing a Box-Cox test on these differences in temperature, we square root transformed our matrix of temperature differences for all subsequent analyses.

We found that the difference in ambient air temperature ($p = 0.039$) and plant community dissimilarity ($p = 0.016$) predicted rewiring (Figure 2.2b). The effect of plant community dissimilarity also included indirect effects of both geographic distance between sites ($p = 0.001$) and temperature difference between site/year aggregates ($p = 0.006$). Geographic distance between sites also significantly predicted temperature differences between sites ($p = 0.025$).

2.5 Discussion

With this study, we show that multiple elements of the biotic and abiotic environment drive rewiring in plant-pollinator communities through both direct and indirect pathways. We found that geographic distance between communities does not have a direct effect on rewiring, despite the existence of a number of studies demonstrating spatial patterns in rewiring. Instead we identified two proximal drivers of rewiring—temperature and plant community composition—through which spatial separation of sites could affect plant-pollinator interactions. Taken together, these results demonstrate roles for both niche and neutral processes in rewiring.

2.5.1 No direct relationship between geography and rewiring

A number of studies have identified geographic patterns in rewiring between plant-pollinator communities (Carstensen et al., 2014; Simanonok & Burkle, 2014; Trøjelsgaard et al., 2015). However, by examining additional pathways through which environmental and biological conditions could influence plant-pollinator interactions, our study suggests that these previously observed geographic trends are likely proxies for more biologically meaningful drivers of plant-pollinator interactions rather than evidence for inherent spatial structure in rewiring patterns. We found that geographic distance was not a significant direct predictor of rewiring when temperature difference, plant community dissimilarity, and pollinator community dissimilarity were included as potential drivers. Despite this lack of direct effect, temperature and plant community composition provided indirect pathways through which geographic distance could influence rewiring in this system.

Spatial patterns in ecology are often thought to be related to underlying environmental patterns (Koenig, 1999; Tuomisto, Ruokolainen, & Yli-Halla, 2003; Zhao, Li, Liu, Peng, & Wang, 2015), and the positive relationship that we observed between temperature difference and geographic distance is consistent with that understanding. While the temperature at a site is undoubtedly also affected by a number of other factors like elevation and aspect, the difference in mean temperatures between sites that was explained by geographic distance in this dataset supports our hypothesis that temperature is one pathway through which spatial separation between communities could yield ecological patterns like rewiring. The positive correlation between geographic distance and plant community dissimilarity is also consistent with a wide range of existing literature showing distance decay in ecological similarity (Nekola & White, 1999; Soininen et al., 2007). This geographic effect on plant community composition could indicate some larger-scale patterns related to dispersal limitations, biogeographic history, and/or community drift that could extend through the composition of plant communities to influence rewiring.

2.5.2 Temperature variation directly and indirectly affects rewiring

Temperature influenced rewiring through both direct and indirect pathways. Our results show that site pairs with less similar similar mean temperatures experienced more rewiring (Figure 2.3a). This direct effect of temperature variation on rewiring illustrates that elements of a pollinator's foraging niche are influenced by abiotic conditions. This is consistent with existing research showing that the quantity and quality of resources produced by flowers change with temperature and that pollinators respond to these thermally mediated changes (McCallum et al., 2013; Scaven & Rafferty, 2013). Thus, sites with similar temperature patterns may also have specific flowers that produce nectar with sugar concentrations, viscosities, or even microbial communities that attract specific insect partners (Russell & McFrederick, 2022). At sites with particularly low average temperatures, pollinators may even seek heat rewards from their partners, providing an additional niche axis that may not exist at warmer sites (Rands & Whitney, 2008).

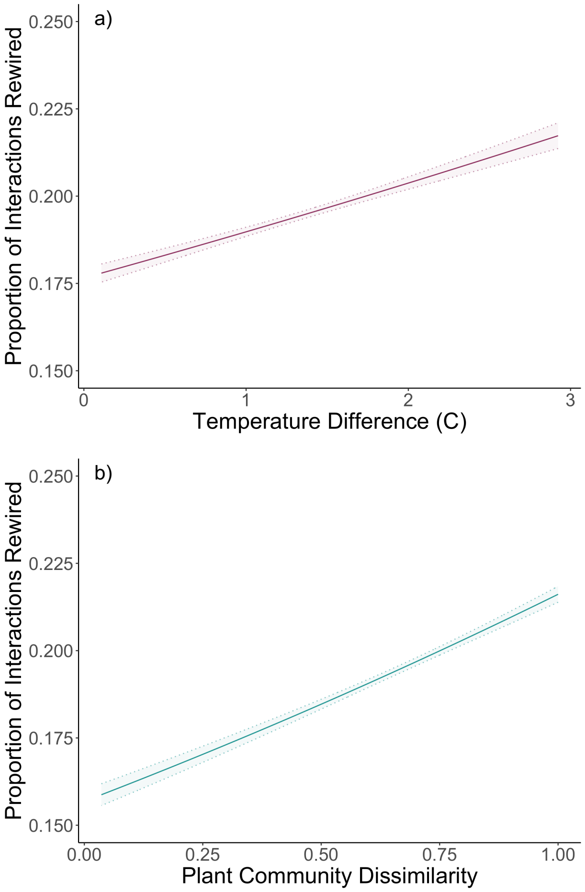


Figure 2.3: Relationships between rewiring and (a) temperature dissimilarity, (b) plant community dissimilarity predicted by our statistical models.

Temperature also indirectly affected rewiring via plant community dissimilarity. Experimental and observational studies have shown that floral abundance and composition both change with temperature (Hegland, Nielsen, Lázaro, Bjerknes, & Totland, 2009). Thus, it is not hard to imagine that sites experiencing large differences in annual mean temperatures could also exhibit dissimilar plant communities, a scenario that is supported by our analysis. Temperature is just one of the many abiotic variables that are likely to influence community composition (Collins et al., 2022; Klanderud, Vandvik, & Goldberg, 2015) and biotic interactions, both within the plant community and across trophic levels, are also likely to influence plant community composition (Klanderud, 2005; Post & Pedersen, 2008). Nonetheless, our results demonstrate that the effect of temperature—and possibly other abiotic variables—on plant-pollinator rewiring is not limited to the direct effects that these variables have on floral resources or pollinator energetics.

2.5.3 Plant community dissimilarity directly affects rewiring

Plant community dissimilarity also directly predicted rewiring in our system, with a stronger effect size than temperature dissimilarity (Figure 2.3b). While similarity in the species present between networks inherently influences the opportunities for rewiring by determining the number of possible interactions among taxa shared between networks (Figure 2.1), we accounted for this numerical effect by including the total number of interactions eligible for rewiring in our binomial models. Thus, the observed effect of plant community dissimilarity on rewiring was not merely a numerical artifact but rather, suggests that contrasting floral resource landscapes lead to variation in plant-pollinator interactions, even among plants and pollinators that co-occur in multiple communities.

The significant relationship that we observed between plant dissimilarity and rewiring is consistent with existing work that finds that pollinator foraging patterns are influenced by the composition of the whole plant community (Arrowsmith, Reynolds, Briggs, & Brosi, 2023). Plants compete for insect pollinators via floral reward, so **similarity** in plant community composition between sites may indicate similarity in these plant-plant competitive dynamics. We thus suspect that our result of increased rewiring (interaction dissimilarity) with increased plant community dissimilarity arises from a difference in the landscape of floral rewards experienced by pollinators in these different communities. For instance, the presence of a highly attractive plant could decrease the frequency of pollinator visits to other plants in a community (Montero-Castaño & Vilà, 2017), so we would expect to see substantial differences in plant-pollinator interaction patterns to the rest of the community when comparing between sites with and without this plant present.

In addition to these niche-level processes, variation in community composition should also yield variation in the encounter rates between particular plants and pollinators (Canard et al., 2014; Carstensen et al., 2014). A plant could be present at two sites, but its relative abundance could vary substantially between the two. Thus, even in the absence of any other site-level differences, we would expect to see an increase in the number and possibly richness of pollinators visiting this focal plant when it comprises a large proportion of the floral community simply because more individual pollinators should encounter it. This explanation for variation in interaction patterns represents an important contrast to the other possible mechanisms of rewiring that we have discussed, since it implies no specific shift in any floral rewards or pollinator foraging behaviors. We do not think that this neutral explanation can be seen as the only mechanism through which plant community composition affects plant-pollinator interaction patterns—the dual direct and indirect effects of temperature, mediated through plant community composition, imply some deterministic shifts in pollinator foraging behavior. However, we also cannot discount a neutral explanation for rewiring and in fact suspect that encounter probabilities do play a role in structuring plant-pollinator interactions.

2.6 Conclusion

With this study, we offer evidence that variation in environmental and biological conditions drives rewiring in plant-pollinator communities. Differences in the mean temperature at sites likely contribute to rewiring via changes in floral rewards and pollinator foraging behavior, while dissimilarity in plant communities could alter both encounter rates and competitive dynamics. We examined a limited number of the possible drivers of rewiring in plant-pollinator communities and there are undoubtedly additional environmental and biological drivers that could be identified with additional work. In particular, we think that further examination of the different environmental conditions that may influence plant-pollinator rewiring patterns, such soil moisture or humidity, will be essential for predicting how climate change may alter these communities. We would also be interested in better understanding the mechanisms through which the proximal drivers of rewiring that we have identified here influence plant-pollinator interaction patterns. For instance, research linking rewiring to variation in the composition of specific floral resources could provide valuable information about the role of temperature variation in this system that this study cannot provide. While the complexities of interspecific interactions guarantee that this study offers just one piece of the puzzle for understanding rewiring patterns across space, we are confident that the relationships that we have identified in this study will provide a strong framework for further research into the drivers of plant-pollinator interaction rewiring.

Chapter 3

Temperature shapes plant-pollinator interaction patterns independently of community composition

3.1 Abstract

Climate change is known to impact species interaction patterns through changes in organisms' spatial and temporal overlap. However, little is known about how fine-scale temperature variation may directly shape species interactions, in part because previous studies have not been able to separate direct effects of temperature from indirect effects of changing community composition. Understanding direct effects of temperature is particularly important for interactions that underlie key ecosystem functions, like pollination. Here, we explicitly disentangle the effects of temperature variation and species turnover on patterns of plant-pollinator interactions to demonstrate that temperature influences interaction patterns independently of any change in species composition. These thermal trends drive dissimilarity in partner choice among pollinator taxa, which may signify changing physiological and behavioral factors like floral resource production or pollinator energetics. Understanding how temperature variation contributes to the structure and function of plant-pollinator communities should equip scientists and conservation professionals with improved information about how climate change may alter patterns of biodiversity.

3.2 Introduction

The structure and function of ecological communities are influenced by the environments in which they exist. Abiotic factors like temperature, precipitation, and land use affect the abundance and distribution of organisms in plant-pollinator communities (Devoto, Medan, Roig-Alsina, & Montaldo, 2009; Ganuza et al., 2022). For instance as temperatures vary, plants and pollinators may shift their geographic distributions or alter the timing of key life history events (e.g., flowering, emergence from overwintering), potentially reducing the times and spaces over which these species overlap. These spatial or temporal mismatches can thus result in the reduction or loss of the plant-pollinator interactions that maintain biodiversity in these communities (Burkle et al., 2013; Hegland, Nielsen, et al., 2009). However, even if organisms continue to co-exist in space and time, environmental conditions like temperature and water availability can affect the quantity or quality of floral resources and the foraging activity of pollinators (Espíndola, Pellissier, & Alvarez, 2011; Scaven & Rafferty, 2013). These physiological and behavioral changes could result in altered interaction patterns even without any changes to the abundance or distribution of organisms.

Several studies have begun to examine the relationship between temperature and interaction patterns in plant-pollinator communities. In observational studies and transplant experiments along elevational gradients, researchers have found an effect of temperature on the rates of interaction occurrence and patterns of specialization between plants and insect pollinators (Classen et al., 2020; Richman, Levine, Stefan, & Johnson, 2020). However, these studies have been understandably limited in their ability to disentangle the physiological and behavioral drivers of these patterns from changes in community composition. Elevational gradients provide predictable thermal variation but also exhibit high rates of species turnover, while transplant experiments involve directly bringing organisms into novel communities in order to observe their response to environmental conditions. Thus, these study designs obscure the extent to which temperature *per se* may influence the interactions between plants and pollinators, independently of the confounding influence of turnover in species composition. Results from a highly controlled experiment that linked temperature and pollinator foraging niches (Fründ, Dormann, Holzschuh, & Tscharntke, 2013) have a contrasting limitation—these findings can be linked to a specific set of plants included in the constructed mesocosms, but extension to natural communities is challenging without additional evidence for temperature-driven niche partitioning in natural systems.

Understanding the effect of temperature variation on plant-pollinator interaction patterns is of urgent importance in an era of unprecedented global environmental change. Animal pollination is a key component of reproduction for around 85% of flowering plants (Ollerton et al., 2011), and these plant-pollinator interactions involve multiple elements that are likely to respond to rising temperatures. The quantity and composition of nectar, a major incentive for pollinator visitation, is expected to shift with abiotic conditions like temperature and water availability (McCombs et al., 2022; Russell & McFrederick, 2022). Pollinator foraging is also dependent on abiotic context, as temperature differences—at least at some scales—will alter the energetic balance that determines when these insects are able to forage and the caloric reward that they seek from floral nectar (Heinrich, 1975; McCallum et al., 2013). While understanding the influence of thermal variation on the composition of species within communities provides some information for how plant and pollinator co-occurrence may be affected by climate change, it is also important to determine whether pollinators' foraging preferences may change under novel temperature regimes, and if so, to develop a mechanistic understanding of how such changes may unfold.

In this study, we examined the relationship between temperature and plant-pollinator interaction occurrence using a multi-year, multi-site dataset of plant-pollinator surveys repeated at regular intervals within distinct sampling days. With community composition thus held constant on individual days, we hypothesized that differing plant resource production and pollinator metabolic needs at different temperatures would lead to changes in the frequency of visits that distinct pollinator taxa made to available flowers. We thus predicted that we would find an effect of temperature on the occurrence of individual plant-pollinator interactions independent of changes to the abundance or distribution of plant species, though we expected that these effects would be highly dependent on the identities of the plant and pollinator involved in each interaction. Our findings provide novel insight into the ways that interactions across trophic levels respond to temperature variation and lay the groundwork for future work understanding and predicting how global environmental change may alter the structure and function of plant-pollinator communities.

3.3 Material and Methods

3.3.1 Data collection

We conducted this study in six subalpine meadows near the Rocky Mountain Biological Laboratory in the Gunnison National Forest (Gunnison County, Colorado, United States). At each meadow, we installed a data logger (HOBO Pendant, UA-001-08) covered by a radiation shield 10-cm above the ground to passively record the temperature at 30-minute intervals during each summer. We surveyed plant-pollinator interactions at four of these sites in the summer of 2021 and at all six sites in the summer of 2022 (Table 3.1). During each summer, we conducted these surveys weekly over the 6—9 weeks during which flowers were blooming. In order to observe pollinators sampling at multiple temperatures within the same plant community, we conducted three sampling rounds on each study day. Sampling rounds were evenly spaced between 10:00–15:00 unless a third sampling round was precluded by inclement weather (18 out of the total 72 sampling days).

For each sampling round, we observed plant-pollinator interactions for 45 minutes of active sampling, evenly distributed across three 1-m by 10-m transects. During this time, we collected any insect that touched the reproductive organs of a flower rooted within our transects for subsequent identification to genus level, with the exception of bumble bees (Apidae:*Bombus*), which we were able to identify in the field. We also surveyed the plant community on each sampling day, noting the species identity and floral abundance of every plant rooted in our transects. We compiled these observations into edge lists for each sampling round with every combination of (1) plant species that was blooming on that day and (2) pollinator taxa (family and genus levels) that was observed actively foraging during that round. We populated the edge lists with counts of the interactions that were observed and inferred an absence of interaction occurrence for any plant-pollinator pairing that was possible due to co-occurrence but not observed during that sampling round.

Year	Site	Days Sampled	Rounds Sampled
2021	Brush Creek Hill	6	16
2021	Kebler Pass	7	20
2021	Rustlers Gulch	7	21
2021	Stupid Falls	7	20
2022	Brush Creek Hill	6	15
2022	Kebler Pass	7	20
2022	Ohio Pass High	7	19
2022	Ohio Pass Low	8	21
2022	Rustlers Gulch	8	23
2022	Stupid Falls	8	21

Table 3.1: Summary of sampling effort.

3.3.2 Data analysis

We conducted all analysis in the statistical software R (R Core Team, 2021). All data used in this analysis are available in Dryad and a fully replicable Rmarkdown of the analysis is available on GitHub (http://github.com/kcarrowsmith).

Temperature variation

This study relied upon our sites experiencing variation in temperature throughout the course of individual days. To test whether we captured this temperature variation during our sampling periods, we used a linear mixed effects model (Bates, Mächler, Bolker, & Walker, 2015) with average temperature during each sampling round as our response variable, sampling date and round as fixed effects, and site as a random intercept. While we expected that day of year also influenced temperature, we did not explicitly test this relationship since our subsequent analysis only compares plant-pollinator interactions that occur on the same dates.

We also tested whether temperature variation affected the number and composition of pollinators actively foraging during our sampling periods in order to determine whether variation in the pollinator community could confound our results. We used a generalized linear mixed effects model to test for an effect of temperature on the total number of pollinators that we observed foraging during each sampling period, regardless of taxon identity. Since our response variable was an overdispersed count variable, we used a negative binomial distribution in the *glmmTMB* package (Brooks et al., 2017). We used linear and quadratic terms describing temperature and a three-level factor for sampling round as fixed effects. We included date and site as random intercepts. We ran this model at three levels of taxonomic resolution—pollinator functional group, family, and genus.

Finally, we used a matrix regression model (Goslee & Urban, 2007) to test for an effect of temperature variation on turnover in pollinator community composition. We controlled permutations by site and round, and we included plant community dissimilarity and difference in day of year as additional predictor matrices to control for anticipated effects of phenological variation when comparing between sampling dates. We calculated plant and pollinator turnover using the Morisita-Horn index in *vegan* (Oksanen et al., 2022). We did not examine any effect of temperature on plant community composition since our subsequent analysis holds plant community composition constant in discrete choice sets.

Pollinator foraging choice

We isolated the effect of temperature from that of plant community composition using a mixed conditional logistic regression from the *mclogit* package (Elff, 2022). This statistical model assessed whether pollinators' foraging choices within the same plant community changed at different temperatures by comparing interaction patterns within "choice sets" of the plants available at a specific site and date. We included temperature as a main effect via both linear and quadratic terms (using the "poly" function in R) because we expected that pollinators' responses to temperature could be non-linear. We also included round number as a fixed effect with three levels, since we knew that floral resources can fluctuate over the day in response to pollinator foraging (Inouye, 1978) and intrinsic daily cycles (Herrera, 1990; Polatto, Chaud-Netto, & Alves-Junior, 2014). We included the taxonomic identity of the plant and insect involved in each interaction as random slopes and intercepts, under the assumption that taxa would exhibit different mean interaction occurrence and respond differently to temperature in moving from that mean. We conducted this analysis at three distinct taxonomic levels, with pollinators grouped at the levels of family, genus, and functional group.

To illustrate thermal trends in pollinator foraging patterns across choice sets, we predicted the interaction probability across all rounds and choice sets for the six most frequently observed pollinator functional groups and their five most common floral partners using the *ggeffects* package (Lüdecke, 2018). We used the same package to plot the shapes of the curves showing the relationship between temperature and interaction probability for the two most common insect families and the single most commonly visited flower.

In order to confirm that any trends identified by our model reflected real variation in pollinator interaction patterns rather than sampling artifacts, we constructed two null models. The first tested how well our observed temperatures predicted pollinator foraging patterns drawn from a temperature-independent distribution of pollinator preferences. To achieve this, we aggregated all of our observed interactions for each pollinator taxon into an overall distribution of preferences. We then re-assigned each observed pollinator's interaction partner from this distribution. The second tested whether randomly sampled temperatures predicted our observed plant-pollinator interaction patterns as well as our observed temperatures. Since our observed temperatures roughly followed a normal distribution, we replaced our empirical temperature values random temperatures drawn from a normal distribution with the mean and standard deviation defined by our empirical values. For both null models, we ran mixed conditional logistic regression models identical to those used for our empirical data. We ran each null model 99 times at the levels of functional group, family, and genus.

3.4 Results

3.4.1 Overall trends

We conducted 201 sampling rounds across two years (Table 3.1) during which we observed a total of 2,585 plant-pollinator interactions. Of these interactions, 2,112 involved insects that we could identify to the family level for a total of 275 unique pairings between 37 insect families and 67 plant species and 1,618 involved insects that we could identify to the genus level for a total of 264 unique pairings between 52 insect genera and 44 plant species.

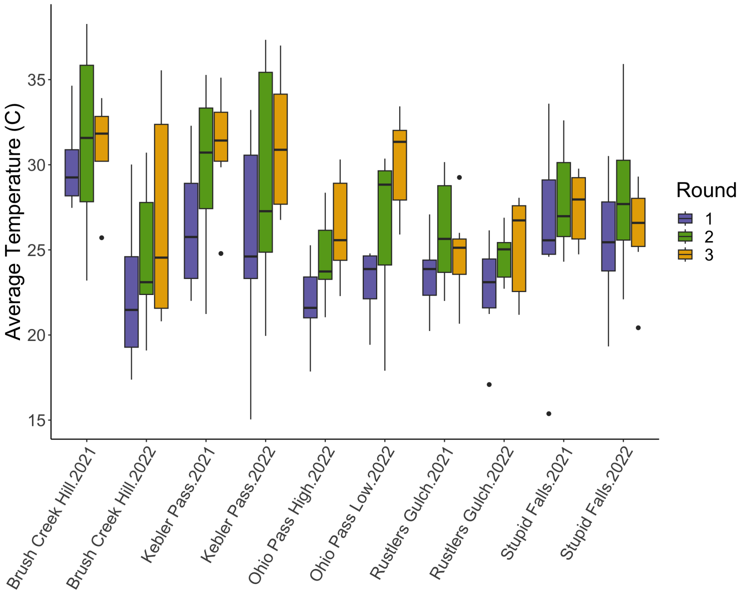


Figure 3.1: Observed relationship between sampling round and average temperature.

Temperatures during our sampling periods ranged from 15.0 °C to 38.3 °C and significantly varied with sampling round ($p < 0.001$; Figure 3.1). We found a small but significant linear relationship between temperature and the number of pollinators actively foraging when we grouped pollinators by functional group ($p = 0.039$) and family ($p = 0.020$; Figure 3.2); however, this effect was not present when interactions were grouped at the genus level ($p = 0.380$). We did not see any significant effect of sampling temperature on the composition of pollinators observed when grouped at any taxonomic level ($p > 0.1$ at all levels).

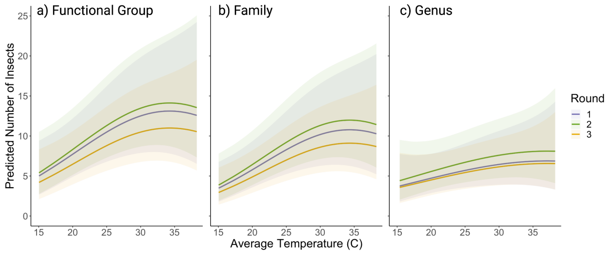


Figure 3.2: Relationship between average temperature and the number of pollinators actively foraging during a 45-minute sampling period.

Pollinator community composition was related to plant community composition ($p < 0.03$) and day of year ($p < 0.002$) at all taxonomic levels; however, these effects would not have confounded our results since our choice model only compared pollinator interaction patterns within a consistent plant community and on individual days.

3.4.2 Relationship between temperature and plant-pollinator interactions

Temperature significantly influenced plant-pollinator interaction patterns at all three levels of taxonomic organization. When insects were pooled by functional group, temperature had significant linear ($p = 0.003$) and quadratic ($p < 0.001$) effects. These temperature effects were maintained when insects were grouped at the family level (linear $p = 0.003$; quadratic $p < 0.001$); however at the genus level, only the quadratic effect of temperature was maintained ($p = 0.01$). In addition to these temperature effects, time of day—which we estimated using three discrete sampling rounds—significantly influenced interaction occurrence at both the functional group ($p < 0.001$) and family ($p = 0.029$) levels. However, the significant effect of sampling round disappeared when interactions were analyzed at the level of insect genera ($p = 0.680$)

3.4.3 Null models

When we selected interactions for every observed insect from a distribution of partner preferences specific to the insect taxon involved but independent of site, date, or round, our null models were not able to replicate the relationships between temperature and plant-pollinator interaction occurrence at any taxonomic level (Figure 3.3). When we selected temperature at the time of foraging from a normally distribution with a mean (26.5 °C) and standard deviation (4.55 °C) that matched our empirical temperature data, our null models were able to predict plant-pollinator interaction occurrence for 2–9 out of 100 simulations, depending on the taxonomic level at which we ran the null model (Figure 3.4). At the level of functional groups, two models met or exceeded the absolute value of the z-score for the linear effect of temperature, while no models met the z-score for the quadratic effect of temperature. At the family level, three models met or exceeded the absolute z-scores of both linear and quadratic effects of temperature. At the genus level, nine models met or exceeded the absolute z-score of the quadratic effect of temperature. In comparison, 23 models met or exceeded the absolute z-score of the linear effect of temperature, which had a non-significant effect in our empirical data.

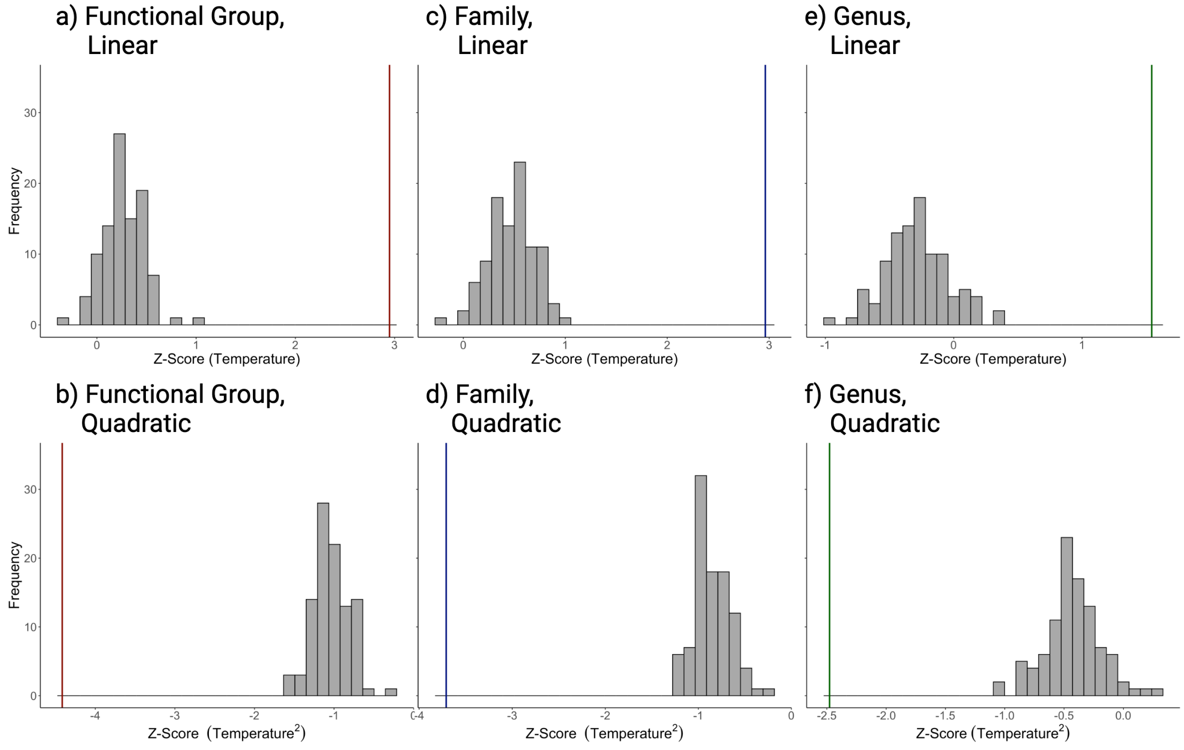


Figure 3.3: Null models testing the ability of our mixed conditional logistic regression to predict plant-pollinator interactions that were specific to the taxon involved, but not to the site, date, or round at which the empirical temperature occurred.

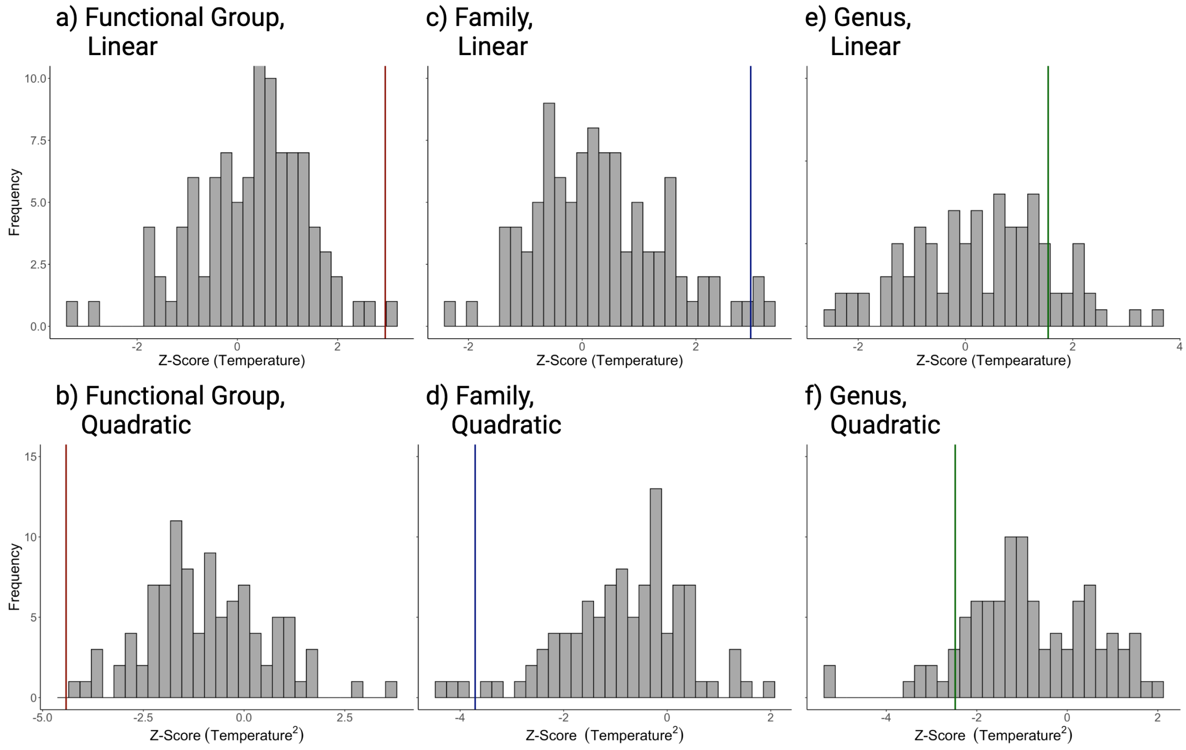
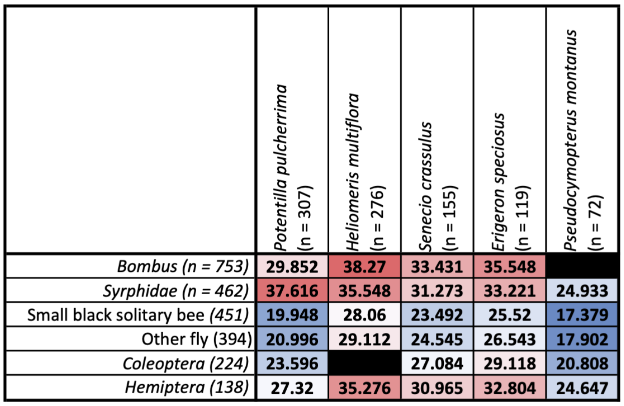


Figure 3.4: Null models testing the ability of our mixed conditional logistic regression to predict our empirical plant-pollinator interactions using random temperatures.

3.5 Discussion

Global environmental change is likely to alter the structure, function, and persistence of ecological communities. We examined how one element of global change—temperature variation—affected plant-pollinator interaction patterns and found that temperature influenced pollinators' foraging patterns even in the absence of changes to community composition. We saw an effect of temperature at all three levels of taxonomic organization that we analyzed, though at the finest taxonomic resolution, we only found a quadratic effect. We also found that time of day affected these interaction patterns, though this effect was not consistent across all levels of taxonomic organization. The relationships between temperature and interaction patterns were not driven by changes to either plant or pollinator community composition, though temperature did have some effect on the overall number of pollinators foraging during an average sampling period. These results indicate that both bottom-up (e.g., floral rewards) and top-down (e.g., pollinator preferences) processes likely influence how plant-pollinator interaction patterns respond to temperature variation and daily cycles. The knowledge that temperature influences patterns of interaction between plants and insect pollinators has a number of important implications for understanding emergent patterns in community ecology, predicting the effects of global environmental change on ecosystem functioning, and implementing effective conservation strategies.

3.5.1 Temperature affects plant-pollinator interaction patterns



	Potentilla pulcherrima (n = 307)	*Heliomeris multiflora* (n = 276)	*Senecio crassulus* (n = 155)	*Erigeron speciosus* (n = 119)	*Pseudocymopterus montanus* (n = 72)
Bombus (n = 753)	**29.852**	**38.27**	**33.431**	**35.548**	■
Syrphidae (n = 462)	**37.616**	**35.548**	**31.273**	**33.221**	**24.933**
Small black solitary bee *(451)*	**19.948**	**28.06**	**23.492**	**25.52**	**17.379**
Other fly (394)	**20.996**	**29.112**	**24.545**	**26.543**	**17.902**
Coleoptera (224)	**23.596**	■	**27.084**	**29.118**	**20.808**
Hemiptera (138)	**27.32**	**35.276**	**30.965**	**32.804**	**24.647**

Figure 3.5: Temperature at which plant-pollinator interactions are most likely for the six most common insect functional groups and their give most common floral partners. Floral partners were selected based on number of interactions in entire dataset.

Our results demonstrate an effect of temperature on the patterns of interactions between plants and their insect pollinators. At each level of taxonomic organization, these trends provide interesting insights into the possible biological processes underlying thermal mediation in plant-pollinator interactions. Among functional groups, we see several examples of distinct relationships between temperature and the probability of occurrence for different plant-pollinator interactions. Our statistical model predicted that the six most common insect functional groups in our system would exhibit "peak" interaction occurrence—defined as the point at which interactions with a particular plant are most probable—at different temperatures depending on the plant involved in that interaction (Figure 3.5). Those different optimal interaction temperatures ranged over a minimum of 8.3 °C (Coleoptera) and a maximum of 12.7 °C (Syrphidae) when we considered interactions involving the five most common plant species. This trend demonstrates that all six functional groups are active at a wide range of temperatures and, to some extent, vary their interactions in relation to temperature.

It is tempting to then wonder if there are plants that are generally more attractive at warmer or cooler temperatures. We do see some such trends—for instance, *Pseudcymopterus montanus* was the floral partner with the coolest optimal temperature for all five of the functional groups that visited it and *Heliomeris multiflora* was the floral partner with the warmest optimal temperature for four of the five functional groups that visited it. However, the other three most common floral partners exhibited substantial variation in the temperatures at which different insects were most likely to visit them. Both *P. pulcherrima* and *Senecio crassulus* were the floral partner with the warmest optimal temperature for one functional group, and all three plants had ranges of optimal temperatures that approached or exceeded 10 °C. Thus, we conclude that the effect of temperature on interaction occurrence was not merely a reflection of the temperatures at which these groups were actively foraging or producing rewards. Instead, we posit that these findings reflect differences in the foraging preferences of individual pollinators at different temperatures.

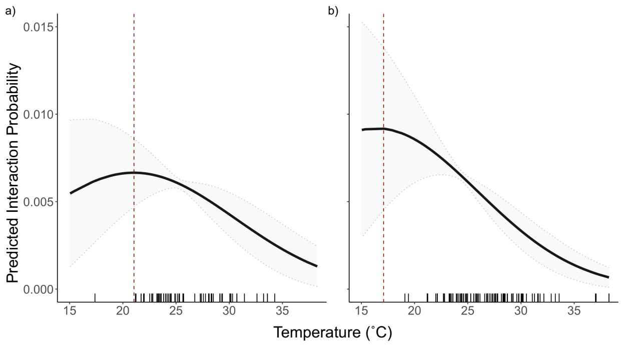


Figure 3.6: Predicted interaction probability for interactions between *Potentilla pulcherrima* and (a) Andrenidae; (b) Halictidae. Rug plot along x-axis shows the temperatures at which each family was observed foraging.

Many of these functional group trends are mirrored at the family level, due in large part to the overlap between functional group and insect family. Nonetheless for functional groups that span multiple insect families, we can use this finer scale of taxonomic resolution to further examine the overall relationships between temperature and interaction occurrence. For instance, we see subtly different optimal temperatures for interactions between *P. pulcherrima* and our two most common families in the "small black solitary bee" functional group—Andrenidae and Halictidae (Figure 3.6). Halictids, which include the common sweat bee *Lasioglossum*, have the highest predicted probability of interaction with *P. pulcherrima* at a lower temperature (17.09 °C) than andrenid bees (21.05 °C). This difference occurs despite overall foraging activity for both families occurring at generally comparable temperatures, so overall variation in activity across temperatures cannot be the main reason for the difference in these optimal interaction temperatures. Rather, we suspect that these trends illustrate family-level thermal niche partitioning in which some element of *P. pulcherrima*'s floral rewards are more attractive to these different families at different temperatures. Since *P. pulcherrima* was the floral partner for 73% of andrenid interactions, we doubt that our data can provide much insight into how Andrenidae partition their interactions across temperatures. However, *P. pulcherrima* was the floral partner for only 20% of halictid interactions, so we think that the prevalence of these interactions at lower temperatures is indicative of a true preference among Halictidae. Though we cannot determine precisely what element of *P. pulcherrima*'s floral rewards are more attractive to halictid bees at lower temperatures, we suspect that the metabolic needs and/or physiological responses of these bees play important roles in this pattern (Bishop & Armbruster, 1999; McCallum et al., 2013; Stone, 1994).

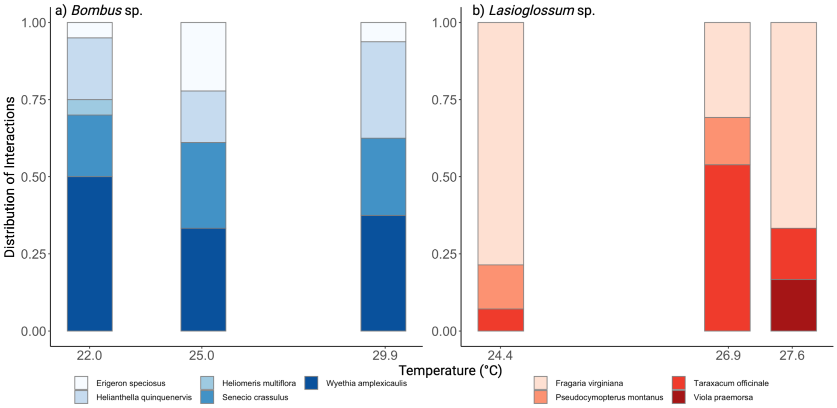


Figure 3.7: Observed distribution of interactions involving the most abundant genus present on two sampling days. (a) Interactions involving *Bombus* at Kebler Pass site on 16 July 2021. (b) Interactions involving *Lasioglossum* at Rustlers Gulch site on 12 June 2022.

Finally, we examined genus-level trends within single sampling days in order to illustrate our general trends of thermal variation in plant-pollinator interaction patterns within distinct plant communities (Figure 3.7). We first looked at the most abundant insect genus in our dataset—*Bombus* (bumble bees)—at a single site in

July 2021. We saw wide variation in the flowers visited by *Bombus* across a temperature range of nearly 8 °C (Figure 3.7a). At 22.0 °C, *Wyethia amplexicaulis* represented nearly 50% of all *Bombus* interactions, while the next most common interactions—with *Helianthella quinquenervis* and *Senecio crassulus* each represented less than 25% of interactions. At 29.9 °C, *Bombus* visited *W. amplexicaulis* at about the same rate as *S. crassulus*, with each flower making up roughly 30% of all *Bombus* interactions. We see similar variation in interaction patterns for *Lasioglossum*, our next most abundant pollinator genus, at a different site in June 2022 (Figures 3.7b). Here, *Taraxacum officinale* made up a greater proportion of *Lasioglossum* visits as the temperature increased from 24.4 °C to 26.9 °C, and interactions involving *Viola praemorsa* only occurred at the highest temperature, 27.6 °C. These differences in just two pollinator genera on two distinct sampling days are illustrative of the broader effect of temperature on pollinator foraging choices in our data. While the specific direction of these trends is highly variable across dates and sites, we rarely see plant-pollinator interactions that are consistent across all temperatures sampled within a single day. This sensitivity to temperature indicates that pollinators' foraging preferences are not static traits but rather, respond to the dynamic biotic and abiotic environment in which these pollinators are foraging.

Temperature-sensitivity in pollinator foraging choices could support dynamic niche partitioning for floral resources. Most pollinators visit a variety of floral resources and most plants receive a variety of insect visitors (Waser, Chittka, Price, Williams, & Ollerton, 1996). This generalization in plant-pollinator systems is expected to increase the stability of pollination services over time and decrease the likelihood of coextinction in the event of partner loss. However, it is also likely to increase competition among pollinators for floral resources and among plants for pollinator visits. Coexistence of these competing organisms could be explained in part by thermal niche partitioning, in which different insects visit these shared floral resources at different temperatures (e.g., Figure 3.6). In addition, dynamic niche partitioning in response to temperature variation could preserve the species-level generalization that imparts resilience to these networks while allowing for more specialized interactions at the level of individual pollinator foraging bouts (Brosi, 2016). Though such an assertion requires far more research to confirm, distinct thermal optima for different pollinators visiting the same plants (Figures 3.5) provide compelling preliminary evidence that such niche partitioning could occur.

3.5.2 Plant-pollinator interactions vary with time of day

In addition to temperature, time of day also affected plant-pollinator interaction patterns in this system. We know that some of that effect is likely related to predictable variation in temperature over the course of a day (Figure 3.1). However, the significant effects of both temperature and round at the functional group and family levels indicate that plant-pollinator interactions may also follow important daily cycles that are independent of temperature variation. A number of the floral resources that attract insect pollinators experience predictable fluctuations during an average day (CaraDonna et al., 2021; Herrera, 1990; Nicolson, Nepi, & Pacini, 2007; Polatto et al., 2014), which may be both a cause and consequence of temporal patterns in pollinator visitation (Howell & Alarcón, 2007; Mallinger & Prasifka, 2017; Nottebrock et al., 2017; Wolff, 2006). The volume of nectar available to pollinators can serve as a predictable bottom-up driver, as flowers often secrete nectar at particular times of day and may even reabsorb it at other times (Corbet, 2003; Pacini, Nepi, & Vesprini, 2003). Additionally, the energetic quality of nectar, which can be inferred from the concentration of sugar, also varies in response to abiotic conditions like temperature and to biological processes like nectar reabsorption (Corbet, 2003).

Pollinators can also act as top-down drivers of daily cycles in interaction patterns by influencing the floral resources available over the course of a day. Nectar and pollen are finite resources that can be over-exploited, with pollinator foraging earlier in the day thus limiting the resources available later in the day. This pollinator-driven variation in floral resources has been demonstrated with nectar standing crop, which is reduced by pollinator visitation such that subsequent pollinator interactions are less likely (Keasar, Sadeh, & Shmida, 2008). Nectar volume can also affect which pollinators can successfully forage upon particular flowers—a lack of pollinator visitation earlier in the day may allow nectar volume to increase to a point that additional pollinators can access it (Inouye, 1978). Some flowers can even be manipulated into producing nectar with higher sugar concentration by the sounds pollinators make while flying (Veits et al., 2019), so variation in pollinator activity over the course of a day could influence nectar sugar content. Since our statistical model found both temperature and time of day to be important predictors of interaction patterns for two of our three taxonomic scales, we must be aware of these non-thermal drivers of interaction variation when considering the effects of temperature on daily patterns of plant-pollinator interactions.

3.5.3 Effects of temperature are context-specific

Since our null models generally failed to replicate our empirical findings, we believe that the relationship between temperature and interaction occurrence is specific to the ecological contexts in which these interactions occur. With our first set of null models, we tested whether our empirical temperatures could predict plant-pollinator interactions that were taxon-specific, but not specific to a particular sampling event. In this model, individual taxa had the same interaction probabilities across all sites, dates, and rounds. With these constant interaction probabilities, our first set of null models were never able to predict these randomly constructed interaction patterns (Figure 3.3). This lack of predictive power indicates that more information than a pollinator's overall foraging niche is necessary to understand the likelihood of a particular plant-pollinator interaction occurring.

With our second set of null models, we examined whether random temperatures drawn from a normal distribution with a mean and standard deviation matching our observed temperature data could predict our empirical plant-pollinator interaction patterns. For all three taxonomic groupings, we found that these random temperatures predicted interaction occurrence as well as our observed temperature data less than 10% of the time, even though the empirical time of day information was retained (Figure 3.4). These results suggest that the relationship between temperature and plant-pollinator interaction patterns is specific to distinct foraging contexts and cannot be estimated simply using seasonal averages. This results also demonstrated that time of day alone cannot adequately predict plant-pollinator interaction patterns—though we retained this empirical information in all of our null models, round number alone rarely mediated the loss of context caused by randomly assigning foraging temperatures or pollinator preferences.

3.5.4 Implications of thermally-mediated plant-pollinator interactions

Increasing temperatures, among other consequences of climate change, are expected to threaten the persistence of many plant and pollinator species (Deutsch et al., 2008; Dormann et al., 2008), making conservation of plants, pollinators, and their interactions a global priority. Many modeling frameworks for predicting the effects of climate change assume that organisms will shift their geographic distributions to follow preferred climatic niches, usually defined by temperature and precipitation. However, our evidence that pollinators change their foraging preferences with temperature presents another important pathway through which pollinators may adapt to climate change—in addition to potentially shifting their ranges, they may also shift their interaction patterns. Effective conservation will need to account for both of these possible responses to global environmental change in order to effectively predict and manage changes to plant-pollinator communities, though the relative importance of each response will depend on the goals of specific conservation action. For instance, conservation of an imperiled insect pollinator might primarily involve planting floral resources that this pollinator is expected to visit under new, warmer temperatures. In contrast, conservation actions focused on protecting pollination function may prioritize regions of a pollinator's suitable habitat in which it is expected to successfully pollinate a particular plant of concern, and possibly even de-prioritize areas of that plant's current habitat where it is likely to lose access to pollination services. Across conservation goals, however, efforts to counter the impacts of climate change on ecological communities will benefit from a stronger understanding of the ways in which temperature might impact not just the presence of plants and pollinators in a particular location but also the patterns of interactions between these groups.

3.5.5 Directions for future research

The results that we present here allow us to begin making some interesting connections between temperature and plant-pollinator interaction patterns. However, our study was not designed in such a way that we can identify the specific mechanisms driving the observed relationships. Building a better understanding of the specific mechanisms driving changes in pollinator foraging preferences and floral resource composition with temperature should be a focus of future work. For example, controlled studies of pollinator foraging choices across temperatures, such as in a temperature-controlled greenhouse, could help elucidate whether pollinators seek higher energy rewards or more water under higher temperatures. Similarly, direct measurements of floral resources like nectar volume and sucrose concentration across temperatures, both in controlled and field settings, would provide valuable insight into how these resources respond to temperature. Ideally, such work would be placed in the context of functional traits or phylogenetics so as to allow for better extrapolation across the variation that we saw with only a few species.

3.6 Conclusions

We find that temperature drives changes in plant-pollinator interaction patterns despite consistency in the plants available for pollinator visitation. This unique perspective on interaction turnover independent of species turnover offers important evidence linking environmental conditions to ecosystem function and community structure. While species range shifts and phenological mismatch will undoubtedly disrupt a vast number of plant-pollinator interactions, pollination in communities that are not disturbed by these spatial or temporal mismatches may still be altered by global environmental change. Pollination services, which rely upon conspecific pollen transfer as pollinators visit multiple individuals of the same species, are likely to be influenced by short-term temperature variation and long-term climatic trends. Conservation of this crucial ecosystem function must therefore consider not only the spatial and temporal overlap of its interacting partners but also the environmental conditions necessary to facilitate consistent interactions.

Chapter 4

Community context mediates effects of pollinator loss on seed production

Note: This chapter has been published in *Ecosphere* under the same title. The full citation can be found in the references under Arrowsmith et al. (2023).

4.1 Abstract

A critical goal for ecologists is understanding how ongoing local and global species losses will affect ecosystem functions and services. Diversity-functioning relationships, which are well characterized in primary producer communities, are much less consistently predictable for ecosystem functions involving two or more trophic levels, particularly in situations where multiple species in one trophic level impact functional outcomes at another trophic level. This is particularly relevant to pollination functioning, given ongoing pollinator declines and the value of understanding pollination functioning for single plant species like crops or threatened plants. We used spatially replicated, controlled single-pollinator-species removal experiments to assess how changes in bumble bee species richness impacted the production of fertilized seeds in a perennial herb—*Delphinium barbeyi*—in the Rocky Mountains of Colorado, USA. To improve predictability, we also assessed how traits and abundances in the plant and bumble bee communities were related to *D. barbeyi* reproductive success. We hypothesized that trait-matching between pollinator proboscis length and *D. barbeyi*'s nectar spurs would produce a greater number of fertilized seeds, while morphological similarity within the floral community would dilute pollination services. We found that the effects of pollinator removal differed depending on the behavioral patterns of pollinators and compositional features of the plant and pollinator communities. While pollinator floral fidelity generally increased *D. barbeyi* seed production, that positive effect was primarily evident when more than half of the *Bombus* community was experimentally removed. Similarly, communities comprising primarily long-tongued bees were most beneficial to *D. barbeyi* seed production in tandem with a strong removal. Finally, we observed contrasting effects of morphological similarity in the plant community, with evidence of both competition and facilitation among plants. These results offer an example of the complex dynamics underlying ecosystem function in multi-trophic systems and demonstrate that community context can impact diversity-functioning relationships between trophic levels.

4.2 Introduction

Biodiversity positively influences many key biological functions, underscoring concerns about the functional impacts of ongoing local and global extinctions (Cardinale et al., 2012; Hooper et al., 2005). Dozens of experiments have confirmed relationships between biodiversity and ecosystem functioning ("BEF"), particularly in primary producer communities involved in functions like biomass production or carbon fixation, to name just two examples (Tilman, Isbell, & Cowles, 2014). However, the evidence for BEF relationships is much more ambiguous for ecosystem functions that are mediated across two or more trophic levels, such as predation, pollination, and seed dispersal (Casula, Wilby, & Thomas, 2006; Loy & Brosi, 2022; Sih, Englund, & Wooster, 1998). For example, in multi-predator/single-prey studies, increased predator richness may positively or negatively impact the biomass of a target prey species, depending on other properties of the system in which these interactions take place (Casula et al., 2006; Sih et al., 1998). Thus, there remains a need to understand what factors shape how biodiversity in one trophic level affects the functional responses in a single species at another trophic level.

Plant-pollinator communities represent one system in which the question of BEF patterns across trophic levels is particularly important. Insect pollinators, which facilitate reproduction in a vast majority of flowering plants, are experiencing widespread population declines (Kremen, Williams, & Thorp, 2002; Potts et al., 2010). Yet BEF studies examining the effects of pollinator losses in these systems have yielded contradictory results (Brosi & Briggs, 2013; Hallett, Mitchell, Chamberlain, & Karron, 2017), limiting ecologists' ability to predict how global patterns of pollinator loss will impact ecosystem function. Removal experiments involving a single pollinator species have shown negative impacts on pollination in some systems (Brosi & Briggs, 2013) but not in others (Hallett et al., 2017), indicating that functional relationships in these systems could be mediated by more than simply species richness. Indeed, while numerical metrics of biodiversity like pollinator richness and abundance are likely to play major roles in pollination function (Tilman et al., 2014; Winfree, W. Fox, Williams, Reilly, & Cariveau, 2015), functional traits within both plant and pollinator communities may also affect the BEF dynamics in these systems (Hooper et al., 2005; McGill, Enquist, Weiher, & Westoby, 2006). For instance trait-matching (or mismatching) is likely to incentivize some plant-pollinator interactions—like pollinators with long tongues visiting flowers with deep corolla tubes—and limit others (Garibaldi et al., 2015; Peralta et al., 2020; Stang, Klinkhamer, Waser, Stang, & van der Meijden, 2009). In this case, successful pollination may rely less on the total abundance of pollinators in a community than on the relative abundance of pollinators morphologically adapted to forage upon these morphologically distinct flowers (Inouye, 1980). Since species richness is likely to interact with several other elements of community composition at both trophic levels involved, it is crucial for future multi-trophic studies of BEF patterns to go beyond investigating species richness *per se* and to instead understand how functional trait distributions in all relevant trophic levels modify the functional effects of biodiversity.

In this study, we examined how plant-pollinator community dynamics influenced the relationship between removals of single bumble bee species (*Bombus* sp.) and pollination services in a target plant species (*Delphinium barbeyi*, Ranunculaceae). We conducted short-term, single-species removal experiments with four of the 11 *Bombus* species present in our study area, precluding identity effects that would arise from consistently removing the same pollinator species. We repeated this experiment over three summers, with true spatial replication across our experimental units. We generally hypothesized that the proportion of specific traits in the plant and pollinator communities, as well as the relative abundance of individuals within each guild, mediated the effects of pollinator removal on plant reproductive function (number of fertilized seeds produced). Specifically, we expected that (i) pollinator community composition that promoted visitation to *D. barbeyi* (high *Bombus* abundance, high proportion of long-tongued bees) would increase the production of viable seeds by this focal plant; (ii) pollinator foraging behavior that promoted conspecific pollen transfer (floral fidelity) would also increase seed production; and (iii) morphological similarity between our focal plant species and other flowers in the community would dilute floral visitation and overall reproduction in our focal plant throughout our experiment via interspecific competition.

4.3 Methods

4.3.1 Study system

We conducted this study over three years (2011, 2013, 2014) in subalpine meadows surrounding the Rocky Mountain Biological Laboratory, (38°57.5′N, 106°59.3′W, 2,900 m above sea level), Gunnison National Forest, Colorado, United States. We replicated our experiment over 14 sampling events distributed across 13 unique sites, with sites separated by at least 1km to ensure independence of the pollinator communities (Darvill, Knight, & Goulson, 2004; Knight et al., 2005). To maintain independence in our data, we sampled each site only once during this study, with the exception of one location that we sampled in both 2011 and

2014. At each site, we established a 20-m by 20-m plot that contained comparable densities of target flowers throughout the study system (Brosi & Briggs, 2013).

To estimate reproductive success, we counted the production of fertilized ovules (seed production) by *Delphinium barbeyi* (Ranunculaceae), a common perennial wildflower in the Rocky Mountains that is visited by a wide range of bumble bee (Hymenoptera: Apidae: *Bombus*) pollinators. *D. barbeyi* is notable for its long nectar spur (10–18mm, Warnock, 1993), which generally results in a high abundance of visits by pollinators with long proboscises like *Bombus* (Inouye, 1980; Pyke, 1982). Previous work in this system has confirmed that nearly all *Bombus* species in the region, including those with relatively shorter proboscises, visit *D. barbeyi* (Brosi & Briggs, 2013; Inouye, 1978).

4.3.2 Field data collection

Assessment of seed production

Prior to each sampling event, we selected 30 focal *D. barbeyi* individuals at which we would assess seed production. We randomly assigned half of these individuals to the control period and the other half to the manipulation. Approximately two days prior to the control survey at each site, we covered racemes of immature floral buds on all focal plants with pollinator exclusion bags to prevent pollination outside of the study period. Then at the beginning of each study period, we removed the pollinator exclusion bags on the assigned half of mature *D. barbeyi* flowers. After four hours, during which our focal individuals were exposed to insect pollinators, we re-bagged the flowers and allowed any fertilized ovules to mature without further pollination. We harvested mature fruits from bagged flowers 7–15 days after the study period and counted the developing seeds produced in each fruit to estimate the reproductive function of each flower during its assigned study period.

Bombus **removal manipulations**

Each of our 14 sampling events comprised two 4-hour study periods. First, we established a control period during which we allowed pollinators to forage without intervention. During this control period, we performed baseline surveys of *Bombus* richness and abundance via timed aerial netting within our 20-m by 20-m plot. Two days after each control period, we performed a manipulation in which we non-destructively removed all individuals of the bumble bee species deemed most abundant from these baseline community compositional surveys. To achieve this removal, we performed targeted aerial netting of every individual of the selected species present in our plot (Inouye, 1978). We kept these removed bumble bees alive in vials in a cooler until the end of the study period, at which time we released them (Brosi & Briggs, 2013). Once we had not observed any individuals of the target *Bombus* species in our plot for one hour, we removed pollinator exclusion bags from our focal *D. barbeyi* individuals and commenced the 4-hour study period. During this study period, field personnel patrolled the perimeter of the plot and removed any individuals of the target *Bombus* species that were seen entering the area, and also repeated surveys of the remaining *Bombus* richness and abundance.

Floral morphological similarity

In order to assess the composition of the floral community around our focal *D. barbeyi* individuals for each sampling event, we surveyed the species identity and number of flowers for each plant in bloom along two 1-m by 20-m transects (Brosi & Briggs, 2013). We then calculated the proportion of the floral community that overlapped with *D. barbeyi* on two morphological characteristics — color (blue/violet) and corolla length (10 – 18mm) (Warnock, 1993). We selected these two characteristics because we expected them to influence the rate of bumble bee visitation to *D. barbeyi* compared to other plants at each site.

Pollinator traits and floral fidelity

We observed the foraging patterns of individual bumble bees during each study period in order to assess how our manipulation affected the number and fidelity of visits to *D. barbeyi*. We followed individual bumble bees foraging within our plots until they either reached 100 foraging visits or left the perimeter of the study area. We recorded a foraging event each time a bumble bee interacted with the reproductive organs (anther or stigma) of a flower. We then calculated floral fidelity as a binary trait for each bee, with an individual considered faithful when all of its visits were made to the same floral species.

Because the morphology of *D. barbeyi* allows long-tongued bees to more efficiently obtain its nectar (Inouye, 1980), we assessed the proportional distribution of this trait in the pollinator communities that we surveyed during each study period. We then calculated the percent of each *Bombus* community that was considered "long-tongued," using accepted expert descriptions (Colla, Richardson, & Williams, 2011; Inouye, 1977; Koch, Strange, & Williams, 2012; P. H. Williams, Thorp, Richardson, & Colla, 2014).

4.3.3 Data analysis

We conducted all analyses in the statistical software R (R Core Team, 2021). A fully reproducible R markdown report of our analyses is available on GitHub.

Relationship between manipulation and *Bombus* community composition

Our experimental manipulation involved the removal of the most abundant *Bombus* species from each site. We assessed whether this removal significantly altered the total *Bombus* abundance and proportion of long-tongued bees across our study system, since such results could have influenced the effects of our experimental removal. To test for an effect of our manipulation on total *Bombus* abundance, we ran a generalized linear mixed effects model (GLMM) with negative binomial errors using *Bombus* abundance as the response variable, the strength of our removal as a fixed effect, and site and year as a random intercepts. To test for an effect of our manipulation on the distribution of tongue-lengths in our community, we used a similar GLMM, but with the proportion of long-tongued bees as our response variable and binomial errors.

Model selection and averaging

To identify the determinants of seed production in *D. barbeyi*, we used an Akaike information criterion (AIC) model selection framework that tested each combination of fixed effects to identify the best set of variables to describe our observed data. Multi-model inference is an effective tool through which a range of likely ecological processes can be assessed simultaneously (Johnson & Omland, 2004; Symonds & Moussalli, 2011). We used this analytical framework because multiple combinations of community ecological factors could affect our outcome of interest — in this case, the production of fertilized *D. barbeyi* seeds. A traditional hypothesis-testing approach would only determine whether a fixed set of explanatory variables significantly affected our response variable, effectively testing our ability to build an *a priori* prediction of the relevant ecological processes in this system. Rather than testing a specific hypothesis, this model selection approach allowed us to evaluate the relevance of a range of community ecological variables on the production of fertilized ovules and build a more robust understanding of the most important ecological processes governing this outcome.

After detecting overdispersion in our data, we built our global model using a GLMM following a zero-inflated negative binomial distribution using the *glmmTMB* package for R (Brooks et al., 2017). We included the number of fertilized ovules collected from each *D. barbeyi* carpel in our models as the response variable. Each *D. barbeyi* flower typically contains three carpels, which resulted in an average of three distinct seed counts per flower. Our fixed effects included strength of our experimental manipulation—measured as the proportion of the total pollinator community that we manually removed—and a series of metrics describing community composition and trait values for both the plant and pollinator communities (Table 4.1). For the plant community, we included the relative abundance of our focal plant at each site (PD) and the proportion of the floral community that shared one of two morphological traits—color (CL) and corolla length (CR)—with *D. barbeyi*. For the pollinator community, we included the total abundance of bumble bees (BA), the proportion of the bumble bees in each community observed engaging in faithful foraging behavior (i.e., visiting only a single plant species; MF), and the relative abundance of long-tongued bumble bees (PL), which we expected to engage in trait-matching with the long corolla spurs of *D. barbeyi*. Since we hypothesized that the experimental *Bombus* removal could have had indirect effects via alteration to the pollinator community, as shown by Inouye (1978), we also examined pairwise interactions between each fixed effect associated with the pollinator community and our manipulation. We included site, *D. barbeyi* plant, and *D. barbeyi* flower as nested random intercepts and year as a separate crossed random intercepts.

We compared the AIC values from models created from every combination of fixed effects that successfully converged. Since our model selection did not yield a single obvious "best" model, we then averaged the coefficients estimated by all models with $\Delta AIC < 2$ (Dormann et al., 2018; Grueber, Nakagawa, Laws, & Jamieson, 2011; Symonds & Moussalli, 2011) to draw general conclusions about the effect plant and pollinator community composition on *D. barbeyi* seed production.

4.4 Results

Over three study years and 14 paired control/manipulation survey periods, we counted 6,816 developing *D. barbeyi* seeds (control: 3,016; manipulation: 3,800) from 679 flowers (334; 345) on 439 unique plants (218; 221). Based on relative abundance during each control period, we removed four different species of bumble bees (*B. appositus*, *B. flavifrons*, *B. kirbiellus*, *B. nevadensis*; Table 4.2). As expected, the removal of the most abundant pollinator species

Table 4.1: Description of fixed effects included in the model selection framework.

Category	Variable Code	Variable description
Experimental	PR	Proportion of the *Bombus* community removed by our manipulation
Pollinator	BA	*Bombus* abundance, scaled to center at 3
Pollinator	PL	Proportion of the *Bombus* community with a long tongue
Pollinator	MF	Proportion of the pollinator community exhibiting floral fidelity during foraging surveys
Plant	PD	Relative abundance of *D. barbeyi* flowers
Plant	CL	Proportion of the floral community with the same color as *D. barbeyi* flowers (blue/violet)
Plant	CR	Proportion of the floral community with a mean corolla length that overlaps with the range for *D. barbeyi* nectar spurs ($10 - 18$mm)
Interaction	PR:BA	Interaction between the experimental removal and *Bombus* abundance
Interaction	PR:PL	Interaction between the experimental removal and the proportion of long-tongued bees in the community
Interaction	PR:MF	Interaction between the experimental removal and floral fidelity
Interaction	PR:PD	Interaction between the experimental removal and the relative abundance of *D. barbeyi*
Interaction	PR:CL	Interaction between the experimental removal and the proportion of the community with color similarity
Interaction	PR:CR	Interaction between the experimental removal and the proportion of the community with corolla-length similarity

resulted in a significant ($p < 0.001$) reduction in *Bombus* abundance when averaged across all sites (control: 66.7 ± 52.9 individuals; manipulation: 53.4 ± 30.1 individuals). For 11 of the 14 manipulation periods, the bumble bee species removed was a long-tongued species (i.e., all removals except for *B. flavifrons*); however, our manipulation did not have a statistically signifiant effect on the relative abundance of long-tongued bees across communities ($p = 0.76$). Though hummingbirds are common pollinators of *D. barbeyi* (C. F. Williams, Ruvinsky, Scott, & Hews, 2001), we observed no hummingbird visits during any of our study periods, likely in response to researcher presence in the plots.

4.4.1 Overview of *Bombus* removal experiment

4.4.2 Model selection and averaging

Model selection yielded 16 models within our threshold range of $\Delta AIC < 2$ (Table 4.3). Because there was no clear best model from this workflow, we performed model averaging on all 16 models using equal weights for all models (Dormann et al., 2018). This workflow yielded

Table 4.2: Summary of *Bombus* removal and *D. barbeyi* relative abundance by site. Tongue categories were long (4 species), medium (5 species), and short (2 species).

Site	Year	PD	PR	Species Removed	Tongue
Brush Creek Fork	2011	0.57	0.52	Bombus appositus	long
Deer Creek Gate 2	2011	0.14	0.36	Bombus nevadensis	long
Gothic Road Grate.2011	2011	0.17	0.46	Bombus appositus	long
Gothic Town	2011	0.44	0.30	Bombus appositus	long
Teocali View	2011	0.19	0.38	Bombus nevadensis	long
Back of Baldy Bend	2013	0.04	0.50	Bombus flavifrons	medium
Emerald Lake 2	2013	0.07	0.49	Bombus kirbiellus	long
Gothic Road 2	2013	0.09	0.60	Bombus appositus	long
Judd Falls TH 2	2013	0.19	0.48	Bombus flavifrons	medium
Past Rustlers	2013	0.17	0.43	Bombus flavifrons	medium
Brush Creek Sign	2014	0.48	0.66	Bombus appositus	long
Cold Springs Ranch	2014	0.71	0.62	Bombus appositus	long
Emerald Lake	2014	0.16	0.53	Bombus kirbiellus	long
Gothic Road Grate.2014	2014	0.23	0.52	Bombus appositus	long

the average model coefficients shown in Table 4.4. All seven main effects and four of the six interactions were included in one or more models in the best set. The two interaction terms that were dropped were the interaction between the removal and color-similarity (PR:CL) and the interaction between the removal and corolla-length similarity (PR:CR). For the main effects, each was present in at least half (8/16) of the best models, while for the interactions, each was present in at least two of the best models. Two fixed effects, both related to the plant community, were present in every model in the set of best models; these were the proportion of the community with flower colors (CL) and corolla lengths (CR) similar to *D. barbeyi*.

After model averaging, our model predicted seed production values for one manipulation period that were more than three times higher than any other survey period. To check whether some element of this site was abnormal and could have skewed our results, we repeated our model selection and averaging protocol with this site (both control and manipulation surveys) removed. This analysis yielded coefficients that were consistent with the model including all sites and plots that were nearly identical, so we chose to report our findings with all 14 sites included.

Table 4.3: Model selection output for models with $\Delta AIC < 2$. Variable names as in Table 1.

ID	AIC	delta	PR	BA	PL	MF	PD	CL	CR	PR:BA	PR:PL	PR:MF	PR:PD
1	6317.69	0.00		-0.29	-1.1		0.18	-0.45	1.46			3.72	
2	6317.99	0.30	3.39	-0.12	-1.52	3.44	0.51	-0.63	1.78	-3.77			
3	6318.00	0.31		-0.17	-1.64	2.58	0.58	-0.67	1.70				
4	6318.27	0.58	3.19			2.89		-0.49	1.58	-3.95			
5	6318.55	0.86	5.73	0.06	-3.01	3.65	1.96	-0.83	1.99	-4.9			-4.79
6	6318.71	1.02		-0.16	-1.8	2.77	0.57	-0.49	1.73		4.18		
7	6318.73	1.04		-0.3	-1.19		0.13	-0.27	1.47		4.06		
8	6318.88	1.19		-0.16	-2.45		1.46	-0.67	1.62			5.84	-3.99
9	6319.14	1.45	3.51		-1.49	3.38		-1.01	1.80	-3.94			
10	6319.28	1.59	3.35	-0.14	-1.38	3.2		-0.83	1.77	-3.79			
11	6319.44	1.75				2.1		-0.61	1.50	-3.62			
12	6319.50	1.81	3.07	-0.31	-0.69		-0.04	-0.37	1.46	-4.11			
13	6319.53	1.84		-0.19	-1.48	2.33		-0.90	1.68				
14	6319.54	1.85	2.97	-0.22		2.63		-0.25	1.55	-3.72			
15	6319.66	1.97	3.53		-1.62	3.59	0.53	-0.78	1.81	-3.9			
16	6319.68	1.99		-0.07	-2.74	2.32	1.64	-0.84	1.80				-3.42

Effect of *Bombus* removal

Contrary to our expectations, our model predicted an increase in the production of fertilized *D. barbeyi* seeds in response to our *Bombus* removal manipulation. Additionally, all three metrics describing *Bombus* community composition interacted with the manipulation to influence the production of fertilized ovules, though the effect of that manipulation generally depended on the relative abundance of the *Bombus* species that we removed. For sites at which the removed bumble bee species represented less than half of the overall *Bombus* community, all three variables related to pollinator community composition had modest negative impacts on the production of *D. barbeyi* seeds that were largely similar to the effects of each variable in the control setting. However, for sites at which more than half of the total bumble bee community was removed in our manipulation, we see dramatically different relationships between all three variables and the production of fertilized seeds (Figure 4.1). In these high-removal settings, overall *Bombus* abundance had a steeply negative effect, while the proportion of long-tongued bees and pollinator floral fidelity both had steeply positive effects (Figures 4.2, 4.3).

Table 4.4: Summary of model averaged from 16 $\Delta AIC < 2$ models. Variable names as in Table 4.1.

Variable	Estimate	Std.Error	Adjusted.SE	z-value	p-value
Intercept	-0.98	1.86	1.86	0.53	0.60
PR	1.74	2.01	2.01	0.87	0.39
BA	-0.13	0.28	0.28	0.49	0.63
PL	-1.34	1.37	1.37	0.98	0.33
MF	2.12	1.88	1.88	1.13	0.26
PD	0.45	1.00	1.00	0.44	0.66
CL	-0.61	1.14	1.14	0.54	0.59
CR	1.66	0.53	0.53	3.13	0.00
PR:BA	-3.86	0.98	0.98	3.94	0.00
PR:PL	0.53	1.43	1.43	0.37	0.71
PR:MF	1.63	2.24	2.24	0.73	0.47
PR:PD	-0.66	1.86	1.86	0.35	0.73

Bombus community composition

In addition to the interactive effects with *Bombus* removal described in the previous paragraph, all three of our pollinator community compositional variables were also identified as important additive predictors of *D. barbeyi* seed production. Pollinator floral fidelity exhibited a positive effect on *D. barbeyi* seed production consistent with previous work in this system (Brosi & Briggs, 2013). However, two other elements of the pollinator community that we also expected to be positively related to *D. barbeyi* reproduction did not show such effects in predictions by our averaged model. The total number of bumble bees in a community and the proportion of those bumble bees that were categorized as "long-tongued" both had negative effects on the production of fertilized seeds in our target plant when assessed as main effects.

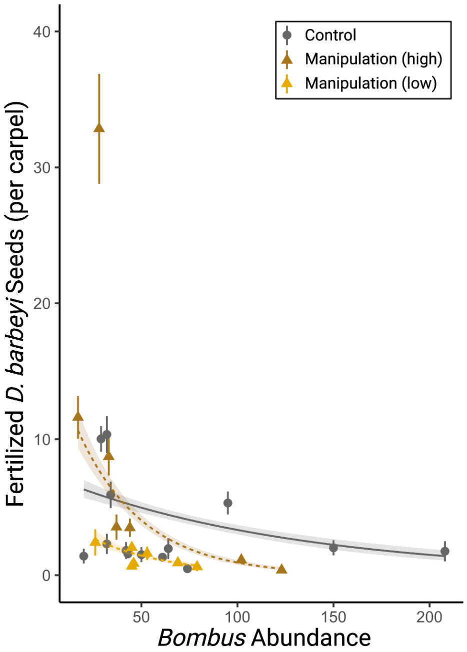


Figure 4.1: Effect of total *Bombus* abundance predicted by our averaged model. Points represent the mean for each survey period and bars represent 95% confidence intervals. Manipulation is plotted in the "high" bin if half or more of the overall *Bombus* community was removed and in the "low" bin if less than half was removed.

Plant community composition

Unsurprisingly, *D. barbeyi* in communities with a higher relative abundance of conspecifics predicted a higher number of fertilized seeds. We also saw an interaction between our experimental manipulation and the relative abundance of *D. barbeyi*, though this interaction was consistent with the overall positive effect of these conspecific flowers in the community. Specifically, sites at which more than half of the *Bombus* community was removed experienced the strongest benefit from high *D. barbeyi* relative abundance, though this variable was also beneficial in the control.

Morphological similarity between *D. barbeyi* and the rest of the floral community had contrasting effects on *D. barbeyi* seed production. *D. barbeyi* yielded more fertilized seeds in communities with a high proportion of flowers with similar corolla lengths, but fewer in communities with a high proportion of similarly colored flowers. This finding partially contradicted our expectation that floral similarity would result in lower reproductive function due to increased heterospecific pollen transfer as pollinators visit different species of flowers with similar morphological traits.

4.5 Discussion

In order to link ongoing biodiversity losses to likely functional outcomes in a way that reflects real-world dynamics—particularly for ecosystem functions that cross trophic levels—ecologists must integrate BEF theory and community ecology. In this study, we do so by examining how the plant and pollinator communities influenced the production of fertilized seeds in a focal plant species—*D. barbeyi*—following the experimental removal of a single *Bombus* species. Our model offers compelling evidence that the effect of a single species removal on *D. barbeyi* reproduction occurs via changes to the behavior and composition of the pollinator community. Three major sets of results stand out from our analysis. First, we found that some elements of pollinator community composition likely influenced the effect of our single-pollinator-species removals on the production of fertilized *D. barbeyi* seeds. Second, we identified patterns in pollinator foraging behavior that also affected reproductive function in this focal plant species. Finally, we found that the plant community exerted both competitive and facilitative effects on *D. barbeyi* reproduction, likely via access to insect pollinators that can transfer conspecific pollen. These findings demonstrate that the relationship between biodiversity and ecosystem function is not always easy to predict and can be influenced by community dynamics in important and unexpected ways.

4.5.1 Pollinator community composition

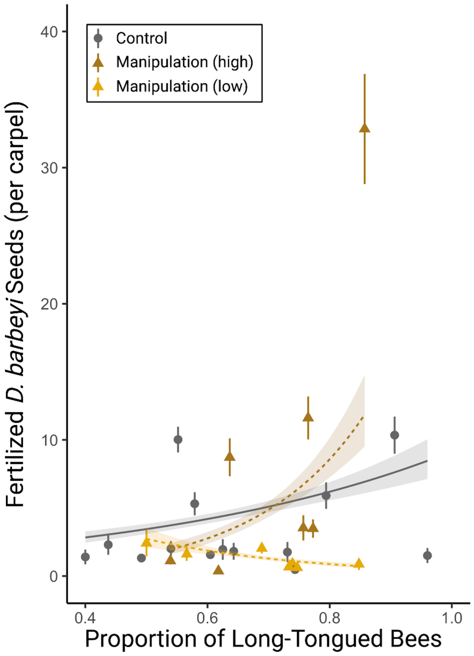


Figure 4.2: Effect of the proportion of the *Bombus* community categorized as "long-tongued," per expert descriptions. Points represent the mean for each survey period and bars represent 95% confidence intervals. Manipulation is plotted in the "high" bin if half or more of the overall *Bombus* community was removed and in the "low" bin if less than half was removed.

The composition of the bumble bee community impacted *D. barbeyi* reproduction, though these impacts did not follow our initial hypotheses. We had hypothesized that access to pollinators represented an important limitation on *D. barbeyi* reproduction and thus, that a community with more bumble bees would support a higher plant reproductive success. However, our averaged model predicted a *negative* effect of *Bombus* abundance on the production of fertilized *D. barbeyi* seeds. While this negative relationship was consistent across our study, it was steepest in our "high" removal scenario in which 50% or more of the overall bumble bee community was removed (Figure 4.1). Since we found a significant negative correlation between our manipulation and *Bombus* abundance, we believe that these negative trends are largely reflective of our removal. However, if correlation between these variables was the only driver of this effect, we would expect that our model selection would have simplified the output to include only one of these two variables. In addition, if the effects of our manipulation on bumble bee abundance were the primary driver of the results on seed production, we would expect to see a different pattern of abundance when examining only the data from the control state of each site (solid, dark gray line in Figure 4.1). Those data, however, are also consistent with a negative relationship of bumble bee abundance on seed production, albeit with a relatively small negative slope. Thus, while we expect that correlation with our manipulation explains a portion of the surprising effect of *Bombus* abundance, we presume that there are other biological processes also underlying this finding that we do not understand.

We also expected that trait-matching between long-tongued bumble bees and *D. barbeyi*'s nectar spurs would make the presence of these bees in a community an important predictor of reproductive function for this focal species. We were again surprised to see that this was not consistently true and that the proportion of long-tongued bees was only strongly beneficial under high-removal scenarios (Figure 4.2). This disparity in functional importance for long-tongued bees could have some relationship to the ability for shorter-tongued species to obtain nectar from these flowers if reduced visitation events allowed nectar to collect in the spurs (Inouye, 1978). Thus, visitation by non-long-tongued species could depend on the overall reduction in pollinators due to our manipulation, which could reasonably have varied depending on the strength of those removals.

The divergent effects of pollinator community composition in low- and high-removal scenarios are interesting as they suggest that the morphological traits and relative abundance of a pollinator jointly contribute to its functional role in a community. When a numerically dominant pollinator is removed from the community, we see that the functional role that is lost is best filled by long-tongued bees. However, in more even communities where the removed bee species represents less than half of the total community, long-tongued bees do not have such an important functional role. Frameworks for understanding BEF relationships that solely focus on functional traits or numerical abundance may therefore overlook important information about how community dynamics shape interspecific interactions.

4.5.2 Pollinator foraging behavior

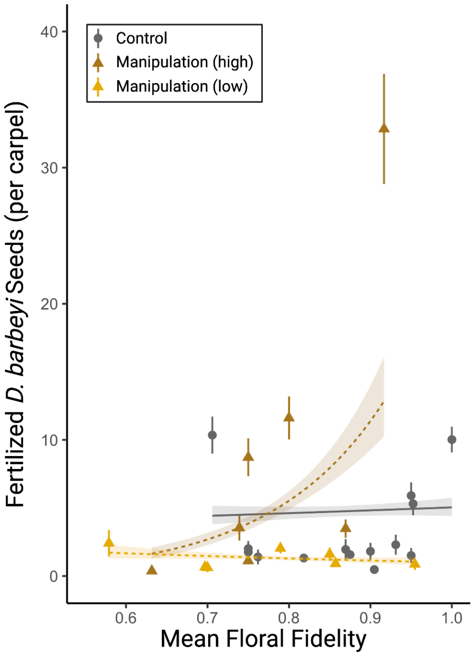


Figure 4.3: Effect of pollinator floral fidelity, averaged across all *Bombus* observed during the represented study period. Points represent the mean for each survey period and bars represent 95% confidence intervals. Manipulation is plotted in the "high" bin if half or more of the overall *Bombus* community was removed and in the "low" bin if less than half was removed.

In addition to having access to a pool of abundant and morphologically suitable pollinators, plant reproduction also requires these pollinators to engage in conspecific pollen transfer. Our model emphasizes the importance of pollinator foraging behavior—specifically floral fidelity—in promoting pollen transfer between plants of the same species. Floral fidelity has been previously identified as an important component of plant reproductive success in the context of plant-pollinator interactions (Amaya-Márquez, 2009; Brosi, 2016; Brosi & Briggs, 2013). Our work here is consistent with that past work, with our averaged model predicting a positive effect of site-level floral fidelity. Interestingly, the effect of floral fidelity is only strongly evident in our high-removal scenario (Figure 4.3). This result suggests that pollinator behaviors that support plant reproduction, like high floral fidelity, could functionally compensate for negative compositional effects, such as the loss of *Bombus* abundance caused by our experimental removal.

At five of our seven "high-removal" sites, *D. barbeyi* produced more fertilized ovules during the manipulation than they did in the control. Interestingly, these included the three sites with the highest seed production during the control period, which indicates that these unexpected trends could have been driven by other site-level variables that promoted the transfer or germination of conspecific pollen. For instance, environmental conditions like soil chemistry or precipitation could have influenced the success of *D. barbeyi* seed fertilization in such a way that overcame the loss of pollinators and floral fidelity at these sites. Other elements of both the plant and pollinator communities also likely influenced these seed production trends, as evidenced by our model selection results. Thus, we take these initial findings as evidence that plant reproductive function cannot be solely determined by pollinator loss or floral fidelity and must be considered in the context of other community-level trends.

4.5.3 Competition and facilitation in the plant community

Our model contains evidence of both competitive and facilitative interactions within the plant communities that we studied. We expected that morphological similarity within the floral community would promote competition among flowers for insect pollinators that are attracted to those traits. By potentially attracting the same pollinators, we surmised that morphological similarity woulfd increase the likelihood of heterospecific pollen transfer and thus, reduce ovule fertilization in our focal *D. barbeyi* individuals. Indeed, we observed a negative relationship between color similarity and *D. barbeyi* seed production that was consistent with our expectation of interspecific competition within the plant community. While many bees are attracted to blue/violet flowers (Briscoe & Chittka, 2001), they may not discriminate well between flowers of the same color. Pollinators may switch floral partners more frequently in the presence of equally attractive, similarly colored flowers and thus increase the chances for heterospecific pollen transfer throughout this floral community. However, we observed more fertilized *D. barbeyi* seeds at sites with a large proportion of other flowers with long corolla lengths, which contradicted our expectation of intraspecific competition on this axis of morphological similarity. Rather than competing for pollen vectors, plants in this community with similar corolla lengths may facilitate pollination of co-flowering individuals. Complex floral morphology like *D. barbeyi*'s long nectar spur may signal increased quantity and quality of floral rewards (Krishna & Keasar, 2018). On the community level, the prevalence of such a trait across multiple plant species may therefore appear more rewarding to pollinators, resulting in facilitation via the attraction of more pollinators to this community (Ghazoul, 2006; Hegland, Grytnes, & Totland, 2009). Pollinators attracted by this trait might also be better suited to forage upon these flowers and through this form of trait-based environmental filtering, provide more consistent pollen transfer services between conspecific individuals (Messier, McGill, & Lechowicz, 2010).

For these traits to truly facilitate plant reproduction, they must not only increase access to pollinators but also increase the overall transfer of conspecific pollen. Because we see a stronger positive effect of corolla-length similarity than we see a negative effect of color (via a larger model coefficient, in variables that are similarly scaled), we suspect that in this plant community facilitation plays a stronger role in plant reproductive function. Specifically, the cost of heterospecific pollen transfer from pollinators switching partners within a pool of similarly colored flowers may be outweighed by the benefits of increased visitation by pollinators that are well suited to transfer pollen between flowers with long corolla tubes (Peter & Johnson, 2008; Wei et al., 2021). Pollinators could also discriminate between similar-shaped flowers along another niche axis, like a flower's nutrient profile or display size, which would increase the likelihood of conspecific pollen transfer. These results also potentially indicate that pollination in this system is more limited by the availability of pollen vectors (pollinators) than of appropriate pollen donors (conspecific plants), which may limit the negative impact of conspecific pollen loss by unfaithful pollinators.

4.5.4 Future directions

As our experiments were conducted at the scale of individual days, we are limited in our ability to draw conclusions about the long-term effects of species loss on ecosystem function. Though our short-term approach had several notable advantages, particularly in ensuring nearly equivalent floral resources between control and manipulation states, facilitating non-destructive bumble bee removals, and allowing us to conduct multiple true replicates over each season, our conclusions could be strengthened by future single-species removal experiments conducted at longer timescales. We also focused our experiment on a single target plant species, which limits our ability to extend our conclusions to other flowering plants in this or other communities. Though the limitations of time and effort likely preclude the inclusion of every flowering plant at a site in such a study, an expanded experiment that examines the effect of pollinator removal on a subset of plants known to compete for pollinator visitation could provide valuable insight into how community context impacts BEF relationships in plant-pollinator communities.

4.6 Conclusion

Biodiversity loss is eroding the economic, social, and biological functions of ecosystems on a global scale (Cardinale et al., 2012; Oliver, Heard, et al., 2015; Oliver, Isaac, et al., 2015). Many important ecosystem functions and services are driven by relationships in which species richness at one trophic level impacts functional outcomes in other trophic levels. However, previous work on such systems has yielded inconsistent diversity-functioning relationships. We show here that in a study system comprising a single plant species visited by multiple pollinator species, single pollinator species removals (with the identity of that removed species changing among replicates) have negative functional impacts on seed production in our focal plant via the effect of this removal on the plant and pollinator communities. This work underscores the need to understand how community context interacts with diversity to mediate biodiversity-ecosystem functioning relationships.

Chapter 5

Conclusion

Plant-pollinator communities are at risk from countless global change pressures. In addition to limiting the times and spaces that plants and pollinators are likely to co-occur, ecological variation can also alter the patterns of interactions between plants and pollinators. Therefore, research into variation in plant-pollinator interactions is an essential element of any effort to conserve plant-pollinator communities. With this dissertation, I examined several important elements of plant-pollinator interaction variation. I explored direct and indirect pathways through which the environment and biological community in which plant-pollinator interactions take place mediate these crucial interactions. I showed that these trends exist at multiple spatial and temporal scales and demonstrated how the disruption of plant-pollinator interactions can translate into decreased reproductive function in the plant community.

The importance of pollination as an ecosystem service and the myriad threats that insect pollinators face make pollinator conservation a crucial task in the 21st century (Brown & Paxton, 2009; Ollerton et al., 2011). However, conservation policies focused on maintaining the ecosystem service of pollination must not only protect the pollinator species providing this service but also the conditions necessary for specific plant-pollinator interactions to occur. By identifying drivers of plant-pollinator interaction turnover and specific pathways for how these drivers may influence pollination, my work helps to identify key environmental and biological conditions necessary to conserve these plant-pollinator interactions.

Acknowledgements

There are so many people who have supported my doctoral journey. I have been extremely lucky to spend five summers at the Rocky Mountain Biological Laboratory and have received valuable logistical and intellectual support from the incredible staff there, especially Jennie Reithel, Ian Breckheimer, and billy barr. Innumerable field and laboratory assistants helped me to collect and analyze the vast amounts of data that went into these projects. In particular, I would like to thank Ashby Baker, Hunter Benkoski, Marcy Cathcart, Manogya Chandar, Sabra Dewey, Daniel Lahn, Therese Lamperty, Annie Schiffer, Caleb Sowers, and Kyra Woytek for providing key support for this work at RMBL. My time at RMBL would not have been the same without the camaraderie of Amelia Litz. So many former mentors helped me to find my wholehearted love of bees, but special thanks belong to Claire Kremen and Hillary Sardiñas for giving me my first taste of pollination research. Lauren Ponisio helped me to build the foundation upon which I have grown as a researcher, and I can trace so many of my research habits and preferences to my time in her lab.

The Brosi lab has been my intellectual home for the past five years, and every member of this group has helped me to become the researcher that I am now. Special thanks to Connor Morozumi, Xingwen Loy, and Donna McDermott for your peer mentorship when I was a brand new graduate student. Madeleine Strait has been not just a top notch labmate but also my fearless catsitter and fellow brewery explorer. Tori Reynolds has been a role model and dear friend whether she's in the next room over or all the way across the globe. Berry Brosi has been the best advisor I could have ever asked for. He has been endlessly compassionate and inspiring, and I hope to emulate his mentorship in my future academic roles. I could not imagine having done this with anyone else.

Finally, and most importantly, my husband Alek has been a constant source of support and encouragement. He has listened to me talk about bees more often than he ever could have expected. He has moved across the country with me four times in pursuit of my career. He has always believed in me and been a constant source of care and love throughout this journey. I love you so much and can't wait to keep telling you the new things I learn about bees.

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