IMPACTS OF SHARED POLLINATORS AND COMMUNITY COMPOSITION ON PLANT-POLLINATOR INTERACTIONS AND THEIR FITNESS CONSEQUENCES

A Dissertation Submitted to the Temple University Graduate Board

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by
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ABSTRACT

The myriad ways species interact with each other have always captivated biologists. These interactions—predation, competition, parasitism, and mutualism—are fundamental to the stability of ecological communities and drive the evolution of species they contain. Some mutualistic systems consist of mutually dependent partners that strongly influence each other's survival, while other mutualistic systems consist of many, diffuse relationships between large assemblages of partners. Critical ecological processes like pollination and seed dispersal are prime examples of such complex systems. Plant-pollinator communities are characterized by extensive pollinator sharing among plant species. My dissertation explores some of the consequences of this reliance on shared pollinators on the structure of plant-pollinator interaction networks, the foraging decisions of pollinators, and the fitness outcomes of plant species. Through several comprehensive field studies, I contribute to our understanding of mutualist interaction patterns at multiple levels of biological hierarchy: the community, species, and individuals. My first chapter examines the forces driving the change in interaction patterns of an entire plant-pollinator community and individual species throughout the flowering season. Nearly all studies of plant-pollinator interaction networks ignore potential intra-annual variation, and in doing so may be missing critical mechanisms contributing to overall community stability. I find that the overall turnover of interactions is high and driven by a process of interaction rewiring in which species frequently shuffle between available partners. Furthermore, I distinguish pollinator species whose interactions are driven by an abundance-based neutral process versus those that change their interactions beyond what is predicted by a neutral, abundance-driven null model.

My second chapter uses a network-based framework to consider the fitness consequences for plants participating in a diffuse plant-pollinator network. I analyze the relationship between plant species' network metrics and pollen deposition. Empirical examples that link patterns of interactions and functional outcomes (e.g., pollination) are scarce, but necessary to establish the utility of characterizing species interaction patterns. My final chapter explores how pollinator composition, local floral neighborhoods, and timing of flowering influence the pollination outcomes of individual *Oenothera fruticosa* flowers. I demonstrate extensive intraspecific variation in receipt of pollen from other species ('heterospecific pollen receipt') and find that this heterospecific pollen has a negative fitness effect if present in sufficiently high amounts. Together, the chapters of my thesis provide novel insights into the consequences of pollinator sharing among co-flowering plant species.

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CHAPTER 1

INTRODUCTION

It is difficult to overstate the importance of the plant-pollinator mutualism to natural and anthropogenic systems. Flowering plants and their pollinators contribute immensely to the generation and maintenance of biodiversity (Ehrlich & Raven, 2006; Thompson, 1999) human food security (Ollerton et al., 2011; Potts et al., 2016), and the stability of ecosystems (Lundberg & Moberg, 2003; Thebault & Fontaine, 2010; Valiente-Banuet et al., 2015). Furthermore, plant-pollinator mutualists have a long history of being model systems for coevolution (Thompson, 1999). There is great concern over the recent declines in pollinator populations (Potts et al., 2016) and insects more broadly (Hallmann et al., 2017), but also research that reveals remarkable resilience in plant-pollinator communities (Landi et al., 2018). Recent decades have seen a upswelling of interest in researching the patterns, dynamics, and ecological and evolutionary consequences of plant-pollinator species interactions (Knight et al., 2018). This dissertation explores just a few facets of this incredibly complex, beautiful, and essential mutualism.

Early studies of plant-pollinator mutualisms focused on highly specialized pairs or groups of species, often which conformed to simplistic pollination syndromes. Highly specialized plant-pollinator mutualisms were thought to be the rule, not the exception. However, several key studies lead to the growing understanding of plant-pollinator interactions as much more generalized than previously thought (Jordano, 1987; Waser et al., 1996). As the shift in scope of pollination research broadened, many researchers started to work at the level of entire communities as opposed to pairs of species. In the last two decades, studies began using network-based frameworks to describe community-level patterns of interactions (Bascompte et al., 2003; Olesen et al., 2007; Vázquez & Aizen, 2004). In these networks, species are represented as nodes and interactions between species are represented as links. Broad measures of community structure derived from webs of interacting species summarize patterns of interactions into quantifiable metrics, species network roles, and network diagrams (Vázquez et al., 2009). Features of mutualistic networks include: a core of generalists more likely to persist across years (Chacoff et al., 2018), nested subgroups of species with progressively more specialized partner choice (Bascompte et al., 2003), asymmetrical reliance of specialists on generalists (Vázquez & Aizen, 2004), and modules which may function as coevolutionary units (Olesen et al., 2007).

Antagonistic interactions are also common in plant-pollinator communities (Mitchell et al., 2009; Morales & Traveset, 2008). First, competition for pollinator visits between co-flowering plant species can occur. Second, pollinator species often compete amongst each other for floral resources (Brosi & Briggs, 2013). Finally, plant species can transfer pollen between species through the process of heterospecific pollen transfer. This exchange of pollen between plant species can be detrimental to the fitness of both the donor and recipient: the donor potentially loses male fitness, while the recipient potentially loses female fitness (Ashman & Arceo-Gómez, 2013; Morales & Traveset, 2008). Research into the causes, occurrence, and fitness consequences of heterospecific pollen transfer has surged in recent years (Moreira-Hernández & Muchhala, 2019). In particular, recent efforts have switched from experimental demonstrations of the impact of the timing (Bruckman & Campbell, 2016b; Caruso & Alfaro, 2000), diversity (Ashman & Arceo-Gómez, 2011), and magnitude (Moreira-Hernández & Muchhala, 2019), of HP receipt to patterns among and within species in entire plant-pollinator communities (Ashman et al., 2020). Heterospecific pollen transfer is a common phenomenon in plant communities and its intensity varies among and within species (Arceo-Gómez et al., 2016; Fang & Huang, 2012; McLernon et al., 1996; Montgomery & Rathcke, 2012; Tur et al., 2016). Furthermore, studies are beginning to explore the fitness consequences of receiving heterospecific pollen in natural systems (Briggs et al., 2016; Parra-Tabla et al., 2020; G. X. Smith et al., 2021; Suárez-Mariño et al., 2019).

Although plant-pollinator communities have garnered the attention and study of many researchers in recent years, much remains to be learned about these remarkable systems. In this dissertation, I explore several topics related to the widespread sharing of pollinators among flowering plants, the temporal turnover of plant-pollinator interactions, and the fitness consequences associated with patterns of pollinator visitation and positions in the plant-pollinator interaction network. My first chapter examines changes in interaction patterns of an entire plant-pollinator community and individual species throughout the flowering season and the forces driving them. This chapter demonstrates extensive intra-annual turnover of species interactions and explains patterns of pollinators' shifting foraging. Furthermore, I determine the relative importance of an abundance-based neutral process and other non-neutral ecological processes in the interaction plasticity of individual pollinator species. My second chapter follows up on this network-based approach and relates plant species' roles in the interaction network to functional outcomes: heterospecific and conspecific pollen deposition. First, I identify relationships between heterospecific and conspecific pollen within a subset of the plant species in my study community. Second, I demonstrate the value of network-based species metrics by linking such metrics with conspecific and heterospecific pollen deposition. My final chapter explores how pollinator composition, local floral neighborhoods, and timing of flowering influence the pollination outcomes of individual *Oenothera fruticosa* flowers. I demonstrate extensive intraspecific variation in heterospecific pollen receipt and find that this heterospecific pollen has a negative fitness effect if present in sufficiently high amounts. Together, this body of work provides novel insights into the structure of pollinators' interactions with a temporally variable plant community and the consequences of these interaction patterns for plant species and individuals.

CHAPTER 2

DRIVERS OF SEASONAL INTERACTION TURNOVER IN PLANT-POLLINATOR COMMUNITIES

2.1 Abstract

The patterns of interactions between pollinators and their plant partners can impact the persistence of species and stability of communities. However, the variability in plant-pollinator community interactions within seasons has largely been ignored. The extent to which species' interaction partners and interaction frequencies with these partners change across short time spans is unclear. Furthermore, the mechanisms responsible for such changes in interaction patterns remain unexplained. In this chapter, I use a series of time slice networks constructed at the scale of individual weeks of plant-pollinator interactions to assess the intra-annual turnover of species interactions. I show that interaction turnover in the plant-pollinator community is high between weekly networks (90.5% interaction turnover, on average) and driven by frequent shuffling of interactions between available partners, i.e., 'rewiring'. Furthermore, I find that interaction rewiring across time at the week-to-week time scale can be explained by a random process (i.e., explained by partner abundance) for approximately one third of pollinator species; but the remaining two thirds of pollinators' interaction shifts cannot be explained by simple abundance tracking alone. These results highlight the temporal volatility of species' interactions across relatively short periods of time and offer novel insights regarding the mechanisms responsible for plant-pollinator interaction plasticity within a season.

2.2 Introduction

Understanding the ecological processes that contribute to biodiversity and community stability is a fundamental goal in ecology (Oliver et al., 2015). Patterns of interactions between plant-pollinator mutualists can influence these processes by facilitating populations of rare species (Benadi & Pauw, 2018; Pedro J. Bergamo et al., 2020; Valdovinos et al., 2013) and strengthening communities' resilience to species loss (Kaiser-Bunbury et al., 2010; Tylianakis et al., 2010) and non-native introductions (Stout & Tiedeken, 2017). Network-based studies of plant-pollinator communities have revealed their overall interaction structure which includes a core of generalist species (Chacoff et al., 2018; Fang & Huang, 2012; Olesen et al., 2007) and asymmetrical dependence of specialists on generalists (e.g., Valdovinos et al., 2016; Miele et al., 2020), i.e., nestedness (Bascompte et al., 2003). Yet, there is a juxtaposition between the apparent stability of the emergent properties of plant-pollinator interaction networks and extensive variability in the individual actors and their interactions across time and space (CaraDonna et al., 2017; Olesen et al., 2007; Petanidou et al., 2008). The mechanisms underlying such interaction turnover, especially over short time spans, remain elusive (Olito & Fox, 2015; Poisot et al., 2012, 2015). Digging below the network surface is essential to identify temporal shifts of interactions between pollination partners, the ecological forces driving these shifts, and the effects of interaction plasticity on community stability and species persistence.

Recent work on plant-pollinator networks has highlighted extensive variability in partner interactions not only across years (CaraDonna et al., 2020) but across finer time scales within a growing season, and the frequencies at which they interact with each partner (Arroyo-Correa et al., 2020; CaraDonna et al., 2017; Olesen et al., 2008; Rasmussen et al., 2013; Simanonok & Burkle, 2014; Souza et al., 2021). This flexibility of interactions should play an important role in generating network structure and the stability it affords the community in the face of species loss, changes in partner relative abundances, or shifting phenologies in response to global climate change (Burkle et al., 2013; CaraDonna et al., 2017; Kaiser-Bunbury et al., 2010, 2011). Interaction turnover can be broadly partitioned into two components: changes among interaction partners caused by species entering or leaving the network (species turnover) and changes due to the switching of mutualistic partners despite both remaining in the network (interaction rewiring; Poisot et al. 2012). The relative importance of interaction rewiring will be greater when examining interaction turnover across shorter temporal scales (Swartz et al. 2020), although both will influence the plasticity of interactions within a season (CaraDonna et al., 2020; Trøjelsgaard & Olesen, 2016). At within-season time scales, species turnover reflects the limited activity periods of species. Seasonal changes are particularly dynamic in plant-pollinator communities, wherein plant species diverge in their start and end flowering times and pollinators have different active periods across a growing season (Elzinga et al., 2007; Olesen et al., 2008). Thus, as species' partners transition out of the network, they need interact with new available partners or cease activity themselves.

The mechanisms responsible for within-season interaction rewiring are less clear, but may be driven by both neutral and non-neutral processes, influenced by species' abundances, learned preferences, shifting nutritional needs, or intra/interspecific competition (CaraDonna et al., 2017; MacLeod et al., 2016; Simanonok & Burkle, 2014). Under a truly neutral foraging process, pollinators would interact randomly with all available plant species, and thus visit plant species directly in proportion to their relative abundance (Vazquez & Aizen, 2004). This random process alone can generate turnover of plant-pollinator interactions through time as plant species' relative abundances shift throughout the season. Alternatively, pollinators may deviate from such abundance-tracking if their diet breadth and foraging is influenced by non-neutral processes (Jordano et al., 2003). For example, adaptive foraging may result in more generalized pollinators visiting specialized, presumably rarer, plant species disproportionate to their abundance if those plant species offer higher per-visit rewards or if it reduces interspecific competition with other pollinators (Valdovinos, 2019). Evolutionary specialization of pollinators for specific partners can restrict a pollinator's diet breadth and reduce interaction plasticity throughout time (Armbruster, 2017; Maglianesi et al., 2014; Weinstein & Graham, 2017). However, specialized pollinators may exhibit substantial interaction rewiring if they sequentially specialize on different species throughout the season, for example by preferentially visiting plant species with the greatest relative abundance in a given time period (e.g., Tur et al., 2014; Szigeti et al., 2019). All of these mechanisms may be at play in the community, although the relative importance of neutral vs. non-neutral processes to interaction turnover in plant-pollinator networks is unclear.

Whether neutral or non-neutral processes drive interaction plasticity is important because these processes can have contrasting impacts on the stability of the community and the persistence of species (Valdovinos et al. 2019). If pollinators are simply tracking abundances, the most abundant plant species would attract most insect visits because just a few species tend to be hyper-abundant. Thus, there would be positive density dependence between plant species abundance and visitation rates, potentially destabilizing communities (Pedro Joaquim Bergamo et al., 2020). Similarly, rare, typically specialized (Fort et al., 2016), pollinator species would face intense competition from more abundant pollinator species for the most generalist plant partners on which they specialize. Abundance-based foraging may also be inefficient for generalist pollinators if they largely ignore rarer plant partners that may have higher quality or underutilized floral resources (Valdovinos et al., 2016). Under such non-neutral foraging, specialized plants would benefit because they would be visited at a higher per capita rate than they would under a neutrality-based process. Valdovinos et al. (2016) offer one explanation for this kind of foraging: generalist pollinators assess per-visit rewards and disproportionately visit specialized plant species that provide greater rewards. If generalist pollinators are selectively visiting specialized plants, rarer pollinators would face less interspecific competition for the most generalist plants and rare plant species would receive greater per capita visitation. Importantly, these visitation patterns could provide an advantage for rarer species in the community, promoting coexistence and the stability of the community.

In this study, I quantify within-season interaction turnover in a temperate plant-pollinator community and evaluate the extent to which shifting interaction patterns are consistent with neutral or non-neutral ecological processes. I construct nine, highly resolved quantitative interaction networks spanning a 17-week growing season and quantify the variation in structural properties of the networks, the amount of interaction turnover, and the contribution of species turnover and interaction rewiring to overall interaction turnover. Second, I determine whether the shifting interaction patterns of individual pollinator species are consistent with a neutral process (i.e., abundance-driven) vs. non-neutral ecological processes using randomization tests. To make inferences about whether the non-neutral foraging patterns seen in this network may promote plant species coexistence, I also quantify pollinator species' interaction diversity to describe their diet breadth. Together this study provides insight into the ecological mechanisms responsible for community interaction turnover, species interaction plasticity, and the generation of important overall interaction network structure.

2.3 Methods and Materials

2.3.1 *Study Site*

I conducted this study at Fort Indiantown Gap, an active National Guard training center, in Annville, Pennsylvania, USA. Approximately 88 ha of training areas and ranges on the base have been designated for researching the plant and animal species that thrive in periodically disturbed grassland habitats. This study focused on a 12ha grassland that contains a diverse assemblage of > 70 flowering plant species. Prior to the study, I established sixteen permanent transects across the grassland that were either 150 m x 2 m (n = 9 transects) or 100 x 2 m (n = 7), depending on where they were placed. All but three transects were located ≥10 m from the edges of the field and separated from each other by 10 m. The remaining three transects were placed along the field margins and were surveyed to incorporate flowering species that were common along edges. I captured plant-pollinator interactions along these transects every other week, beginning on May 14th, 2018 through September 7th, 2018, resulting in 9, 1-week long interaction networks.

2.3.2 ***Plant Abundance Surveys***

Plant abundance surveys were conducted on the first day of each new sampling period by walking each transect and counting the number of flowering individuals per species in each plot. For two species with dense patches of creeping colonial growth, *Securigera varia* and *Rubus flagellaris*, I estimated individual counts based on ground cover (1 square foot = 1 individual per species). Most plants were identified to species using a field guide (Newcomb, 1977), but a handful of plants could only be identified to genus (n = 5). Most notably, several *Solidago* species are present in the community, but readily hybridize and are difficult to distinguish in the field (Nesom, 1989).

2.3.3 ***Pollinator Observations***

I recorded plant-pollinator interactions using HD video cameras (Sony, San Diego, California, USA) during periods of pollinator activity (0800 – 1600) on warm, sunny days. Approximately 10 focal individuals per plant species per sampling period were selected to be filmed. I randomly chose individuals to film based on each species' spatial distribution in transects. I counted the number of flowers (or floral units, depending on inflorescence structure) on each filmed individual (mean ± SD; 24.5 ± 15.9 individuals/species) to later calculate flower number per plant per species. To film pollinator visits to focal plants, cameras were mounted on tripods placed approximately 1 meter away. Most films focused on one to several floral units, such as one *Cirsium* inflorescence or 1-3 *Oenothera* flowers, to optimize the clarity of flower visitors while capturing as many visits to an individual plant as possible. I filmed 62 flowering plant species throughout the season; several species detected in extremely low abundance (*Asclepias viridiflora, Cuphea viscosissmia, Impatiens capensis,* and *Polygonum sp.*) were not filmed. I filmed each species for a cumulative ~10 hours per sampling period. However, to account for variation in exact timing of the footage, I scaled interactions by the number of hours each plant species was filmed. Film was first processed using 'MotionMeerkat' software (Weinstein, 2015) to screen out periods of inactivity. Because not all flower visitors function as pollinators (King et al., 2013), I only considered visits where insects contacted anthers or stigmas as legitimate interactions and refer to these legitimate flower visitors as pollinators. I also excluded interactions involving some groups of flower visitors that are not typically involved in pollination (e.g., Hemipterans, spiders, and ants). Based on images captured during filming, I identified pollinators to the lowest taxonomic level possible by using field guides or consulting expert taxonomists (see Acknowledgements). I was able to identify pollinators representing the majority of interactions to species level (68.1%). Of the remaining interactions, I identified 18.6% to genus, 7.6% to family, and 5.7% to order. In total, these networks include 93 pollinator species plus 79 higher order morphospecies (hereafter, "species"; Appendix A).

2.3.4 *Network Construction and Metrics*

I constructed quantitative interaction networks for each of the 9 sampling periods using the 'bipartite' package (Dormann et al., 2009) in R (R Core Team, 2022). The interaction strength of each network link for quantitative networks was calculated as the observed interaction frequency divided by the number of hours a plant species was filmed during a given sampling period.

2.3.5 *Interaction Turnover*

I used a modified version of the ‘betalink’ package (Bartomeus, 2019) to quantify interaction turnover across pairs of networks following the framework of Poisot et al. (2012). This quantitative calculation is based on the Ruzicka distance coefficient (quantitative Jaccard index) and the following formula to calculate quantitative interaction turnover (Legendre, 2014).

$$\beta_{int} = \frac{A + B + C}{(2A + B + C)/2} - 1$$

Where B and C each represent the interaction frequencies of interactions unique to one of two adjacent sampling periods, and A represents the interaction frequencies of interactions common to both sampling periods. Values for this index range from 0, indicating perfectly overlapping interactions with no turnover, to 1, indicating entirely non-overlapping interactions with complete interaction turnover between two networks. β_{int} can be partitioned into two additive components: β_{st} and β_{rw} which represent the contributions of species turnover and interaction rewiring to overall interaction turnover, respectively. The rewiring component, β_{rw}, is calculated using the same generic formula as for β_{int}, above, but using only the interactions of species present in both adjacent sampling periods to determine *a, b,* and *c*. Then, the values of β_{int} and β_{rw} can be used to solve:

$$\beta_{st} = \beta_{int} - \beta_{rw}$$

β_{st} and β_{rw} can be interpreted as the relative proportions of overall interaction turnover attributed to species entering and exiting the network across the season and interaction rewiring.

2.3.6 *Null Model for Abundance-driven Rewiring*

To assess the extent to which network rewiring from the pollinator perspective is driven by changes in the relative abundance of open flowers per species across time, I modified the null model analysis of MacLeod et al. (2016). I focus on pollinators that (1) were present in the network for at least two consecutive sampling periods and (2) participated in at least 20 interactions in total across the season (MacLeod et al., 2016). These criteria resulted in inclusion of 54 pollinator species. First, I quantified each pollinator species' interaction dissimilarity across networks within the season using the Morisita-Horn dissimilarity index (Horn 1966). This dissimilarity metric represents the chance that a two randomly selected visits across two sampling periods will be to different plant species and is insensitive to varied sampling sizes (Barwell et al., 2015). Species with low Morisita-Horn dissimilarity values change interaction partners little throughout the season (and represent partner fidelity), while species with high values change partners frequently throughout the season (and represent rewiring between partners). Then, for each pollinator, I calculated the arithmetic mean of between-week dissimilarity index values to represent its mean interaction dissimilarity throughout the season.

To assess whether each pollinator species' interaction dissimilarity could be explained by an abundance-based neutral process, I used plant species floral abundances to conduct a null model randomization test. To calculate each plant species' floral abundance during sampling periods, I multiplied each species' individual abundance counts by the average floral units per individual. To create the null models, I first constructed separate matrices for each pollinator species with all plant species that flowered in the community at any time across the growing season represented as rows, each sampling period the pollinator is present represented as columns, and the number of interactions between that pollinator and each plant species during a given sampling period filling each cell. I then generated 1000 null networks for each pollinator species according to the following conditions: (1) constrain the number of observations for each pollinator species during each sampling period (column totals), (2) use the relative log floral abundances of each plant species that occurred during a given sampling period to weight interaction probabilities. These null model matrices represent expected interaction frequencies per pollinator if pollinators visited plants in proportion to their relative floral abundance. I use log transformed abundance data to weight pollinator interaction probabilities because pollinators are more likely to respond to coarse differences in floral abundance (e.g., Benadi and Pauw, 2018).

To create a confidence interval of dissimilarity values expected under my neutral model, I calculated the same Morisita-Horn dissimilarity for each pollinator's 1000 null networks. I score pollinators with mean dissimilarity values above the 97.5 percentile as having 'actively switched' between partners beyond simple abundance tracking and those below the 2.5 percentile as having ‘fidelity’ to partners (MacLeod et al., 2016). For species with mean dissimilarity values inside the 95% confidence interval, we cannot reject the null hypothesis and thus interpret them as ‘abundance tracking’ according to a random (neutral) process.

To further infer the ecological mechanisms, I separated pollinator species into three groups: those active switching, those remaining faithful, and those tracking abundance. Active switching can arise either when a pollinator visits rarer species at disproportionately greater rates or common species more than expected. The former situation would promote the maintenance of rare species, whereas the latter could lead to their exclusion. Similarly, different foraging behaviors can place a pollinator in the fidelity group; a pollinator may either be faithful to few species throughout the season or faithful to many species throughout the season. To distinguish among these possibilities, I calculated interaction diversity, as Shannon’s diversity index, for each pollinator species to determine their diet breadth. I conducted a one-way ANOVA to test for differences in interaction diversity among the three pollinator categories and use post hoc Tukey HSD tests for pairwise comparisons of these three groups to characterize their diet breadth and evenness of visitation to partners. I expect species that actively switch partners to have the highest interaction diversity because their interactions will incorporate many rarer partners. Species exhibiting fidelity to specific partners will interact with the fewest partners and have low values for interaction diversity.

All analyses, unless otherwise noted, were conducted using R 4. 1. 2 (R Core Team, 2022).

2.4 Results

2.4.1 *Community Structure and Interaction Turnover*

I captured 8986 interactions between 58 plant species and 172 pollinator species cumulatively across the nine plant-pollinator networks. There was near complete interaction turnover across sequential sampling periods (mean 90.5% ± 3.4% SD). On average, interaction rewiring accounted for 91.4% ± 3.6% SD of interaction turnover, varying from 83.6% to as high as 95.5% of interaction turnover across sampling periods. Consequently, the contribution of species turnover to overall interaction turnover was consistently low (mean 8.6% ± 3.6% SD).

2.4.2 *Pollinator Species' Interaction Patterns*

I was able to reject the null model of abundance-tracking for most (67%) pollinator species examined (Figure 2.1). For these species, changes in interactions across time were disproportionate to partner availability. Specifically, 17 species changed interaction partners more than expected (active switching) and 17 species changed their interactions less than expected (fidelity). We could not reject the null model of abundance tracking to explain changing interaction patterns for the remaining 37% of pollinator species.

I found significant differences in interaction diversity ($F = 13.92$, $df = 2$, $P < 0.001$) among pollinator groups (Figure 2.2).

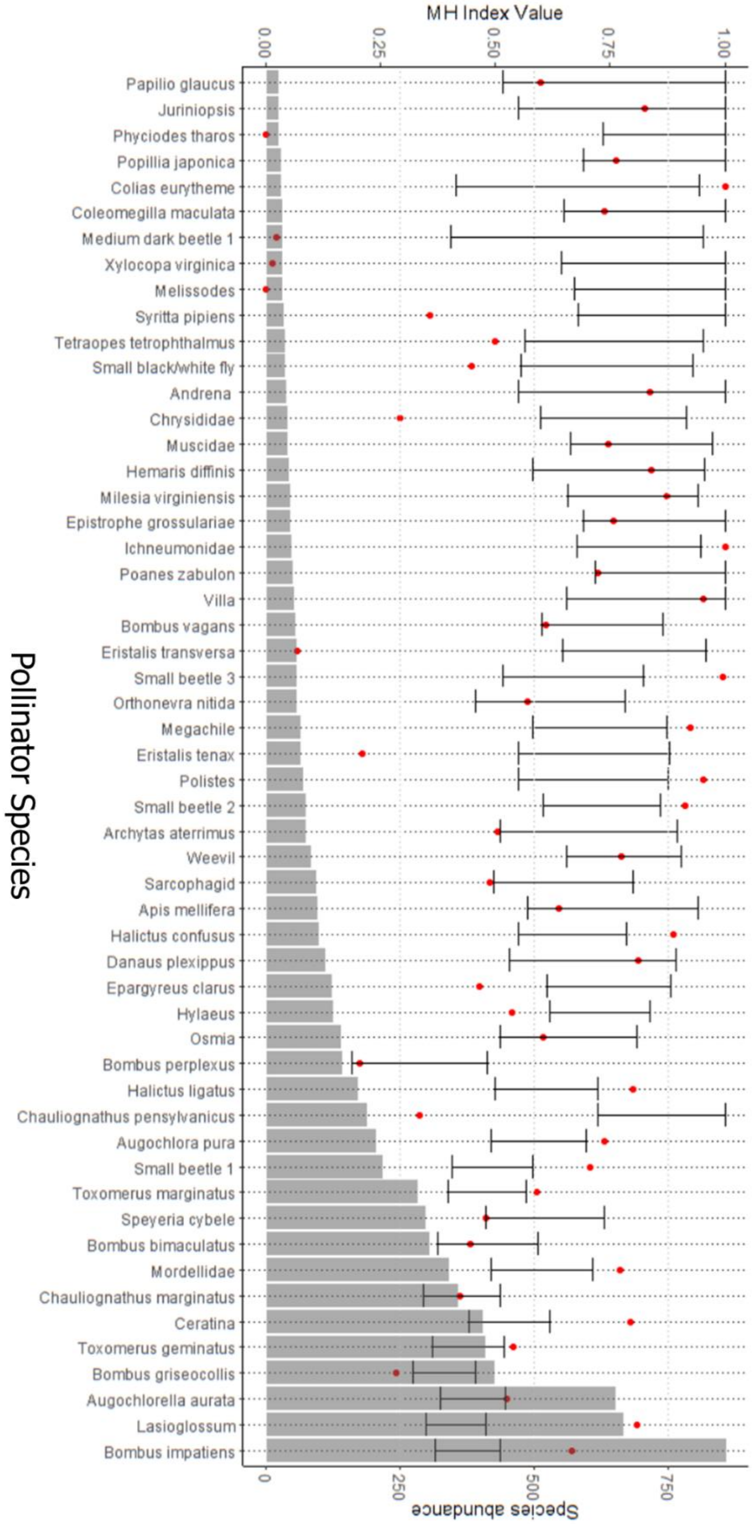
MH Index Value
0.00
0.25
0.50
0.75
1.00
Pollinator Species
Papilio glaucus
Juriniopsis
Phyciodes tharos
Popillia japonica
Colias eurytheme
Coleomegilla maculata
Medium dark beetle 1
Xylocopa virginica
Melissodes
Syritta pipiens
Tetraopes tetrophthalmus
Small black/white fly
Andrena
Chrysididae
Muscidae
Hemaris diffinis
Milesia virginiensis
Epistrophe grossulariae
Ichneumonidae
Poanes zabulon
Villa
Bombus vagans
Eristalis transversa
Small beetle 3
Orthonevra nitida
Megachile
Eristalis tenax
Polistes
Small beetle 2
Archytas aterrimus
Weevil
Sarcophagid
Apis mellifera
Halictus confusus
Danaus plexippus
Epargyreus clarus
Hylaeus
Osmia
Bombus perplexus
Halictus ligatus
Chauliognathus pensylvanicus
Augochlora pura
Small beetle 1
Toxomerus marginatus
Speyeria cybele
Bombus bimaculatus
Mordellidae
Chauliognathus marginatus
Ceratina
Toxomerus geminatus
Bombus griseocollis
Augochlorella aurata
Lasioglossum
Bombus impatiens
0
250
500
750
Species abundance

Figure 2.1 Morisita-Horn dissimilarity values and null model dissimilarity predictions for 54 pollinator species. For each species, red dots represent mean interaction dissimilarity, error bars indicate 95% confidence intervals from the abundance-based null mode, and grey bars show abundance.

Interaction diversity of actively switching species was significantly greater than that for species tracking abundances (Tukey HSD, active switcher – abundance tracking: P = 0.010, 95% CI = 0.084, 0.722) or remaining faithful (Tukey HSD, active switcher – fidelity: P < 0.001, 95% CI = 0.392, 1.06). Furthermore, interaction diversity of faithful species was significantly lower than that of species that tracked abundances (Tukey HSD, abundance tracking – fidelity P = 0.049, 95% CI = 0.035, 0.211).

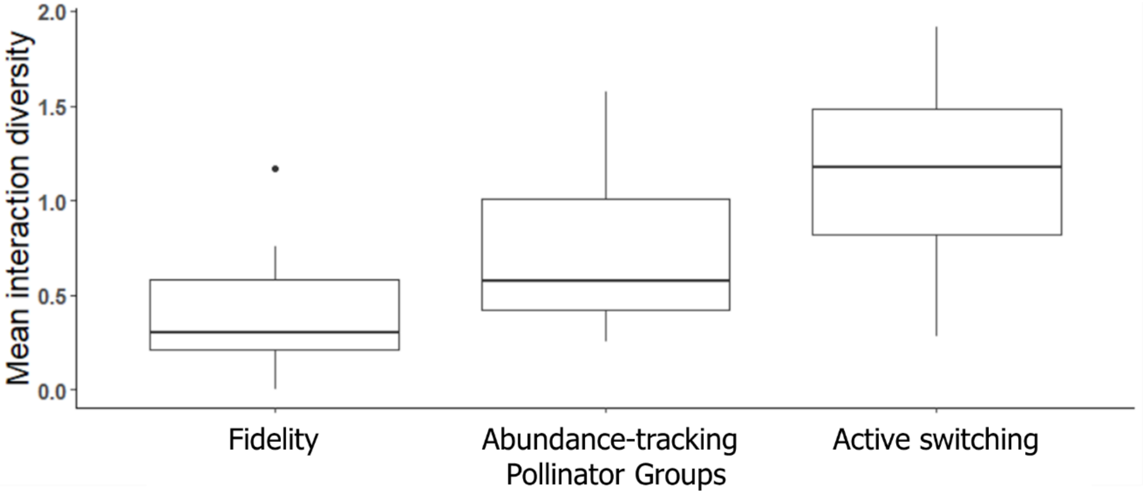

Figure 2.2 Boxplots of the interaction diversity for three groups of pollinators: showing fidelity (n = 17), abundance-tracking (n = 20), and active switching (n = 17). An ANOVA and Tukey HSD tests revealed that all three groups had significantly different values of interaction diversity.

2.5 Discussion

This study highlights the importance of within-season temporal dynamics in plant-pollinator interactions and the need to consider temporally variable interaction patterns to better understand the ecological mechanisms contributing to network properties. Specifically, I demonstrate substantial within-season interaction turnover within a plant-pollinator community—on par with interaction turnover found across years in other studies—and reveal that the high turnover over week-to-week time spans is primarily driven by interaction rewiring (Arroyo-Correa et al., 2020; CaraDonna et al., 2017; Noreika et al., 2019; Simanonok & Burkle, 2014; Souza et al., 2021). Furthermore, I show that changing interaction patterns of pollinators throughout the season are attributable primarily to non-neutral mechanisms that should reinforce networks stability.

I found that within-season, community-wide interaction turnover was high and driven by interaction rewiring with species turnover playing a reduced role, consistent with findings of other studies examining turnover across similar time spans within seasons (Arroyo-Correa et al., 2021; CaraDonna et al., 2017; Souza et al., 2021). Certainly, the relative importance of these processes will depend on temporal scale examined (CaraDonna et al., 2020). Indeed, in this dataset alone there is a positive linear relationship between temporal distance between networks and the contribution of species turnover to interaction rewiring between those networks ($t = 4.816$, $df = 34$, $p < 0.001$). All but one plant species present in the first sampling period had left the network by the third sampling period, resulting in a complete turnover of the interactions between sampling period one and three. Nevertheless, the high degree of partner shifting I've documented emphasizes foraging plasticity of pollinator species and highlights the generalized nature of plant-pollinator interactions.

What causes species to rewire their connections is an open question, in part because it is difficult to gather sufficiently thorough quantitative data on the frequencies of species interactions in addition to independent estimates of floral abundance per species, especially over short periods of time (Chacoff et al., 2012; MacLeod et al., 2016). I reveal that temporal shifts in floral abundances can account for shifting interactions in approximately one third the pollinator species I analyzed, such that interaction patterns for these species are indistinguishable from random encounters. Yet, this result also means that the interaction patterns of the two thirds are not simply a by-product of abundance tracking and underscores that pollinator foraging decisions are non-random. Instead, processes such as evolutionary specialization and adaptive foraging primarily shape the overall network (Hervías-Parejo et al., 2020; Junker et al., 2013; Valdovinos et al., 2016), emphasizing that a diffuse plant-pollinator network need not mean weak ecological interactions based primarily on partner availability.

The pollinator species that we determined remained faithful to certain partners through time had the lowest interaction diversity of the groups of species considered, suggesting these are specialists in the traditional sense, relying heavily on one or few specific plant partners consistently throughout their activity periods. In fact, this group includes pollinators already known to exhibit strong partner preferences, such as *Mellisodes desponsus* (*Cirsium* specialist), *Xylocopa virginica* (targets flowers for nectar robbing), and *Eristalis transcersa (Rudbeckia hirta* specialist). Although less common, another pattern of specialized interactions I observed was sequential specialization (Szigeti et al., 2019). For instance, *Bombus griseocollis* foraged almost exclusively on *Asclepias syrica* for 3 sampling periods (5 to 6 weeks), then switched to near exclusive foraging on *Monarda fistulosa* for 2 sampling periods (3 to 4 weeks) when the former was no longer available. The Great Spangled Fritillary, *Speyeria cybele*, also sequentially specialized on different three different *Asclepias* species across different sampling periods. This pattern of specialization on different partners throughout the season is necessary given the turnover of partners and highlights the importance of considering temporal dynamics of even specialized species.

On average, pollinators that actively switched foraging patterns had the greatest interaction diversity, indicating visits were more evenly distributed visits across many partners and consistent with greater per capita visits to rare species than expected. One possible explanation for this pattern of interaction plasticity is that these generalists are assessing floral rewards and adapt their foraging to maximize per visit resource intake (Valdovinos et al., 2016). This adaptive foraging of generalists has been suggested to promote biodiversity of plant-pollinator communities by facilitating the pollination of specialist, and most likely also rare, plants. Furthermore, if generalist pollinators focus on specialist plants, specialist pollinators also benefit from reduced interspecific competition for the generalist plants they symmetrically depend on (Valdovinos et al., 2013). Alternatively, competition between certain species might also drive abundant generalists to switch interactions throughout the season. Species who actively switch interaction partners had the broadest diet breadths which may be a response to interspecific competition between pollinators that forces species to visit less desirable partners (Inouye, 1977). For instance, Fründ et al. (2013) suggested that interspecific competition among bee species in experimental communities drove some species to forage from less desirable floral partners. However, the effects of interspecific competition between pollinator species for floral resources may vary when focusing on the interactions of individuals or entire populations (Brosi, 2016). Many species that I found switching to less abundant partners were highly abundant and a complementary explanation could be that intraspecific competition was driving individuals to widen their diet breadth (Heinrich, 1979; Tur et al., 2014). Finally, other factors such as species' morphological traits (e.g., Kaiser-Bunbury et al., 2014; Maglianesi et al., 2014) and sexually dimorphic foraging (G. P. Smith et al., 2019) can also contribute to the complicated shifting of plant-pollinator interactions across short time scales. Regardless, my results suggest that the interaction plasticity of abundant generalists may enable the persistence of rare species and provide stability to the plant-pollinator community.

I demonstrate the necessity to consider fine-scale temporal dynamics in the study of plant-pollinator interactions and suggest these dynamics make important contributions to overall network topology. In particular, the extreme plasticity of plant-pollinator interactions across very short time spans signifies a capacity of these communities to adapt to disturbances. Furthermore, the plasticity arising from the dynamic and putatively active foraging decisions of generalist pollinators may facilitate the pollination and persistence of rare plants and promote coexistence with specialized pollinators.

CHAPTER 3

LINKING SPECIES-LEVEL NETWORK ROLES TO FUNCTIONAL POLLINATION OUTCOMES

3.1 Abstract

In plant-pollinator communities with diffuse interactions, the widespread sharing of pollinators by flowering plants can provide fitness benefits but is also associated with costs. In particular, pollinator sharing can lead to frequent pollen exchange between plant species (i.e., heterospecific pollen transfer), which can depress individual fitness. Recent studies find great variability in heterospecific pollen transfer across species, but the factors determining which species suffer the greatest, the links between heterospecific pollen and conspecific pollen receipt, and how these relate to a given plant species' position in a diffuse plant-pollinator network is unclear. Indeed, studies are only just beginning to link pollination and fitness outcomes to network-based metrics used to characterize species' structural positions in plant-pollinator interaction networks. In this study, I examine the extent to which species' network position is related to fitness benefits (conspecific pollen receipt) and costs (heterospecific pollen receipt). I find strong evidence linking pollen receipt to network metrics, but interestingly that heterospecific and conspecific pollen receipt are associated with different aspects of plant species' network role. Consistent with this result, I also find that relationships between conspecific and heterospecific pollen within each of the 13 studied species are either absent or weak. This work presents novel evidence linking network roles to functional outcomes in plant-pollinator networks and furthers our understanding of the importance

of interaction network roles by associating such metrics with pollination outcomes in the field.

3.2 Introduction

Plant-pollinator relationships are important drivers of species' evolution, contribute to the maintenance of biodiversity, and represent an important ecosystem service (Ehrlich & Raven, 2006; Thompson, 1999; Winfree et al., 2015). Many studies have used a network-based framework to efficiently describe patterns of community-wide interactions and the interaction patterns of individual species in plant-pollinator communities (Knight et al., 2018). However, there is a paucity of empirical studies linking patterns of species interactions in a network-based framework to functional consequences (e.g., pollen deposition and seed production for plants) and their underlying mechanisms (Ashman et al., 2020). Thus, a fundamental gap remains in our understanding of community-wide plant-pollinator interactions.

The study of community-wide interactions between pollinators and plants has primarily been conducted via the observation pollinators visiting flowering plants. Different efforts have been made to distinguish "pollinators" that actually contact plant reproductive parts and transfer conspecific pollen ('CP'), from ineffective visitors (Ballantyne et al., 2015). This requirement and the observation that greater variability exists in interaction frequencies compared to variation in per-interaction effects (e.g., CP deposited per visit; (Sahli & Conner, 2006; Vázquez et al., 2005, 2012) have been used to justify the use of visits as an acceptable proxy of interaction outcomes. However, quantifying interactions based on visitation alone omits certain features of the quantitative nature of plant-pollinator relationships (Minnaar et al., 2019). For instance, the incorporation of single-visit deposition data and pollen collected from pollinators' bodies can reveal substantive differences in pollinator quality (Ballantyne et al., 2015; King et al., 2013) and affect overall network metrics (e.g., Ballantyne et al., 2017; Souza et al., 2021). Observations of pollinators visiting plants clearly provide informative data, but more studies are needed to explore the quantity of CP delivered per visit to better distinguish meaningful links from insubstantial ones (Vázquez et al., 2012). Furthermore, quantifying the amount of CP transferred during pollinator interactions crucially links patterns of visitation and pollination outcomes for plants.

From a plant's perspective, interactions with pollinators would ideally result in the deposition large amounts of pure CP loads, but the generalized foraging of most pollinator species makes this scenario unlikely (Morales & Traveset, 2008). Indeed, the receipt of pollen from another species, heterospecific pollen ('HP') receipt, is a common occurrence among co-flowering plants (Arceo-Gómez et al., 2016; Fang & Huang, 2013; McLernon et al., 1996; Tur et al., 2016). Although these studies provide evidence that HP receipt is widespread in communities, we still lack a thorough understanding of the magnitude of HP receipt among and within species (Arceo-Gómez et al., 2016, 2018; Ashman et al., 2020). Furthermore, little is known about what factors influence the diversity of HP received, HP load size, and the relationships between the accrual of CP and HP (Ashman et al., 2020). Nonetheless, the importance of HP receipt has been documented by extensive experimental studies that have demonstrated HP receipt can reduce fruit or seed production (Morales & Traveset, 2008). Furthermore, several recent field studies have found significant fitness effects associated with the receipt of HP (Briggs et al., 2016; Parra-Tabla et al., 2020; G. X. Smith et al., 2021; Suárez-Mariño et al., 2019), suggesting that HP receipt can be an influential process in wild plant populations. Research on the drivers of HP receipt and its relationship to CP receipt provide an important step towards understanding the consequences of HP receipt for plant species coexistences and trait evolution (Moreira-Hernández & Muchhala, 2019).

Even though patterns of CP and HP deposition are both related to pollinator activity, the extent to which CP and HP receipt are correlated is largely unknown (Ashman et al., 2020). Importantly, there may be different drivers of variation in CP vs. HP receipt. Pollinator composition may be of particular importance to both CP and HP receipt because pollinators are the proximate cause of pollen transferring between flowers (but see Parra-Tabla et al., 2020). However, the drivers of HP need not be related, as different pollinators may separately contribute to HP and CP receipt. It has been hypothesized that the receipt of HP is related to the frequency of visitation from pollinators and the quality of pollen loads they deposit (Arceo-Gómez et al., 2016; Ashman et al., 2020). For example, a positive linear relationship between CP and HP deposition would be expected if most pollinators carried similar proportions of HP. Nonlinear relationships are also possible if pollinators vary in the proportions of HP and CP they carry and in their visitation frequencies. Finally, there may be no, or only weak, relationship between HP and CP if HP is only brought by stochastic visits of poor pollinators. Other factors can also influence the variable receipt of CP and HP among species in a community including morphological traits, native status, and the composition of floral neighborhoods (Arceo-Gómez et al., 2016; Johnson & Ashman, 2019; Parra-Tabla et al., 2020; G. X. Smith et al., 2021). The prevalence of these patterns among co-flowering species remains unclear and studies linking pollinator effectiveness and evenness to pollen deposition are needed (Arceo-Gómez et al., 2016).

Because network metrics can be used to describe direct and/or indirect interactions between plant species and their pollinators, network metrics could predict CP and HP receipt. For instance, the number of direct relationships a species has with pollinators, measured as normalized degree in networks, indicates its degree of pollinator sharing and likelihood of interacting with shared pollinators bringing HP. Visitation by a more diverse assemblage of pollinator species may also bring more CP (Albrecht et al., 2012), although the relationship between pollinator richness and CP receipt remains unclear (Loy & Brosi, 2022). In one of the few studies linking species-level metrics to pollination or plant fitness, Arceo-Gómez et al. (2020) found that a plant species' specialization in the plant-pollinator interaction network was positively related to the amount of HP it received. While initially counterintuitive, this association makes sense in the context of nested networks because specialized plants will interact with a generalized pollinator partner. Measures of network centrality may also be positively related to HP receipt because centrality describes the length of direct and indirect paths of a species to all others in the network. Species that occupy central roles in the network likely share pollinators with many other plants and might receive higher amounts of HP. However, there is some evidence suggesting that occupying central roles can benefit reproductive output of plant species (Lázaro et al., 2020), so they may receive comparably low proportions of HP, even if they receive an increased number of HP grains. One potential difficulty in linking plant species' network metrics to fitness outcomes is that plant-pollinator networks are not static through time, even within a season, such that a species' role in the network itself changes (Miele et al., 2020; Mora et al., 2020). Consequently, resolving interaction networks and pollen receipt over relatively short portions of a season may be key to deciphering the functional outcomes of interaction networks for plant species.

In this study, I examine functional outcomes for plants in a plant-pollinator community and link these to species' network roles across time within a flowering season. I quantified CP and HP receipt across a flowering season for 13 species within the plant-pollinator networks characterized in Chapter 1. First, I examine the relationships between CP and HP receipt for each species and characterize which type of relationship (or lack thereof) is most common for species in this study community. Second, I test whether receipt of CP and HP are associated with fundamental species' network metrics (normalized degree, *d',* closeness centrality, and network role) in the plant-pollinator interaction network. Associations between these network metrics and pollen deposition will reveal their usefulness in relating patterns of pollinator visitation to pollen deposition.

3.3 Materials and Methods

3.3.1 *Study Site and Design*

This study was conducted at Fort Indiantown Gap, an active National Guard training center, in Annville, Pennsylvania, USA. Approximately 88 ha of training areas and ranges on the base have been designated for researching the plant and animal species that thrive in periodically disturbed grassland habitats. The study consists of two parts: (i) constructing plant-pollinator networks describing the plant-pollinator community in this grassland and (ii) qualifying and quantifying pollen loads per plant species in the network. For the former, I use the networks constructed in Chapter 1. Briefly, I observed plant-pollinator interactions and conducted plant abundance surveys along 16 permanent transects across a 12ha grassland every other week during the 2018 growing season. During plant abundance surveys, I also sampled stigmas from individuals in bloom along transects. In this study, I focus stigma collection from 13 plant species (Table 3.1) sampled between June 12th, 2018, through August 24th, 2018. In total, these data were collected over six, single-week sampling periods.

3.3.2 *Stigma Collection and Processing*

Stigmas were sampled from 13 flowering species along the six transects between June and August 2018. Because stigmas sampled later in the day would have more time to accumulate pollen, I sampled along transects in a randomly determined order, starting after 12:00 pm. During each sampling period, I collected stigma samples from unique

Table 3.1 Summary statistics for stigma samples of 13 plant species surveyed for patterns of pollen deposition. Table includes the coefficient of variation (CV) for conspecific (CP) and heterospecific pollen (HP), mean CP and HP per stigma sample, and mean proportion of HP per stigma.

Species	**Samples**	**CV of CP**	**CV of HP**	**Mean CP**	**Mean HP**	**Mean % HP**
Achillea millefolium	120	103.42	126.71	39.95	2.99	0.070
Anemone virginiana	70	199.98	201.86	64.87	2.61	0.039
Daucus carota	87	155.76	254.88	33.79	5.20	0.133
Erigeron annuus	78	155.65	217.02	35.16	2.29	0.061
Leucanthemum vulgare	31	119.52	297.88	36.84	2.94	0.074
Lotus corniculatus	35	129.64	199.22	327.08	1.59	0.005
Melilotus albus	63	286.30	235.90	29.38	2.56	0.080
Monarda fistulosa	49	147.89	177.83	4.09	7.87	0.658
Oenothera fruticosa	74	119.92	156.88	484.53	476.84	0.496
Penstemon digitalis	24	87.79	158.80	422.19	5.46	0.013
Prunella vulgaris	119	131.54	153.47	23.75	3.30	0.122
Rudbeckia hirta	154	127.45	258.79	88.91	8.01	0.083
Securigera varia	38	163.99	175.27	197.97	2.47	0.012

individuals approximately every 30-50 meters along transects. Because some species were rare, I sampled whenever they were encountered along transects. All stigma samples were taken from different individual plants and typically consisted of a single mature stigma, but approximately 10% of samples contained multiple stigmas from the same plant. Stigma samples were collected with clean forceps and immediately stored in microcentrifuge tubes containing 70% ethanol until processing. Depending on the thickness of species' stigmas, samples were either soaked in 8M NaOH to soften them or processed through acetolysis to completely dissolve the stigmatic material and stain the pollen grains (Kearns & Inouye, 1993). Afterwards, softened stigmas and pollen grains were stained with Fuchsin dye and mounted on slides with glycerol following standard procedures (Kearns & Inouye, 1993). I examined the slides with a compound light microscope (Nikon Optiphot-2; Tokyo, Japan) and counted all conspecific and heterospecific pollen grains on each sample, distinguishing HP from CP using a pollen reference library made from anther samples of the focal species. In total, I analyzed pollen deposition on 942 stigma samples collected from 13 plant species. I counted pollen grains on an average of 72.5 (range: 24 – 154) stigma samples per plant species across the entire season.

3.3.3 *Network Metrics*

I use the ‘bipartite’ package (Dormann et al., 2009) in R (R Core Team, 2022) to create binary and quantitative interaction networks and calculate species-level network metrics using interactions observed across all plant species in the community. I calculate the following species-level metrics to characterize each plant species’ network position and role. Normalized degree, a qualitative measure of the number of partners a species interacts with, normalized by the total number of species in the network. Specialization, *d’*, represents the deviation of a species’ partner use from interacting with species according to their abundance and ranges from 0 to 1, with larger values representing species’ preferences for partners disproportionate to their availability. Closeness centrality, a quantitative measure that reflects the proximity of a species to the center of a network based on the weighted shortest path of direct and indirect links between itself and all other species in the network. Finally, I characterized each species’ role in the network according to Olesen et al. (2007)'s framework. First, overall network modularity (Q) was calculated by running the DIRTLPAwb+ algorithm (Beckett, 2016) 100 times and selecting the iteration with the highest modularity value (Q). Next, each species' standardized within-module degree (z) and among-module connectivity (c) are computed and used to assign it a role based on whether z and c exceed critical values. A species with both measures below the critical values is considered a 'peripheral' species. Species with a c value above and a z value below critical values are termed 'connectors' because they are involved in interactions with species distributed across several modules. Species with a z value above and a c value below critical values are 'module hubs' which are highly connected within their module. Finally, species with z and c values both exceeding critical values are 'network hubs' because of their many connections within their module and connections to other modules. Species assigned the latter three roles, connectors, module hubs, and network hubs, are scored as having important network roles, while peripheral species are designated as having an unimportant role (Olesen et al., 2007). I note that the data used to build each network and the stigma samples were collected at the same time.

3.3.4 *Statistical Analyses*

I evaluated the relationship between HP deposition and CP deposition for each of the 13 study species. For these analyses, each stigma sample is a data point of both HP and CP counts per stigma. I considered both linear and nonlinear relationships in order to relate the shape of the pattern to different hypotheses about the ecological mechanisms driving HP and CP receipt (Arceo-Gómez et al., 2016). I conducted separate general linear models including a linear-response model (untransformed data), log-linear model (HP or CP is log-transformed), and log-log model (HP and CP are log-transformed). If multiple models were significant for a species, I present the model with the highest adjusted R^2 value. I added one to HP and CP grain number before log-transformation because there were zeros present in the dataset. These and all subsequent analyses were conducted in R 4. 1. 2 (R Core Team, 2022).

To evaluate the relationship between species' network position and pollen deposition, I used generalized linear mixed effect models. The response variables of CP, HP, and proportion of HP were calculated as the average value for each measure for each species during each sampling period. I use these models to test whether interaction network-based species-level metrics predicted the number HP and CP grains and proportion HP. For these analysis, all species are included in the same model because I am interested in asking the relevance of species network roles broadly, regardless of the specific traits of each species. All models included normalized degree, closeness centrality, and specialization (*d'*) as continuous predictor variables and whether a species occupied a structurally important network role as a categorical binary predictor variable (1 for yes, 0 for no). I included species identity and sampling period as random effects. I modeled HP and CP counts with a Poisson error distribution and log link function and modeled proportion HP as following a binomial error distribution with logit link function, weighted by total pollen count. I only included species for which I had ≥ 10 stigma samples during a given sampling periods. This resulted in a total of 45 data points representing 13 total species. These mixed model analyses were conducted using the ‘lme4’ package (Bates et al., 2015).

3.4 Results

3.4.1 *Among Species CP and HP Deposition*

CP and HP receipt were highly variable across species (CV of CP receipt range: 87.7 – 298; CV of HP receipt range: 127 – 298). The mean count of HP grains per stigma was generally low (for 12 of 13 species mean HP < 9), except for one species, *Oenothera fruticosa*, which received nearly 60-fold more HP than the next closest species (mean HP = 477). *O. fruticosa* also received the highest average CP per stigma (mean CP = 485), but this value was not as extreme (all other species mean CP = 73.5). The proportion of HP received per stigma was also highly variable among species (mean = 14.2%; range 0.48% - 65.8%).

For most species, I did not detect a significant relationship between the amount of CP and HP received (Table 3.2). However, I detected a significant relationship between CP and HP receipt for four of the 13 species (~30%). CP and HP receipt were linearly related in *Melilotus albus* (Figure 3.1A; t = 7.013, df = 61, p <0.001, R^2 = 0.437). This relationship remained significant (t = 2.001, df = 60, p = 0.049) when a single leverage point was removed, but became weak (R^2 = 0.047). For *Anemone virginiana*, the relationship was log-linear relationship (HP ~ logCP) (Figure 3.1B; t = 2.113, df = 68, p = 0.038, R^2 =0.048). Finally, two species had significant log-log relationships: *Oenothera*

Table 3.2 Results from linear and non-linear regressions between HP and CP receipt for 13 flowering species. Significant regressions are bolded. If multiple regressions were significant for a species, the relationship with the highest adjusted R^2 was used.

Species	Model	t value	df	P value	*Adj-R^2*
Achillea millefolium	HP ~ CP	1.089	118	0.278	0.002
Achillea millefolium	log(HP + 1) ~ CP	1.314	118	0.191	0.006
Achillea millefolium	HP ~ log(CP + 1)	0.084	118	0.933	-0.008
Achillea millefolium	log(HP + 1) ~ log(CP + 1)	0.539	118	0.591	-0.006
Anemone virginiana	HP ~ CP	1.536	68	0.129	0.019
Anemone virginiana	log(HP + 1) ~ CP	0.922	68	0.360	-0.002
Anemone virginiana	**HP ~ log(CP + 1)**	**2.113**	**68**	**0.038**	**0.048**
Anemone virginiana	log(HP + 1) ~ log(CP + 1)	1.515	68	0.134	0.018
Daucus carota	HP ~ CP	-0.206	85	0.837	-0.011
Daucus carota	log(HP + 1) ~ CP	0.301	85	0.764	-0.011
Daucus carota	HP ~ log(CP + 1)	0.123	85	0.903	-0.012
Daucus carota	log(HP + 1) ~ log(CP + 1)	0.663	85	0.509	-0.007
Erigeron annuus	HP ~ CP	1.046	76	0.299	0.001
Erigeron annuus	log(HP + 1) ~ CP	1.687	76	0.096	0.023
Erigeron annuus	HP ~ log(CP + 1)	0.681	76	0.498	-0.007
Erigeron annuus	log(HP + 1) ~ log(CP + 1)	1.099	76	0.275	0.003
Leucanthemum vulgare	HP ~ CP	0.289	29	0.775	-0.032
Leucanthemum vulgare	log(HP + 1) ~ CP	1.302	29	0.203	0.023
Leucanthemum vulgare	HP ~ log(CP + 1)	0.309	29	0.760	-0.031
Leucanthemum vulgare	log(HP + 1) ~ log(CP + 1)	0.467	29	0.644	-0.027
Lotus corniculatus	HP ~ CP	0.225	33	0.824	-0.029
Lotus corniculatus	log(HP + 1) ~ CP	-0.156	33	0.877	-0.030
Lotus corniculatus	HP ~ log(CP + 1)	0.473	33	0.639	-0.023
Lotus corniculatus	log(HP + 1) ~ log(CP + 1)	0.011	33	0.991	-0.030

Table 3.2 (continued)

Melilotus albus	**HP ~ CP**	**7.013**	**61**	**0.000**	**0.437**
Melilotus albus	**log(HP + 1) ~ CP**	**4.580**	**61**	**0.000**	**0.244**
Melilotus albus	**HP ~ log(CP + 1)**	**2.361**	**61**	**0.021**	**0.069**
Melilotus albus	**log(HP + 1) ~ log(CP + 1)**	**2.271**	**61**	**0.027**	**0.063**
Monarda fistulosa	HP ~ CP	0.176	47	0.861	-0.021
Monarda fistulosa	log(HP + 1) ~ CP	0.291	47	0.772	-0.019
Monarda fistulosa	HP ~ log(CP + 1)	0.093	47	0.927	-0.021
Monarda fistulosa	log(HP + 1) ~ log(CP + 1)	-0.751	47	0.456	-0.009
Oenothera fruticosa	HP ~ CP	1.828	72	0.072	0.031
Oenothera fruticosa	**log(HP + 1) ~ CP**	**2.187**	**72**	**0.032**	**0.049**
Oenothera fruticosa	HP ~ log(CP + 1)	1.716	72	0.090	0.026
Oenothera fruticosa	**log(HP + 1) ~ log(CP + 1)**	**2.937**	**72**	**0.004**	**0.095**
Penstemon digitalis	HP ~ CP	-0.466	22	0.646	-0.035
Penstemon digitalis	log(HP + 1) ~ CP	0.395	22	0.696	-0.038
Penstemon digitalis	HP ~ log(CP + 1)	-0.789	22	0.438	-0.017
Penstemon digitalis	log(HP + 1) ~ log(CP + 1)	0.183	22	0.856	-0.044
Prunella vulgaris	HP ~ CP	1.785	117	0.077	0.018
Prunella vulgaris	log(HP + 1) ~ CP	1.847	117	0.067	0.020
Prunella vulgaris	HP ~ log(CP + 1)	1.834	117	0.069	0.020
Prunella vulgaris	log(HP + 1) ~ log(CP + 1)	1.807	117	0.073	0.019
Rudbeckia hirta	HP ~ CP	1.177	152	0.241	0.003
Rudbeckia hirta	**log(HP + 1) ~ CP**	**3.591**	**152**	**0.000**	**0.072**
Rudbeckia hirta	HP ~ log(CP + 1)	2.218	152	0.028	0.025
Rudbeckia hirta	**log(HP + 1) ~ log(CP + 1)**	**5.414**	**152**	**0.000**	**0.156**
Securigera varia	HP ~ CP	-0.680	36	0.501	-0.015
Securigera varia	log(HP + 1) ~ CP	-0.525	36	0.603	-0.020
Securigera varia	HP ~ log(CP + 1)	-1.010	36	0.319	0.001
Securigera varia	log(HP + 1) ~ log(CP + 1)	-0.600	36	0.552	-0.018

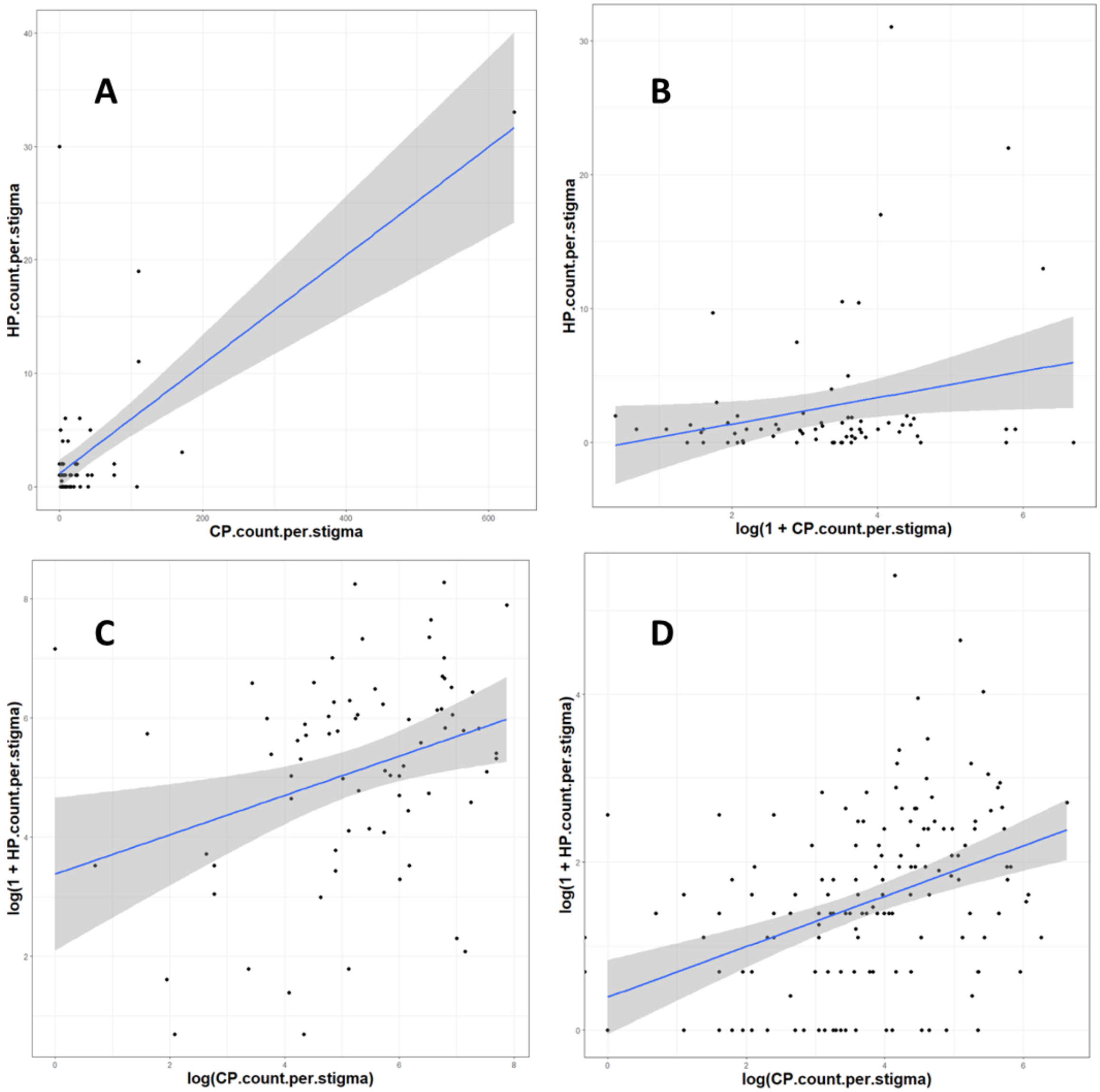

Figure 3.1 Significant linear and non-linear relationships between HP and CP receipt for four species: *Melilotus albus*, n=63 (A), *Anemone virginiana*, n=70 (B), *Oenothera fruticosa*, n=74 (C), and *Rudbeckia hirta*, n=154 (D). Blue lines represent regressions

fruticosa (Figure 3.1C; t = 2.937, df = 72, p = 0.004, R^2 = 0.095) and *Rudbeckia hirta* (Figure 3.1D; t = 5.414, df = 152, p < 0.001, R^2 = 0.156).

3.4.2 *Network Metrics and Pollen Deposition*

I found that several network metrics significantly predicted pollen receipt, but different metrics significantly influenced CP and HP receipt. Normalized degree and closeness centrality significantly predicted mean CP grains per stigma, whereas species' network role and specialization had no effect (Table 3.3). However, species' specialization significantly predicted mean HP receipt (both number and proportion of grains), with more specialized species receiving higher amounts of HP (Table 3.3). Species' network role, normalized degree and closeness centrality had no detectable influence on HP.

3.5 Discussion

Our study clearly illustrates the links between community-level network metrics and pollination outcomes and furthers our understanding of the functional outcomes of pollinator sharing among a diffuse set of mutualists. I further reveal that HP receipt is very common among co-flowering plant species, but that the magnitude of HP received varies greatly across species. Interestingly, CP and HP receipt were unrelated for most species. This result is echoed in the finding that different network metrics predicted HP between either HP and CP (see axes for transformations) and shading is 95% confidence intervals and CP receipt for species. I interpret these results in the context of how each network metric summarizes related, but different ecological processes.

Table 3.3 Results of GLMMs testing the effects of plant species' network role and position on pollen deposition. N = 45.

	Response variables											
	Proportion Heterospecific Pollen				Heterospecific Pollen Count				Conspecific Pollen Count			
Fixed effects	df	Estimate	χ^2 value	P value	df	Estimate	χ^2 value	P value	df	Estimate	χ^2 value	P value
Module Role	1	-0.225	0.863	0.353	1	-0.232	0.832	0.362	1	0.023	0.041	0.840
Specialization	**1**	**1.781**	**9.083**	**0.003**	**1**	**1.493**	**5.441**	**0.020**	1	-0.114	0.162	0.688
Normalized degree	1	-2.163	1.272	0.259	1	-1.131	0.434	0.510	**1**	**-2.785**	**9.685**	**0.002**
Closeness centrality	1	-6.472	0.613	0.434	1	5.660	0.420	0.517	**1**	**21.338**	**26.173**	**<0.001**

The patterns of incidence and intensity of HP receipt in this study concur with findings of other studies characterizing the community-wide HP receipt in plant-pollinator communities (Fang & Huang, 2013; McLernon et al., 1996; Parra-Tabla et al., 2020; Suárez-Mariño et al., 2019; Tur et al., 2016). I found that HP receipt was a common phenomenon (76.8% of stigma samples contained HP), but the overall intensity of HP receipt was low (median = 2 HP per stigma). This widespread low-intensity deposition of HP is likely a byproduct of the generalized nature of species relationships in plant-pollinator communities (Waser et al., 1996). Nearly all plants are expected to be visited by pollinators that are shared by other species, so some level of HP receipt is unavoidable. Interestingly, I find that one species, *Oenothera fruticosa*, receives a tremendous amount of HP (mean = 476.8 HP pet stigma; 46.68% HP per stigma). Other surveys of HP deposition among species similarly find that most species receive relatively low averages of HP grains, but a few species receive very high amounts of HP (Fang & Huang, 2013; McLernon et al., 1996; Suárez-Mariño et al., 2019). This divergence in patterns of HP receipt may reflect species' different adaptive strategies to deal with HP receipt. Specifically, species may evolve to either avoid HP receipt, or tolerate HP receipt's deleterious effects (Ashman & Arceo-Gómez, 2013). Fang et al. (2019) found that species tend to receive consistent amounts of HP across years, suggesting that some species have effectively adapted to avoid HP receipt, while others consistently receive high amounts of HP receipt. Indeed, Smith et al. (2021) found similarly high levels of HP deposition (mean ± SD; 319.9 ± 362.8) for *O. fruticosa* at the same site a year after this study. Certain floral traits such as small and concealed stigmas, restrictive floral morphologies, and shorter styles may facilitate more precise pollen deposition enable the avoidance of HP (Arceo-Gómez & Ashman, 2014; Ashman et al., 2020; Montgomery & Rathcke, 2012). Whereas species adopting a tolerance strategy might benefit from larger stigmas and styles – to reduce pre-fertilization effects of HP receipt (Galen & Gregory, 1989; Randall & Hilu, 1990) – or larger floral displays to increase pollinator visitation and CP receipt to neutralize the detrimental impacts of HP (G. X. Smith et al., 2021).

Conspecific pollen and HP receipt were ostensibly independent in the majority of species I studied. Even for those with positive relationships, the relationships were weak in all but one case: *Melilotus albus.* These results suggest that variation in the fitness benefits and costs of shared pollination are driven by different processes (Arceo-Gómez et al., 2016; Ashman et al., 2020). Such a scenario might be due to HP being brought by haphazard visits from poor pollinators that bring mostly HP, while CP is brought by more specialized pollinators bringing nearly pure CP loads. For example, I previously demonstrated that HP pollen receipt remains steady across the season for *O. fruticosa*, whereas CP receipt changes (Smith et al., 2021; Chapter 4). My results contrast with the findings of Arceo-Gómez et al. (2016) which found that most species had either positive linear relationships or exponential decreasing relationships between HP and CP receipt. I acknowledge that the number of stigmas sampled in total for some of the study species may have been too low to detect relationships, especially weak ones. It remains unclear how many stigmas are necessary to characterize HP-CP deposition relationships (Arceo-Gómez et al., 2018), but the variability of HP and CP received by individual flowers suggests larger sampling efforts are necessary (Arceo-Gómez et al., 2016). My sampling effort varied between species (mean ±SD, 72.5 ± 39.4 stigmas), but was comparably high compared to some other community-wide surveys of HP receipt (Fang & Huang, 2013; McLernon et al., 1996). Nevertheless, the widespread independence of HP and CP receipt is consistent with the finding that HP and CP receipt are best predicted by different aspects of a species' role in the plant-pollinator network.

Normalized degree, the proportion of partners a species interacts with in a network, and closeness centrality, species proximity to the center of the network, significantly influenced CP receipt on average across species. Closeness centrality has previously been associated with increased plant species reproductive output (Lázaro et al., 2020). Furthermore, studies that created individual-based networks find that plant individuals occupying central roles had higher fitness (Arroyo-Correa et al., 2021; Gómez & Perfectti, 2012). These studies have hypothesized that more central plant species and individuals are visited by a greater number of pollinator species which leads to better pollination and subsequent reproduction. While I find a significant, positive relationship between normalized degree and closeness centrality (r = 0.559, df = 43, P <0.001), I find a negative effect of ND on CP receipt. One possible explanation is ND is a binary measure of partner usage and closeness centrality incorporates link weights. Thus, a species with high normalized degree may have many weakly connected partners, but higher values of closeness centrality depend on having stronger relationships (i.e., more frequent) with more pollinator species.

In contrast, specialization influences HP receipt. The positive relationships between plant species specialization and the amount and proportion of HP received were also found in the only other study that tested for relationships between network metrics and HP deposition (Arceo-Gómez et al., 2020). One explanation for this effect is that specialist species tend to interact with generalist partners in plant-pollinator communities because interactions follow a nested pattern (Bascompte et al., 2003). Thus, a single generalist pollinator visits multiple specialized plant species and functions as their primary pollinator. Consequently, these generalist pollinators may carry more diverse pollen loads because they are visiting many different species. Forager constancy (Waser, 1986) should reduce the amount of pollen transferred between plant species as individual pollinators of a generalist species consecutively visit specific plants (Goulson, 2003; Tong & Huang, 2016; Tur et al., 2014). However, this consistent foraging behavior is not absolute and individual foragers often inspect other floral resources to assess their quality (Pyke, 1978). Other studies have found the opposite trend of increasing HP load size and diversity with increased generalization (Arceo-Gómez et al., 2016; Fang & Huang, 2013), so further investigations of the relationships between plant species specialization and HP receipt are needed.

I illustrate how different aspects of species' roles in a plant-pollinator network can uniquely affect benefits and costs of participating in a shared, diffuse mutualism. These results can also explain in part why HP and CP receipt are only loosely related for species in this study community. Thus, plant species may rely on specific important pollinators for bringing CP, while others may be responsible for the deposition of HP. Future studies should also consider how species' network position relates to functional outcomes to further validate the importance of interaction network metrics. In sum, this study helps close the gap in our knowledge between observations of plant-pollinator interactions and pollination outcomes.

CHAPTER 4

CAUSE AND CONSEQUENCES OF HETEROSPECIFIC POLLEN RECEIPT IN *OENOTHERA FRUTICOSA*

4.1 Abstract

Heterospecific pollen transfer, the transfer of pollen between species, is common among co-flowering plants, yet the amount of pollen received is extremely variable among species. Intraspecific variation in heterospecific pollen receipt can be even greater, but we lack an understanding of its causes and fitness consequences in wild populations. I examined potential drivers of variation in heterospecific pollen receipt in *Oenothera fruticosa*. I evaluated the relationship between heterospecific and conspecific pollen receipt and considered how visitation by different pollinator groups, local floral neighborhood composition, and flowering phenology affect the total amount and proportion of heterospecific pollen received. Finally, I tested whether variation in heterospecific pollen receipt translated into lower seed production. Heterospecific pollen is ubiquitous on *O. fruticosa* stigmas, but the amount received is highly variable and unrelated to conspecific pollen receipt. Heterospecific pollen receipt depends on pollinator type, the proportion of nearby conspecific flowers, and flowering date. Significant interactions reveal that the effects of pollinator type and neighborhood are not independent, further contributing to variation in heterospecific pollen. Naturally occurring levels of heterospecific pollen were sufficient to negatively impact seed set, but large amounts of conspecific pollen counteracted this detrimental effect. Although selection could act on floral traits that attract quality pollinators and promote synchronous flowering in *O. fruticosa*, the risk of heterospecific pollen is equally dependent on local

floral context. This work highlights how extrinsic and intrinsic factors contribute to intraspecific variation in heterospecific pollen receipt in wild plants, with significant fitness consequences.

4.2 Introduction

Over 80% of angiosperms rely on animal pollinators for successful pollination (Ollerton et al., 2011). Yet because most plants share pollinators with several to a few dozen other co-flowering species (Jordano, 1987; Waser et al., 1996), this success can be compromised by pollinator-mediated competition among plant species (Brown et al., 2002; Lopezaraiza-Mikel et al., 2007). Plants can compete directly for visitation by shared pollinators (Waser, 1978) or indirectly when shared pollinators transfer pollen between plant species, termed heterospecific pollen transfer ('HPT'; Morales and Traveset, 2008). HPT is extremely common in co-flowering communities, and its incidence and intensity vary markedly across species (Arceo-Gómez et al., 2016; Fang & Huang, 2013; McLernon et al., 1996; Tur et al., 2016), with implications for the evolution of floral morphology, species divergence, and community structure (Moreira-Hernández & Muchhala, 2019). Even as our understanding of the forces driving such interspecific variation grows (Arceo-Gómez et al., 2016; Ashman & Arceo-Gómez, 2013), investigations into the extent of variation in HPT within species and its underlying causes have scarcely begun (Ashman et al., 2020; Moreira-Hernández & Muchhala, 2019). Indeed, few studies sample thoroughly enough to comment on within-species variation in HPT (Arceo-Gómez et al., 2018). Strikingly, one study indicates that intraspecific variation in stigmatic loads of heterospecific pollen (‘HP’) can be greater than interspecific variation in HP loads (Arceo-Gómez et al., 2016).

Most plant species are visited by a suite of generalist pollinator species (Waser et al., 1996). Indeed, studies examining the interactions within entire plant-pollinator communities reveal broadly generalized interaction networks (Bascompte et al., 2003; Vázquez & Aizen, 2004). Visitation by the entire suite of pollinators need not be distributed evenly among individuals of a given plant species; instead, individuals—and even individual flowers—will be visited by some subset of pollinators (Bruckman & Campbell, 2014; Kuppler et al., 2016; Tur et al., 2014), contributing to intraspecific variation in pollination success. The effectiveness of any given pollinator depends in part on its morphology (e.g., body size), foraging behavior, and its visitation frequency (Ballantyne et al., 2015; King et al., 2013; Koski et al., 2018; Page et al., 2019; Sahli & Conner, 2007). Differences in these factors result in the common observation that floral visitors are not equally desirable as pollinators with respect to conspecific pollen (hereafter, ‘CP’) deposition (Bruckman & Campbell, 2014; King et al., 2013; Koski et al., 2018). Pollinators are also known to vary in the quality of pollen they deliver (Herrera, 1989), but pollinator effectiveness with respect to HP deposition is less studied. Furthermore, fidelity to a given plant species within a foraging bout (floral constancy; Waser, 1986) is not necessarily widespread among pollinator taxa (Amaya-Márquez, 2009; Gross, 1992). Consequently, pollinators should also vary in the amount of HP and/or the proportion of HP relative to CP they transfer. In fact, the relationship between

HP and CP deposited within species may be driven in part by pollinator assemblage (Arceo-Gómez et al., 2016).

The probability of HP receipt for any given plant might also be a problem of circumstance, specifically a plant’s local neighborhood. Greater numbers of conspecific individuals within a local neighborhood are often associated with greater CP receipt (Ghazoul, 2005), at least up to a point (Benadi & Pauw, 2018), and can be more important than population-level abundance measures in determining reproductive success (Roll et al., 1997; Spigler & Chang, 2008). However, the impacts of co-flowering species in local floral neighborhoods on HP receipt are unclear (Cariveau & Norton, 2009; Charlebois & Sargent, 2017; Ha & Ivey, 2017; Morales & Traveset, 2008). For example, co-flowering neighbors may facilitate pollinator visitation (Mitchell et al., 2009; Morales & Traveset, 2009; Tur et al., 2016), but negatively affect the purity of pollen loads transported (Bell et al., 2005; Brown et al., 2002), leading to a positive relationship between HP and CP receipt (Thomson et al., 2019). However, precisely because pollinators diverge in foraging patterns and those patterns are often governed by the abundance and distribution of flowering plants at small spatial scales (Feinsinger et al., 1991; Ghazoul, 2005; Goulson, 2003), the impact of the neighborhood on HP receipt likely varies with pollinator type.

HP receipt might also change predictably across the flowering season or be stochastic. During the flowering period of a given species, large scale changes in the diversity, relative abundance, and composition of plant species in the flowering community typically occur (CaraDonna et al., 2017; Kantsa et al., 2018). Meanwhile, the flowering schedules of individual plants are not evenly distributed across the season, and early versus late flowering plants could have different pollination outcomes (Chen et al., 2017; Kitamoto et al., 2006; Stone et al., 1998). In addition, synchronous flowering of individuals within a population can be positively associated with successful pollination because it increases the absolute number of available mates and can boost signals to pollinators against the backdrop of the flowering community (Bartkowska & Johnston, 2014; Elzinga et al., 2007). If this results in pollinators temporarily specializing on a given species during its peak flower, individuals may not only receive more CP but less HP. On the other hand, if HP receipt is steady or stochastic as a consequence of the background ‘noise’ of the community, then the *proportion* of HP may instead change across the season with variable CP deposition. Currently, we know little about intra-seasonal variation in HP receipt and the relationship between HP receipt and flowering phenology.

Interest in HPT is driven in large part because it is anticipated to have major fitness consequences for plants, though current evidence is equivocal, revealing a mixture of negative and neutral fitness effects (Ashman & Arceo-Gómez, 2013; Morales & Traveset, 2008; Moreira-Hernández & Muchhala, 2019). Most examples of costs associated with HP receipt are from experimental hand pollinations of flowers with mixes of CP and HP (Moreira-Hernández & Muchhala, 2019) to test and isolate the effects of factors such as the arrival of HP relative to CP (Bruckman & Campbell, 2016b; Caruso & Alfaro, 2000), HP diversity (Ashman & Arceo-Gómez, 2011), and HP donor identity (Arceo-Gómez et al., 2019; Arceo-Gómez & Ashman, 2016). Fewer have investigated the fitness consequences of naturally occurring levels of HP receipt in wild populations (Briggs et al., 2016; Parra-Tabla et al., 2020; Suárez-Mariño et al., 2019). The documentation of potential HP impacts on pollen tube development and extreme within-species variability in HP receipt (Arceo-Gómez et al., 2016, 2018; Briggs et al., 2016; Fang et al., 2019) suggests an opportunity for natural selection on traits that better allow plants to avoid HP receipt, but only if such variation is associated with fitness differences in natural populations.

In this study, I examined variation in HP receipt and its potential causes and consequences in the perennial *Oenothera fruticosa* L. (Onagraceae). Specifically, I asked the following sets of questions. (1) How much variation is there in HP receipt among *O. fruticosa* individuals, and what is the relationship between CP and HP receipt? (2) To what extent do pollinator identity, local floral neighborhood composition, and timing of flowering influence HP receipt? And, does the impact of local neighborhood depend on pollinator type? Finally, to address the significance of natural variation in HP receipt for plant fitness and its implications for microevolutionary dynamics, I asked (3) to what extent does this natural variation translate into variation in seed set?

4.3 Materials and Methods

4.3.1 *Study Species and Site*

Oenothera fruticosa is an herbaceous perennial native to eastern North America that occurs in open habitats such as fields and meadows. The species is hermaphroditic and blooms from June through July, producing showy yellow flowers that provide nectar as a floral reward. Individuals typically have 1-3 flowers open at a time; each flower contains an average of 150 ovules, opens in the morning, and lasts approximately 24 hours. *O. fruticosa* is self-incompatible, and thus requires outcross pollination from insects (Silander & Primack, 1978). Pollinated flowers develop into dehiscent capsules that mature around 3 weeks after pollination.

I conducted the study at Fort Indiantown Gap, an active National Guard training center, in Annville, Pennsylvania, USA. Approximately 88 ha of training areas and ranges on the base have been designated for researching the plant and animal species that thrive in periodically disturbed grassland habitats. In June 2019, I tagged 122 *O. fruticosa* plants distributed across a 12ha grassland that contains a diverse assemblage of >70 flowering plant species. Focal *O. fruticosa* plants were at least 2m apart and tagged prior to flowering such that their blooming times were randomly distributed across the entire flowering season. From June to August, I randomly selected one flower per plant to follow from date of anthesis through to fruit set, quantifying pollinator visitation, pollen deposition, and seed production. I recorded diurnal insect visitation on each flower for approximately 1 hour using high-definition video cameras (Sony, San Diego, California, USA) during pollinator activity (0800 -- 1600) on warm, sunny days, capturing a total of 130h of film. I note that although many *Oenothera* species are visited by both diurnal and nocturnal floral visitors, the importance of these groups as pollinators varies across species (Antoń & Denisow, 2018; Krakos & Austin, 2021; Rhodes et al., 2017). Prior work has demonstrated that *O. fruticosa* is visited by diurnal pollinators including, butterflies, bees, and beetles, with no record of nocturnal visitors (Krakos & Austin, 2021; Primack & Silander, 1975; Silander & Primack, 1978). From the video footage, I identified pollinators and determined pollinator visitation rates of each, only considering insects that were observed contacting the reproductive structures of the flowers. Pollinators captured on film were almost exclusively bees (*Bombus, Ceratina, Halictus, Lasioglossum, Augochlorini*) and flies (*Milesia virginica*, *Toxomerus*); there were no visits from lepidopterans and only two visits from coleopterans, representing 0.7% of total visits. Therefore, I considered and quantified pollinator visitation rates of four functional groups: large bees, small bees, large flies, and small flies, consistent with pollinator groupings used in other studies of HP and CP deposition (e.g., Bruckman & Campbell, 2016a; Koski et al., 2015). Large and small bees were the most common (40.8% and 41.8%, respectively), followed by small flies (11.4%) and large flies (6.0%).

4.3.2 ***Composition of Local Floral Neighborhoods***

I quantified the composition of the local floral neighborhood within a 1m radius of each focal plant on the date visitation was filmed. This scale was chosen because it has been demonstrated to significantly influence pollinator visitation, conspecific pollen receipt, and seed production in a number of other grassland systems (e.g., Roll et al., 1997; Spigler and Chang, 2008; Cariveau and Norton, 2009). Within each neighborhood, I counted the number of open *O. fruticosa* flowers and open flowers (or floral units) of heterospecifics. I then calculated the proportion of conspecific flowers per neighborhood as the abundance of conspecific flowers divided by total flower number within the neighborhood. Floral units of heterospecifics were defined depending on the inflorescence structure of each species. For species with singular flowers, I was able to count individual flowers; for composite flowers or compact inflorescences with small flowers (e.g., asters) I considered each inflorescence as a floral unit. The most common co-flowering species across the focal neighborhoods were *Securigera varia* (40% of heterospecific flowers), *Erigeron annuus* (22%), and *Leucanthemum vulgare* (19%); the remaining 20 species in bloom each represented 5% or less, though still occurred in up to 37% of neighborhoods.

4.3.3 *Pollen Deposition*

I collected stigmas of all filmed flowers the day after filming, when flowers were wilted, using clean forceps, and stored them in 70% ethanol until processing. Because *O. fruticosa* has relatively large stigmas, I used an acetolysis processing step (Kearns and Inouye, 1993) to remove stigmatic tissue and retain only pollen grains. I mounted post-acetolysis pollen samples in ethanol and examined them under a compound light microscope (Nikon, Tokyo, Japan) to count conspecific and heterospecific grains. *O. fruticosa* pollen grains have a unique triangular shape and are especially large (>100µm height), making them easily distinguishable from pollen grains of all other species in the community. I considered that pollen grains could be lost during wash steps of the acetolysis procedure. I captured the wash and quantified pollen lost in these steps for a subset of samples. The amount of pollen grains lost was minimal (mean pollen lost <1% per sample), such that I effectively have complete counts per stigma.

4.3.4 *Fruit Collection and Seed Set*

Finally, I collected mature fruits approximately three weeks after flowering and stored them in coin envelopes until processing. In total, I was able to collect 93 fruits from the 122 flowers which were filmed. Some of the fruits went missing in the field or experienced herbivory and were not included in the analyses. Each fruit was carefully examined under a dissecting microscope to count fully developed seeds and unfertilized ovules. I calculated seed set as the number of developed seeds divided by total ovule number (the sum of developed seeds and unfertilized ovules).

4.3.5 Statistical Analyses

To evaluate the relationship between HP and CP deposition I considered both linear and nonlinear relationships given these patterns may reflect different ecological mechanisms (Arceo-Gómez et al., 2016). I evaluated separate general linear models including a linear-response model (untransformed data), log-linear model (HP is log-transformed), and log-log model (HP and CP are log-transformed), the latter two of which would indicate a non-linear relationship between HP and CP. Due to a single zero-value for HP grains I added one to HP grain number before log-transformation. I note that I also considered generalized linear models: a Poisson error distribution, which proved to fit poorly based on residuals and the dispersion parameter (results not shown), and a negative binomial, which proved better but with qualitatively similar results to the general linear models (results not shown).

I evaluated the contributions of different pollinator groups, local floral neighborhood composition, and flowering phenology to both the number and proportion of HP grains received using a linear model and a generalized linear model, respectively. In the former, I used the square root of HP grain number per stigma as the response variable to conform to model assumptions. In the latter, I modeled the proportion of HP received with a binomial error distribution and logit link function weighted by the total number of grains per stigma. Six of the flowers filmed were excluded from these analyses because of partially missing data ($n = 116$). In both models, visitation by each of the four main pollinator groups (large bees, small bees, large flies, and small flies) were included as independent binary predictor variables because of extreme overdispersion in the distribution of pollinator visitation rates. I also included as predictor variables: proportion of neighborhood conspecific flowers and date (Julian day) of flowering. Both predictors were z-transformed to allow for comparisons between their dissimilar scales. Finally, to test whether the impact of pollinators is context dependent, I included interactions between each visitor group variable and proportion of conspecific flowers. However, to maximize power of my analyses to detect main effects, interactions were retained only when significant.

To test whether increasing HP receipt translates to lower reproductive output of *O. fruticosa*, I used a generalized linear model to model seed set following a binomial error distribution, weighted by total ovule number. This model included fixed effects for CP grain number, HP grain number, and their interaction. Julian day was included as a covariate to account for differences in seed set across the season. Each predictor was z-transformed to allow for comparisons between their dissimilar scales. All analyses, unless otherwise noted, were conducted using R 4. 0. 2 (R Core Team, 2021).

4.4 Results

4.4.1 *Conspecific and Heterospecific Pollen Receipt*

HP deposition on *O. fruticosa* flowers at this site was ubiquitous: all but one of the 122 flowers I examined received HP. On average, *O. fruticosa* flowers receive 319.9 (±362.8 SD; range 0 – 2030) HP grains compared to an average of 998.7 (± 938.9 SD; range 2 – 5016) CP grains. Both are highly variable; when scaled by the mean, HP receipt is more so (CV = 113) than CP receipt (CV = 94). Mean proportion HP per stigma is 0.30 (± 0.25 SD) and highly variable as well (range 0 – 0.98). I did not detect a linear relationship between HP and CP grain number (i.e., untransformed data; F = 0.89, df = 1, P = 0.35) nor a significant log-linear relationship (F = 3.01, df = 1, P = 0.09). The log-log relationship between HP and CP grain number is significant (F = 8.10, df = 1, P = 0.005), but this relationship is weak (R^2 = 0.06; Figure 4.1) and hinged on two data points (removed: F = 1.34, df = 1, P = 0.25).

Both the number of HP grains and proportion HP grains decrease significantly as the proportion of conspecific flowers increases in the neighborhood (Table 4.1). However, for proportion HP, significant interactions between proportion conspecific flowers and each pollinator group reveal that the effects of neighborhood composition and pollinator type depend on each other (Table 4.1, Figure 4.2).

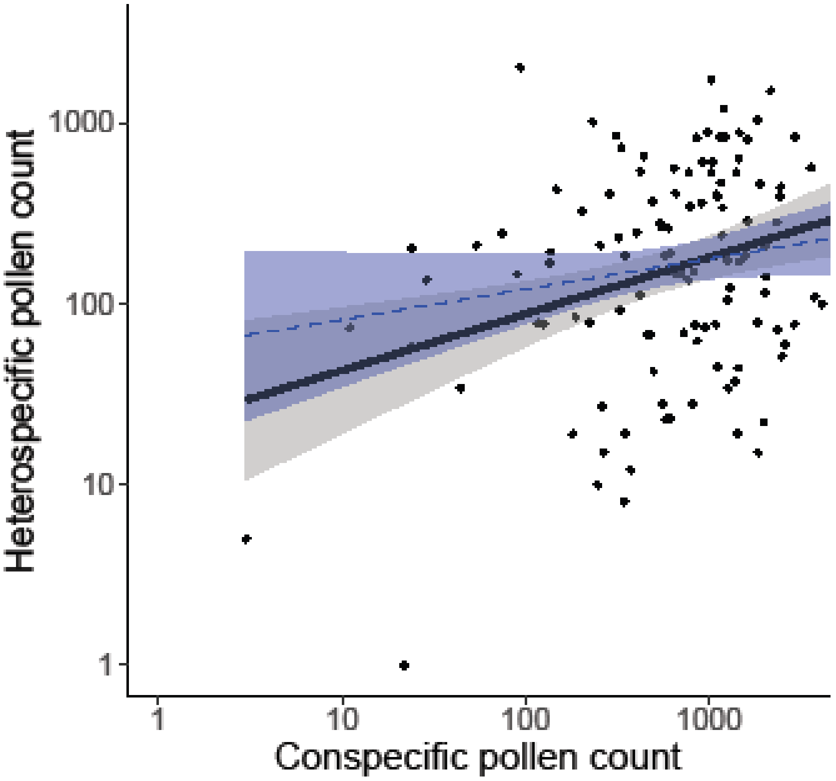

Figure 4.1 Linear regression of the relationship between heterospecific pollen and conspecific pollen deposited on *Oenothera fruticosa* stigmas. Both heterospecific pollen counts and conspecific pollen counts were log transformed for analysis and are plotted here on a log scale. Shading indicates 95% confidence intervals for regression line. Black solid line and grey shading are for the entire dataset (n = 122); blue dashed line and blue shading are for the dataset with two overly influential points removed (n = 120).

Table 4.1. Results from linear and generalized linear models on the effects of insect visitation, local neighborhood composition, and Julian day on heterospecific pollen receipt in *Oenothera fruticosa*. %Con is the proportion of conspecific flowers in local floral neighborhoods. The heterospecific pollen count model uses square root transformed counts of heterospecific pollen as a response with a Gaussian distribution. The proportion heterospecific pollen model uses a binomial distribution. Visitation by each pollinator group is coded as a binary predictor variable. %Con and Julian day are z-transformed. Significant model parameters are shown in bold. n = 116. Only significant interactions were retained for final models.

		Response variables					
		Count of HP received			Proportion of HP received		
Fixed effects	df	Estimate	F value	P value	Estimate	χ^2 value	P value
Large bee visitation	1	0.628	0.151	0.698	**-0.167**	**155.80**	**<0.001**
Small bee visitation	1	**4.149**	**5.911**	**0.017**	**0.341**	**623.14**	**<0.001**
Large fly visitation	1	-3.502	1.342	0.249	**0.642**	**104.30**	**<0.001**
Small fly visitation	1	0.933	0.213	0.645	0.033	3.46	0.063
%Con	1	**-2.696**	**12.11**	**0.001**	**-0.401**	**758.69**	**<0.001**
Julian day	1	-1.710	2.607	0.109	**0.442**	**1788.88**	**<0.001**
Large bee x %Con	1	-	-	-	**-0.327**	**464.34**	**<0.001**
Small bee x %Con	1	-	-	-	**0.198**	**177.75**	**<0.001**
Large fly x %Con	1	-	-	-	**2.179**	**484.12**	**<0.001**
Small fly x %Con	1	-	-	-	**-0.178**	**102.78**	**<0.001**

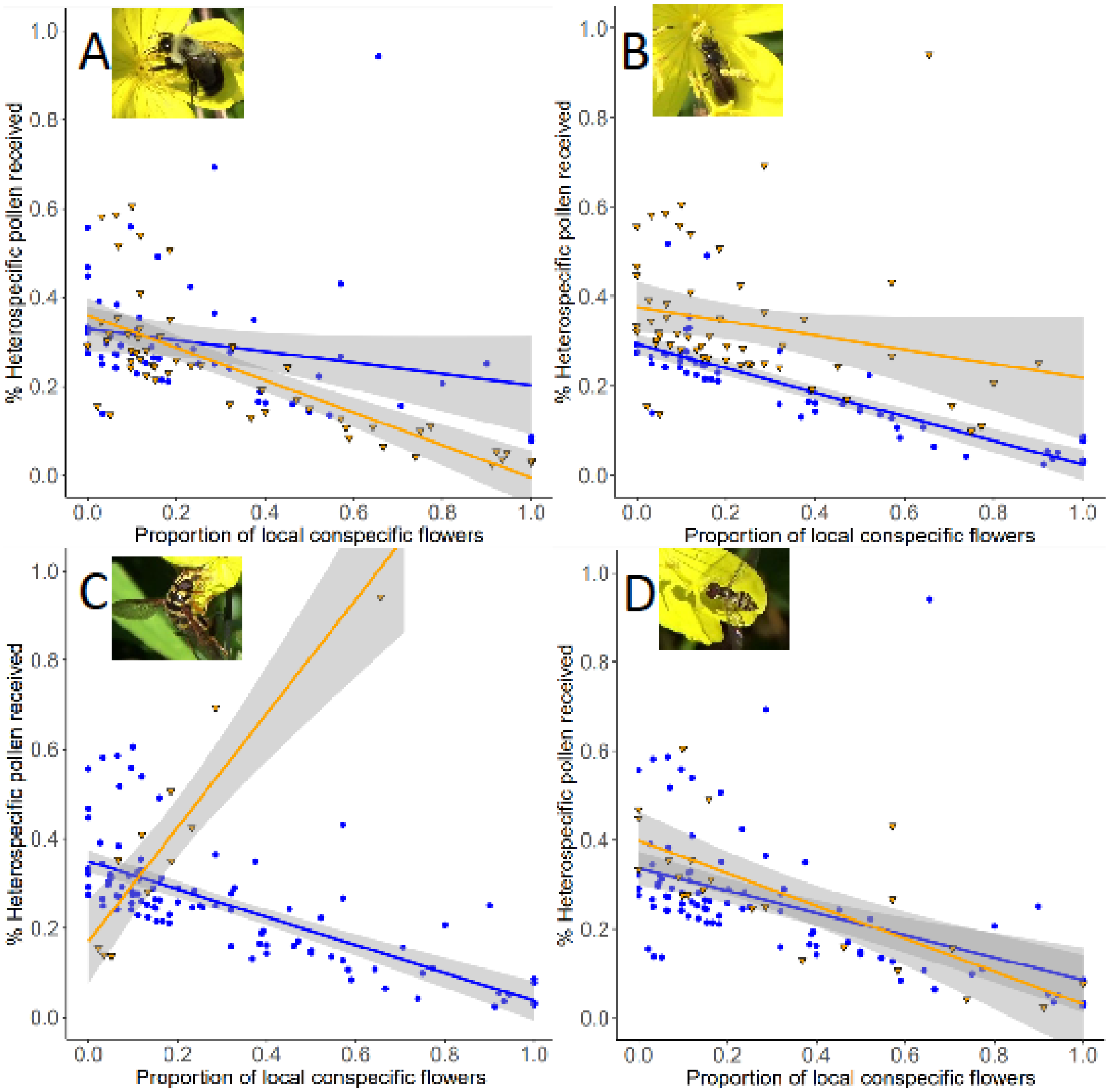

Figure 4.2 Scatterplots of the proportion heterospecific pollen received by the proportion of conspecific flowers in local floral neighborhoods. n = 116. This relationship is mediated by the visitation of various pollinator groups: (A) large bees, (B) small bees, (C) large flies, and (D) small flies. Yellow triangles and lines represent flowers that were visited by each group, while blue circles and lines represent flowers not visited by each group. Shading indicates 95% confidence intervals of each line.

The negative relationship between proportion HP received and proportion local conspecific abundance is significantly steeper when plants are visited by large bees and small flies (Figure 4.2A&D). For flowers visited by large bees, this amounts to similar proportions of HP deposited in neighborhoods where conspecific flowers range from low density to absent, regardless of whether large bees visited (Figure 4.2A). Yet as conspecific density increases, visitation by large bees becomes increasingly important, such that the proportion of HP received is significantly lower for plants visited by large bees. Plants visited by small bees consistently receive greater proportions of HP regardless of conspecific floral density. In this case, the interaction arises because the benefit of conspecific neighbors is substantially weakened for plants visited by small bees, i.e., proportion HP deposited is relatively constant under small bee visitation (Figure 4.2B). Surprisingly, visitation by large flies reverses the relationship between proportion HP receipt and percent conspecific flowers (Figure 4.2C). This pattern remains significant even when I remove the data point representing the highest proportion HP received under large fly visitation (results not shown). In contrast to the results for proportion HP deposition, only small bee visitation significantly influences the number of HP grains per stigma, increasing HP deposition independently of neighborhood composition (Table 4.1).

Additionally, I find that flowers opening later in the season receive significantly greater proportions of HP (Table 4.1, Fig. 4.3A). The number of HP grains, however, is not significantly affected by phenology (Table 4.1, Fig. 4.3B). Thus, despite constant HP receipt across the season, an increase in proportion HP arises because CP deposition declines later in the season (F = 25.80, df = 1, P < 0.001; Fig. 4.3C).

4.4.2 *The Effect of Pollen Receipt on Seed Production*

Seed set is highly variable (39.3% ± 27.5%, mean ± SD) and significantly influenced by the composition of pollen deposited and phenology (Table 4.2). I found that seed set is negatively related to the number of HP grains per stigma, positively related to CP grain number, and declines across the season (Table 4.2). However, there is also a significant interaction between HP and CP deposition (Figure 4.4). In particular, the negative impact of HP deposition is strongest at low levels of CP, below the mean (z-scaled mean = 0 in Figure 4.4) and then begins to dissipate as the number of CP grains on the stigma increases. At the highest levels of CP receipt, there appears to be even a positive impact of HP, though I caution that this area of the contour map is not well supported, based on a few data points of excessively high CP and HP. With respect to HP loads, I see that greater CP receipt can rescue seed set of plants with heavy HP. Ultimately, these data support that the proportion of heterospecific as well as the total number of conspecific and HP received influences seed set.

4.5 Discussion

This study identifies extrinsic and intrinsic factors shaping intraspecific variation in HP receipt. I reveal how the interaction between pollinator identity and local floral neighborhood, together with a plant’s flowering phenology, affect HP deposition on *O. fruticosa* flowers. HP receipt is largely independent of CP receipt and sufficient to

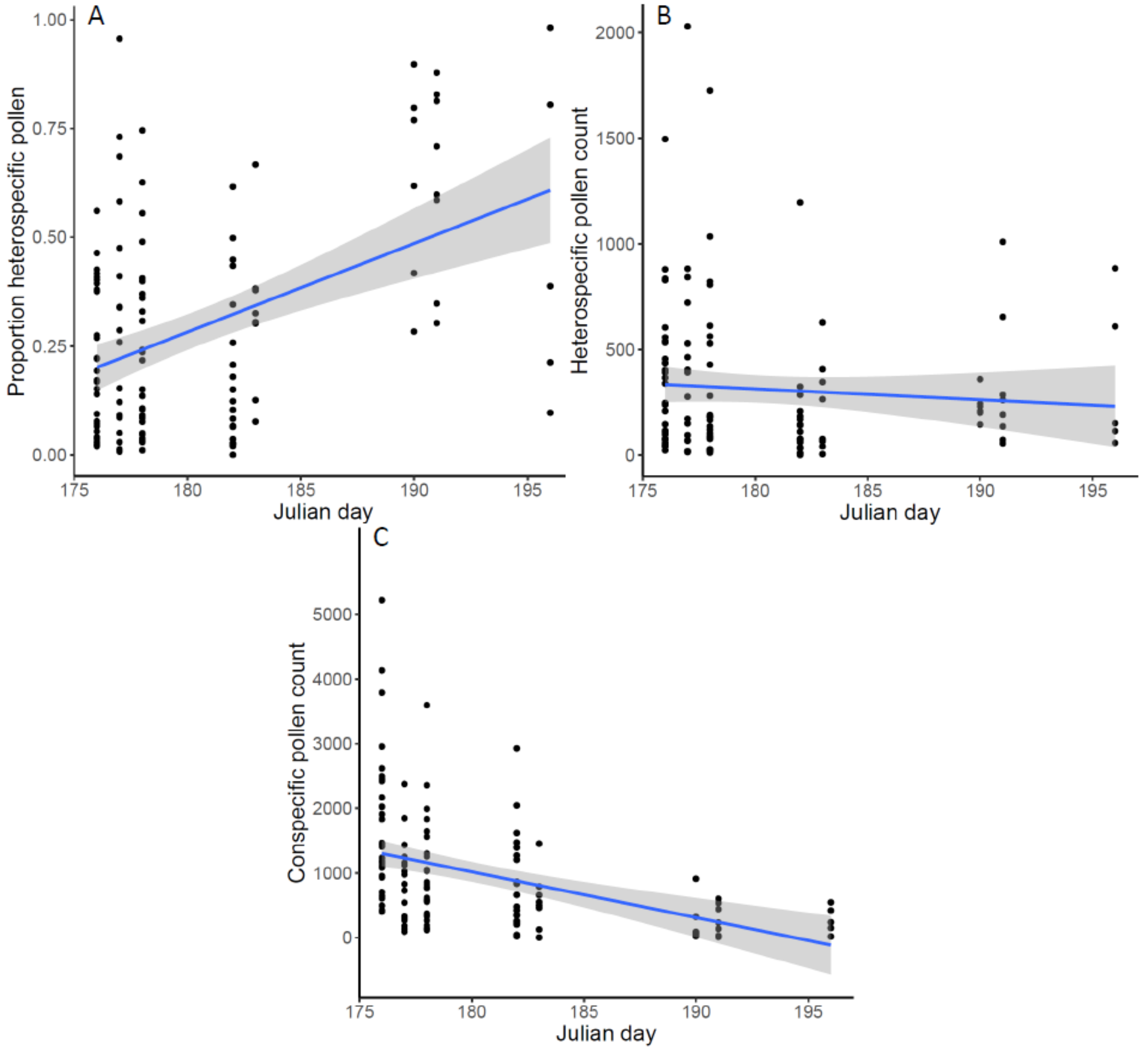

Figure 4.3 Scatterplots of the proportion of heterospecific pollen (A), count of heterospecific pollen (B), and count of conspecific pollen (C) for individual *Oenothera fruticosa* flowers through the season. n = 122. Blue lines depict linear regressions with 95% confidence intervals.

Table 4.2 Results from generalized linear model on the effects of pollen receipt and Julian day on seed set in *Oenothera fruticosa*. CP count and HP count are counts of conspecific and heterospecific pollen deposited on stigmas, respectively. This model uses seed set as a response with a binomial distribution. All predictor variables were z-transformed. Significant model parameters are shown in bold. n = 93

Fixed effects	df	Estimate	χ^2 value	P value
CP count	1	**0.226**	**57.62**	**<0.001**
HP count	1	**-0.194**	**38.42**	**<0.001**
Julian day	1	**-0.270**	**111.84**	**<0.001**
CP count x HP count	1	**0.289**	**175.77**	**<0.001**

negatively impact seed set. This study joins only one other study demonstrating the consequences of HP loads for seed production in wild populations (Briggs et al., 2016). I discuss the results and their implications for natural selection to avoid HP receipt and its constraints.

Pollinators notoriously vary in their effectiveness at depositing CP (Ballantyne et al., 2015; Herrera, 1989; King et al., 2013), but their effectiveness with respect to CP need not correlate with their probability of depositing HP (Arceo-Gómez et al., 2016; Ashman et al., 2020; Mitchell et al., 2009). In general, I found that HP and CP deposition stigmas are not related—or at best only weakly so—in *O. fruticosa*. Arceo-Gómez et al. (2016) suggested that such independence could arise if one or few high-quality

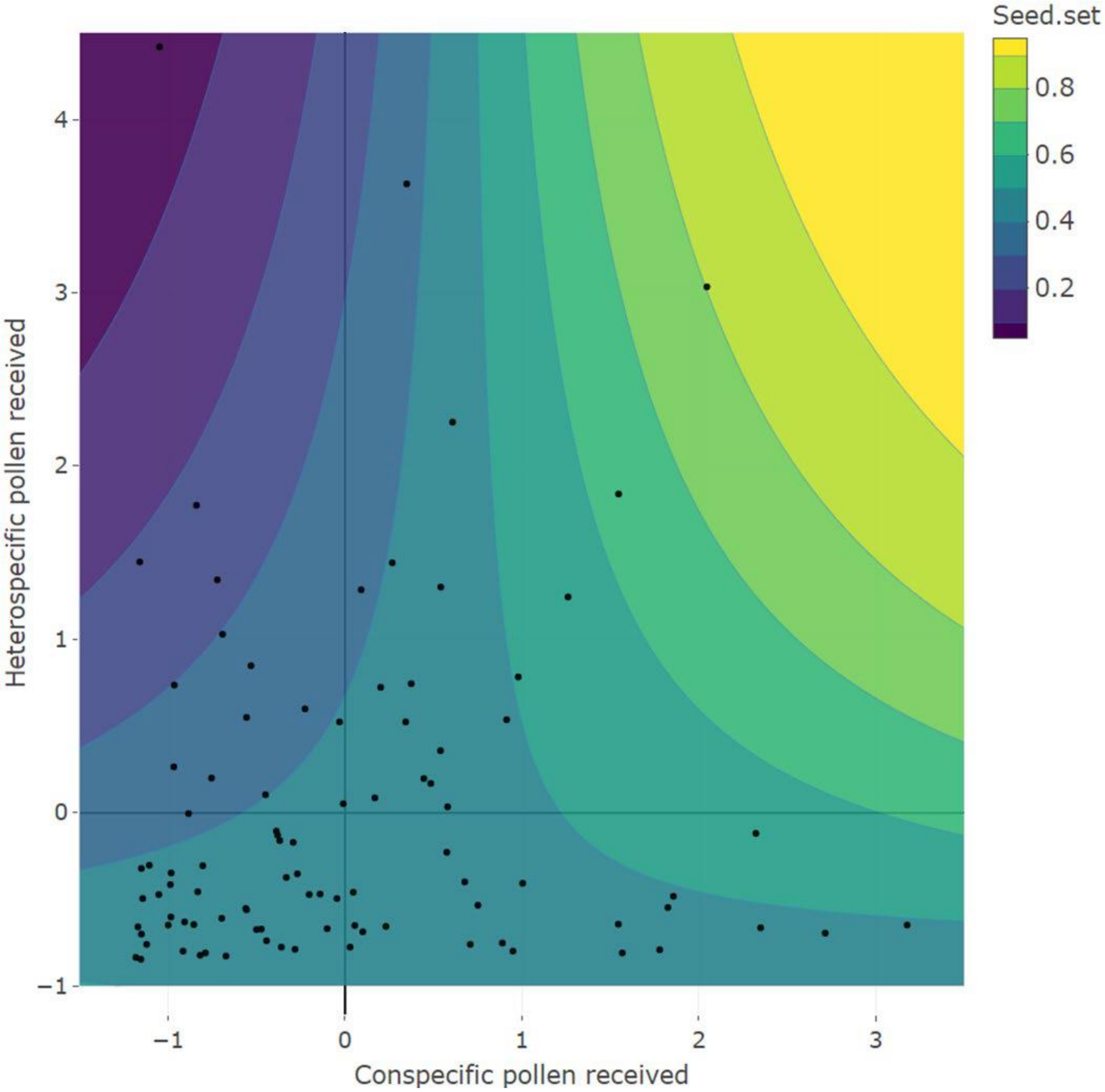

Figure 4.4 Contour plot representing predicted seed set values for given amounts of heterospecific and conspecific pollen deposited. n = 93. Pollen counts are z-transformed; 0 represents the mean observed value on each axis. Black dots represent data points used in the model. The detrimental fitness impact of receiving heterospecific pollen occurs in stigmas that received fewer than the mean value of conspecific pollen grains but disappears for moderate/high CP values.

pollinators deliver nearly pure CP loads, while HP is brought stochastically by ineffective pollinators. The number of HP grains received by *O. fruticosa* varied only with visitation by small bees, but visitation by all pollinator groups influenced the *proportion* of HP received. Thus, all these pollinator groups likely bring varying amounts of CP with relatively consistent HP loads, resulting in a gradient of pollinator effectiveness. Likewise, Fang et al. (2019) suggests that species receiving high average HP loads are likely to be visited by pollinators that carry relatively high, consistent amounts of HP.

Nearly half of the visits to *O. fruticosa* flowers in the population under study were by small bees, which are inefficient pollinators in some systems (Gorenflo et al., 2017; Konzmann et al., 2019; Koski et al., 2018). Indeed, two previous studies of pollinator importance in other *Oenothera* species found that small bees functioned as poor pollinators or pollen thieves (Artz et al., 2010; Rhodes et al., 2017). I show that both the number and proportion of HP are greater for *O. fruticosa* flowers visited by small bees. In contrast, visitation by large bees, which comprised a similar proportion of visits as small bees, has no influence on the number of HP grains but is associated with a lower proportion of HP grains. Thus, the large bees deposit higher amounts or proportions of CP, relative to the less effective pollinators in this system. Comparison of HP loads from small and large bees is consistent with this interpretation; flowers that were visited by small bees *only* during observation periods received slightly more HP and slightly less CP on average than those visited solely by large bees, and the proportion HP received was significantly lower for flowers visited by only large bees ($\chi^2 = 1419.1$, df = 1, $P < 0.001$). The well-documented floral constancy of *Bombus* pollinators during foraging bouts may be responsible for this effect (Goulson, 2003). Indeed, multiple studies have reported that large amounts of CP are lost when pollinators switch between species during a foraging bout (Flanagan et al., 2009; Muchhala & Thomson, 2012). For constant pollinators, the transfer of CP grains may be more strongly influenced by pollinator behavior than the transfer of HP grains, which appears to be a more consistent byproduct of pollinator sharing. Nocturnal visitors have not been documented previously for *O. fruticosa*, but if present in this population, could also have contributed to HPT. Nevertheless, though I cannot empirically exclude their importance, I do see strong patterns of HPT related to diurnal visitors alone.

I further show that HP deposition in *O. fruticosa* is context dependent, influenced by a plant's local floral neighborhood and the way pollinators interact with this neighborhood. Individuals in local neighborhoods with high proportions of conspecific flowers received fewer HP grains and a lower proportion of HP relative to CP. The strength and even the direction of impact of floral neighborhood, however, was mediated by pollinator group. Theoretical models predict changes in pollinator foraging behavior with changes in the relative densities of species in mixed plant assemblages (Goulson, 1994; Kunin & Iwasa, 1996). I found a steeper decrease in the proportion of HP received per increase in proportion of local conspecific flowers for flowers visited by large bees and small flies compared to flowers they did not visit. The difference in slope is strongest for large bees, which might be expected for constant foragers, either because the absolute increase in local CP availability in neighborhoods with greater *O. fruticosa* relative abundance enables constant pollinators to bring in greater amounts of CP relative to HP or because their constancy (Waser, 1986) or preference (Smithson, 2001) increases with *O. fruticosa* density. The latter cases would lead to these pollinators visiting *O. fruticosa* disproportionately as its floral density increases. Flowers visited by small bees exhibited a weaker negative relationship between proportion of HP received and proportion of conspecifics in their floral neighborhoods. In neighborhoods with higher *O. fruticosa* abundance, this translates to a greater proportion of HP on flowers visited by small bees compared to those that were not. This pattern is again consistent with small bees depositing pollen loads generally of poorer purity (more HP) and suggests that small bees foraging behavior is influenced less by the composition of local floral neighborhoods than that of other visitors.

Surprisingly, when large flies visited, the proportion of HP increased as local conspecific relative abundance increased. I suggest caution in interpreting this curious finding, given visits by *M. virginica* were relatively rare during this study. Still, I speculate that such a pattern could arise if large flies exhibit negative-frequency dependent foraging (Smithson, 2001) on *O. fruticosa*, i.e. visitation to *O. fruticosa* is inversely related to *O. fruticosa* abundance. Negative-frequency dependent foraging can occur when rarer pollinators must compete with more common pollinators for access to the more common resource, assuming that other plant species are equally rewarding (Eckhart et al., 2006). Alternatively, I observed the large fly, *M. virginica*, consuming pollen from *O. fruticosa* anthers and even the stigma. If instead these pollinators increase visitation to *O. fruticosa* when *O. fruticosa* is abundant, they might remove more CP from stigmas while still depositing HP. Consideration of neighbor identity might help unpack the interactions causing patterns involving large flies as well as those of the other pollinator groups. For example, co-flowering species with similar floral morphologies and colors (Bell et al., 2005; Liao et al., 2011; Stout et al., 1998) or substantially more attractive and rewarding flowers (Randle et al., 2018; Thomson et al., 2019) may encourage pollinators to switch between species more frequently during a given foraging bout and transfer more pollen between species.

Timing of flowering is well known to influence HPT dynamics and competition for pollinators more generally among species. In fact, these dynamics can lead to character divergence and shape community structure (Mosquin, 1971; Pleasants, 1980; Rathcke, 1983). These results illustrate the significant role of flowering time in determining intraspecific variation in HP receipt. Specifically, the proportion of HP found on *O. fruticosa* flowers increases predictably with flowering date across the season, and the strength of this effect is as great as the effect of local neighborhood. This increase in proportion HP occurred even though the *number* of HP grains received did not change with flowering time or at best declined weakly, but rather because CP receipt declined significantly across the same period. Thus, as the flower season progresses, fewer individuals remain in bloom, reducing the amount of CP available for transport in the population. This shortage of CP and the consistent receipt of HP leads to increased proportions of HP for late-flowering individuals. Additionally, lower CP deposition rates at the end of the season are not due to decreased pollinator visitation ($F = 14.88$, $df = 1$, $P < 0.001$; $R^2 = 0.11$). Rather, the seasonal change in proportion HP deposition highlights the importance of flowering synchronously against a backdrop of steady HP arrival.

Synchronous flowering increases amount of CP available for transport in the population (Elzinga et al., 2007; Méndez & Díaz, 2001). Since *O. fruticosa* population-level flower abundance is positively skewed and leptokurtic in the study population (Smith et al., unpublished), early flowering *O. fruticosa* plants are likely more synchronous with the population. Thus, synchronous flowering can not only benefit plants by increasing CP receipt, but also by reducing the proportion of HP received.

Both the number and proportion of HP on stigmas influenced seed production, demonstrating a clear detrimental fitness effect of HP receipt in *O. fruticosa*. Although the fitness impacts of receiving HP have been shown in many hand-pollination experiments, recent work has shown similar negative impacts on CP tube growth (Parra-Tabla et al., 2020; Suárez-Mariño et al., 2019) and seed set (Briggs et al., 2016) in wild plant populations. There are many mechanisms by which HP can cause reproductive interference including stigma clogging (Galen & Gregory, 1989), pollen allelopathy (Murphy & Aarssen, 1995), style clogging (Randall & Hilu, 1990), and the usurpation of ovules (Burgess et al., 2008). I suspect that interference by HP in this system likely took place on the stigmatic surface, given HP tube growth in the style and via ovule usurpation is unlikely between distantly related species (Moreira-Hernández & Muchhala, 2019) and no confamilial species bloomed during this study. Perhaps key is that I found that the interaction between the amount of CP and HP influences seed set; seed set is the lowest for flowers that receive low amounts of CP and high amounts of HP. Low CP deposition alone, however, is unlikely the cause because seed set is relatively constant across the range of CP amounts seen in this study, so long as HP is also low (Figure 4.4). Proportion

HP on stigmas has been found to influence seed set in other systems as well (Briggs et al., 2016; Thomson et al., 1982) and is more suggestive of neutral dilution or allelopathic effects of HP that reduce pollen germination rates and pollen tube growth of CP (Parra-Tabla et al., 2020; Suárez-Mariño et al., 2019). High levels of CP may be able to effectively neutralize the negative impacts of HP. Although detailed experiments are needed to identify the mechanism, these results suggest that there may be a threshold level of CP receipt *O. fruticosa* flowers can reach to avoid the detrimental impacts of HP receipt.

Given the substantial fitness impacts of HP, the question is whether and how selection could act for plants to avoid the detrimental impacts of HP receipt. It has been hypothesized that species may adopt alternative strategies of HP avoidance or tolerance to minimize the negative effects of HP receipt (Ashman & Arceo-Gómez, 2013; Fang et al., 2019). Extrinsic factors such as a plant's local floral neighborhood cannot be controlled, which combined with a constant background threat of HP deposition seen in this study, limits the ability of *O. fruticosa* to avoid HP. Instead, these findings suggest that the best defense against HP in *O. fruticosa* is to increase CP deposition. I expect to see selection for earlier flowering synchrony, which is associated with receiving lower proportions of HP relative to CP. In addition, although I did not measure stigma sizes in this study, if the large stigmas of *O. fruticosa* allow it to tolerate HP, as has been suggested for other species (Arceo-Gómez & Ashman, 2014; Montgomery & Rathcke, 2012), then selection could also favor plants with larger stigmas in this population. Finally, there is the opportunity for selection on floral traits in *O. fruticosa* that influence attraction of pollinators bringing low proportions of CP. For instance, Kuppler et al. (2016) found the composition of pollinators visiting individual *Sinapis arvensis* plants varied with floral phenotypes and influenced reproductive output. Other studies also find that pollinator groups discriminate between individuals based on floral traits like flower color (Briggs et al., 2018), floral scents (Parachnowitsch et al., 2012), and flower size (Conner & Rush, 1996; Mothershead & Marquis, 2000). Individual *O. fruticosa w*ould benefit from attracting fewer small bees because they bring more HP grains and higher proportions of HP.

CHAPTER 5

SUMMARY

This dissertation extends our understanding of some of the dynamics of and consequences from plant-pollinator interactions. The structure and dynamics of plant-pollinator community interactions are immensely complex and there is still much work required to fully understand the forces that drive patterns of interactions, how aggregated metrics of species interactions should be interpreted, and the ecological and evolutionary consequences of the way mutualists interact. This work contributes to our understanding in several novel ways and raises further questions for future work to consider.

My second chapter explores the driving forces of temporal turnover of species interactions in my study community throughout the season. I find that turnover between interaction networks separated by a week is high and driven by the rewiring of interactions between species present across both time points. The dynamism of plant-pollinator community interactions has been demonstrated across space, environmental gradients, and years, but this chapter contributes to the increasing appreciation of within-season turnover of plant-pollinator interactions. This intra-annual turnover has important implications for the evolution of plant and pollinator species, the conservation of such communities, and their contribution to biodiversity. Furthermore, this chapter quantifies the contribution of a neutral abundance-based process to the variable foraging choices of pollinator species throughout the season. Partner abundances are a useful, but incomplete explanation for the foraging decisions of pollinator species. Other processes such as adaptive foraging, intraspecific competition, interspecific competition and evolutionary specialization play important roles in shaping how plant-pollinator interactions change throughout the season. Importantly, some of these non-neutral mechanisms facilitate the pollination of rare plant species and floral resource availability of rare pollinator species, promoting biodiversity in plant-pollinator communities. These patterns of interactions and the generalized nature of the interactions provide stability to the community from disturbances such as species loss, non-native introductions, and phenological uncoupling from climate change.

The widespread use of network analysis has contributed to our understand plant-pollinator community interactions, but the gap in research that links such network analyses to pollination outcomes motivated Chapter 3. Specifically, I investigated the relationship between species-level network metrics, which characterize species' position and role in the network, and HP and CP receipt. I find that separate metrics of plant species' network role are related to pattern of HP and CP deposition: plant species' specialization is positively related to HP receipt, CP receipt is positively related to species centrality, and CP receipt is negatively related to normalized degree. Future studies should continue to look for such relationships to validate the usefulness of network metrics as proxies for meaningful pollination and fitness outcomes. Additionally, this chapter provides data on the relationship between HP and CP accrual for 13 flowering plant species in the study community. I find few significant relationships between the accrual of HP and CP, suggesting that the receipt of each may be related to different groups of pollinators. It is also possible that there are true relationships to be found between HP and CP receipt, but that these relationships are weak when aggregated as the level of species and across a species' phenology. Furthermore, I confirm that HP receipt is a relatively common, but a small proportion of pollen received by many species. One notable exception in my study community is *Oenothera fruticosa,* which receives tremendous amounts of pollen and is investigated further in chapter 4. Together, the findings of this chapter suggest that HP receipt and CP receipt may be unrelated processes, that are related to different aspects of species' network roles.

Finally, chapter 4 zooms in to determine the importance of pollinator sharing, local floral communities, and phenology on the pollination of individuals. My study system, *Oenothera fruticosa,* is an herbaceous perennial native to eastern North America with a penchant for receiving large amounts of heterospecific pollen. I leverage its short flowering duration to study the effects of ecological factors on its receipt of conspecific and heterospecific pollen. I demonstrate that the proportion of conspecific individuals in a flower's immediate area (1m radius) reduce the proportion of heterospecific pollen it receives. Furthermore, I document how interactions with different pollinators modulate this relationship. Some effective pollinators appear to be especially effective pollinators in highly conspecific neighborhoods, specifically, large bees (*Bombus spp.*) and small Syrphid flies (*Toxomerus spp.*). These pollinators likely bring purer conspecific pollen loads to *O. fruticosa*, possibly because of foraging constancy. Furthermore, small bee species function as poor pollinators of *O. fruticosa,* regardless of the floral neighborhood context. The exact reasons for their inadequacy as pollinators are unclear but may involve bringing pollen loads with relatively more HP or even being more effective at removing CP, thus, causing less CP to be deposited. This chapter also relates patterns of HP deposition to seed set. This novel finding of wild plants suffering fitness effects from HP receipt had only been documented by one other study (Briggs et al., 2016). However, studies linking HP receipt to fitness effects in natural populations represent a crucial step in understanding the importance of the phenomenon of heterospecific pollen transfer. Without demonstrations of fitness costs, the receipt of HP would remain an abstract phenomenon without substantial meaning for affected individuals. This chapter demonstrates how some intrinsic forces, flowering phenology, and the attraction of pollinators, in addition to extrinsic factors, both affect the pollen receipt and fitness of wild plants. The combination of these factors offers an explanation of why heterospecific pollen receipt is so ubiquitous among flower plants, despite its detrimental impacts.

Although plant-pollinator communities have received abundant attention from researchers in recent decades, there is still much to learn. Plant-pollinator communities are dynamic systems comprised of many interconnected species playing a variety of roles. Species interactions in these communities represent relationships than range widely from facilitative to competitive, central to peripheral, and generalized to specialized. Research into the functioning of these systems will continue to refine our understanding of the ecological and evolutionary dynamics of these systems and secure their well-being for the future.

BIBLITOGRAPHY

Albrecht, M., Schmid, B., Hautier, Y., & Müller, C. B. (2012). Diverse pollinator communities enhance plant reproductive success. *Proceedings of the Royal Society B: Biological Sciences*, *279*(1748), 4845–4852. https://doi.org/10.1098/rspb.2012.1621

Amaya-Márquez, M. (2009). Floral constancy in bees: a revision of theories and a comparison with other pollinators. *Revista Colombiana de Entomología*, *35*(2), 206–216.

Antoń, S., & Denisow, B. (2018). Pollination biology and breeding system in five nocturnal species of *Oenothera* (Onagraceae): reproductive assurance and opportunities for outcrossing. *Plant Systematics and Evolution*, *304*(10), 1231–1243. https://doi.org/10.1007/s00606-018-1543-y

Arceo-Gómez, G., Abdala-Roberts, L., Jankowiak, A., Kohler, C., Meindl, G. A., Navarro-Fernández, C. M., Parra-Tabla, V., Ashman, T. L., & Alonso, C. (2016). Patterns of among- and within-species variation in heterospecific pollen receipt: The importance of ecological generalization. *American Journal of Botany*, *103*(3), 396–407. https://doi.org/10.3732/ajb.1500155

Arceo-Gómez, G., Alonso, C., Ashman, T. L., & Parra-Tabla, V. (2018). Variation in sampling effort affects the observed richness of plant–plant interactions via heterospecific pollen transfer: implications for interpretation of pollen transfer networks. *American Journal of Botany*, *105*(9), 1601–1608.

https://doi.org/10.1002/ajb2.1144

Arceo-Gómez, G., & Ashman, T. L. (2014). Heterospecific pollen receipt affects self pollen more than outcross pollen: Implications for mixed-mating plants. *Ecology*, *95*(10), 2964–2973.

Arceo-Gómez, G., & Ashman, T. L. (2016). Invasion status and phylogenetic relatedness predict cost of heterospecific pollen receipt: implications for native biodiversity decline. *Journal of Ecology*, *104*(4), 1003–1008. https://doi.org/10.1111/1365-2745.12586

Arceo-Gómez, G., Barker, D., Stanley, A., Watson, T., & Daniels, J. (2020). Plant–pollinator network structural properties differentially affect pollen transfer dynamics and pollination success. *Oecologia*, *192*(4), 1037–1045. https://doi.org/10.1007/s00442-020-04637-5

Arceo-Gómez, G., Kaczorowski, R. L., Patel, C., & Ashman, T. L. (2019). Interactive effects between donor and recipient species mediate fitness costs of heterospecific pollen receipt in a co-flowering community. *Oecologia*, *189*(4), 1041–1047. https://doi.org/10.1007/s00442-019-04379-z

Armbruster, W. S. (2017). The specialization continuum in pollination systems: diversity of concepts and implications for ecology, evolution and conservation. *Functional Ecology*, *31*(1), 88–100. https://doi.org/10.1111/1365-2435.12783

Arroyo-Correa, B., Bartomeus, I., & Jordano, P. (2021). Individual-based plant–pollinator networks are structured by phenotypic and microsite plant traits. *Journal* *of Ecology*, *May*, 2832–2844. https://doi.org/10.1111/1365-2745.13694

Arroyo-Correa, B., Burkle, L. A., & Emer, C. (2020). Alien plants and flower visitors disrupt the seasonal dynamics of mutualistic networks. *Journal of Ecology*, *108*(4), 1475–1486. https://doi.org/10.1111/1365-2745.13332

Artz, D. R., Villagra, C. A., & Raguso, R. A. (2010). Spatiotemporal variation in the reproductive ecology of two parapatric subspecies of *Oenothera cespitosa* (Onagraceae). *American Journal of Botany*, *97*(9), 1498–1510. https://doi.org/10.3732/ajb.1000086

Ashman, T. L., Alonso, C., Parra-Tabla, V., & Arceo-Gómez, G. (2020). Pollen on stigmas as proxies of pollinator competition and facilitation: complexities, caveats and future directions. *Annals of Botany*, *125*(7), 1003–1012. https://doi.org/10.1093/aob/mcaa012

Ashman, T. L., & Arceo-Gómez, G. (2011). Heterospecific pollen deposition: does diversity alter the consequences? *New Phytologist*, *192*, 738–746.

Ashman, T. L., & Arceo-Gómez, G. (2013). Toward a predictive understanding of the fitness costs of heterospecific pollen receipt and its importance in co-flowering communities. *American Journal of Botany*, *100*(6), 1061–1070. https://doi.org/10.3732/ajb.1200496

Ballantyne, G., Baldock, K. C. R., Rendell, L., & Willmer, P. G. (2017). Pollinator importance networks illustrate the crucial value of bees in a highly speciose plant community. *Scientific Reports*, *7*(1), 1–13. https://doi.org/10.1038/s41598-017-

08798-x

Ballantyne, G., Baldock, K. C. R., & Willmer, P. G. (2015). Constructing more informative plant - pollinator networks: visitation and pollen deposition networks in a heathland plant community. *Proceedings of the Royal Society B*, *282*(1814), 20151130. https://doi.org/10.1098/rspb.2015.1130

Bartkowska, M. P., & Johnston, M. O. (2014). The sexual neighborhood through time: competition and facilitation for pollination in *Lobelia cardinalis*. *Ecology*, *95*(4), 910–919. https://doi.org/10.1890/13-0447.1

Bartomeus, I. (2019). *No Title*. https://doi.org/10.5281/zenodo.2577565

Barwell, L. J., Isaac, N. J. B., & Kunin, W. E. (2015). Measuring β-diversity with species abundance data. *Journal of Animal Ecology*, *84*(4), 1112–1122. https://doi.org/10.1111/1365-2656.12362

Bascompte, J., Jordano, P., Melian, C. J., & Olesen, J. M. (2003). The nested assembly of plant-animal mutualistic networks. *Proceedings of the National Academy of Sciences*, *100*(16), 9383–9387. https://doi.org/10.1073/pnas.1633576100

Bates, D., Mächler, M., Bolker, B. M., & Walker, S. C. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, *67*(1). https://doi.org/10.18637/jss.v067.i01

Bell, J. M., Karron, J. D., & Mitchell, R. J. (2005). Interspecific competition for pollination lowers seed production and outcrossing in *Mimulus ringens*. *Ecology*, *86*(3), 762–771.

Benadi, G., & Pauw, A. (2018). Frequency dependence of pollinator visitation rates suggests that pollination niches can allow plant species coexistence. *Journal of Ecology*, *106*(5), 1892–1901. https://doi.org/10.1111/1365-2745.13025

Bergamo, Pedro J., Streher, N. S., Wolowski, M., & Sazima, M. (2020). Pollinator-mediated facilitation is associated with floral abundance, trait similarity and enhanced community-level fitness. *Journal of Ecology*, *December 2019*, 1334–1346. https://doi.org/10.1111/1365-2745.13348

Bergamo, Pedro Joaquim, Streher, N. S., Traveset, A., Wolowski, M., & Sazima, M. (2020). Pollination outcomes reveal negative density-dependence coupled with interspecific facilitation among plants. *Ecology Letters*, *23*(1), 129–139. https://doi.org/10.1111/ele.13415

Briggs, H. M., Anderson, L. M., Atalla, L. M., Delva, A. M., Dobbs, E. K., & Brosi, B. J. (2016). Heterospecific pollen deposition in *Delphinium barbeyi*: linking stigmatic pollen loads to reproductive output in the field. *Annals of Botany*, *117*(2), 341–347. https://doi.org/10.1093/aob/mcv175

Briggs, H. M., Graham, S., Switzer, C. M., & Hopkins, R. (2018). Variation in context-dependent foraging behavior across pollinators. *Ecology and Evolution*, *8*(16), 7964–7973. https://doi.org/10.1002/ece3.4303

Brosi, B. J. (2016). *Tansley insight Pollinator specialization : from the individual to the community*. *1996*.

Brosi, B. J., & Briggs, H. M. (2013). Single pollinator species losses reduce floral fidelity and plant reproductive function. *Proceedings of the National Academy of Sciences*, *110*(32), 13044–13048. https://doi.org/10.1073/pnas.1307438110

Brown, B. J., Mitchell, R. J., & Graham, S. A. (2002). Competition for pollination between an invasive species (purple loosestrife) and a native congener. *Ecology*, *83*(8), 2328–2336. http://ejournals.ebsco.com/direct.asp?ArticleID=44ECA54B1BB531D494EC

Bruckman, D., & Campbell, D. R. (2014). Floral neighborhood influences pollinator assemblages and effective pollination in a native plant. *Oecologia*, *176*(2), 465–476. https://doi.org/10.1007/s00442-014-3023-6

Bruckman, D., & Campbell, D. R. (2016a). Pollination of a native plant changes with distance and density of invasive plants in a simulated biological invasion. *American Journal of Botany*, *103*(8), 1458–1465. https://doi.org/10.3732/ajb.1600153

Bruckman, D., & Campbell, D. R. (2016b). Timing of invasive pollen deposition influences pollen tube growth and seed set in a native plant. *Biological Invasions*, *18*(6), 1701–1711. https://doi.org/10.1007/s10530-016-1113-6

Burgess, K. S., Morgan, M., & Husband, B. C. (2008). Interspecific seed discounting and the fertility cost of hybridization in an endangered species. *New Phytologist*, *177*(1), 276–284. https://doi.org/10.1111/j.1469-8137.2007.02244.x

Burkle, L. A., Marlin, J. C., & Knight, T. M. (2013). Plant-pollinator interactions over 120 Years: loss of species, co-occurrence, and function. *Science, 339*(6127), 1611–

1615. https://doi.org/10.1126/science.1232728

CaraDonna, P. J., Burkle, L. A., Schwarz, B., Resasco, J., Knight, T. M., Benadi, G., Blüthgen, N., Dormann, C. F., Fang, Q., Fründ, J., Gauzens, B., Kaiser-Bunbury, C. N., Winfree, R., & Vázquez, D. P. (2020). Seeing through the static: the temporal dimension of plant–animal mutualistic interactions. *Ecology Letters*, 149–161. https://doi.org/10.1111/ele.13623

CaraDonna, P. J., Petry, W. K., Brennan, R. M., Cunningham, J. L., Bronstein, J. L., Waser, N. M., & Sanders, N. J. (2017). Interaction rewiring and the rapid turnover of plant-pollinator networks. *Ecology Letters*, *20*(3), 385–394. https://doi.org/10.1111/ele.12740

Cariveau, D. P., & Norton, A. P. (2009). Spatially contingent interactions between an exotic and native plant mediated through flower visitors. *Oikos*, *118*(1), 107–114. https://doi.org/10.1111/j.1600-0706.2008.16705.x

Caruso, C. M., & Alfaro, M. (2000). Interspecific pollen transfer as a mechanism of competition: effect of *Castilleja linariaefolia* pollen on seed set of *Ipomopsis aggregata*. *Canadian Journal of Botany*, *78*(5), 600–606. https://doi.org/10.1139/cjb-78-5-600

Chacoff, N. P., Resasco, J., & Vázquez, D. P. (2018). Interaction frequency, network position, and the temporal persistence of interactions in a plant–pollinator network. *Ecology*, *99*(1), 21–28. https://doi.org/10.1002/ecy.2063

Chacoff, N. P., Vázquez, D. P., Lomáscolo, S. B., Stevani, E. L., Dorado, J., & Padrón,

B. (2012). Evaluating sampling completeness in a desert plant-pollinator network. *Journal of Animal Ecology*, *81*(1), 190–200. https://doi.org/10.1111/j.1365-2656.2011.01883.x

Charlebois, J. A., & Sargent, R. D. (2017). No consistent pollinator-mediated impacts of alien plants on natives. *Ecology Letters*, *20*(11), 1479–1490. https://doi.org/10.1111/ele.12831

Chen, L., Zhang, B., & Li, Q. (2017). Pollinator-mediated selection on flowering phenology and floral display in a distylous herb *Primula alpicola*. *Scientific Reports*, *7*(1), 13157. https://doi.org/10.1038/s41598-017-13340-0

Conner, J. K., & Rush, S. (1996). Effects of flower size and number on pollinator visitation to wild radish, *Raphanus raphanistrum*. *Oecologia*, *105*(4), 509–516. https://doi.org/10.1007/BF00330014

Dormann, C. F., Frund, J., Bluthgen, N., & Gruber, B. (2009). Indices, graphs and null models: analyzing bipartite ecological networks. *The Open Ecology Journal*, *2*(1), 7–24. https://doi.org/10.2174/1874213000902010007

Eckhart, V. M., Rushing, N. S., Hart, G. M., & Hansen, J. D. (2006). Frequency-dependent pollinator foraging in polymorphic *Clarkia xantiana* ssp. *xantiana* populations: implications for flower colour evolution and pollinator interactions. *Oikos*, *112*(2), 412–421. https://doi.org/10.1111/j.0030-1299.2006.14289.x

Ehrlich, P. R., & Raven, P. H. (2006). Butterflies and plants: A Study in coevolution. *Evolution*, *18*(4), 586. https://doi.org/10.2307/2406212

Elzinga, J. A., Atlan, A., Biere, A., Gigord, L., Weis, A. E., & Bernasconi, G. (2007). Time after time: flowering phenology and biotic interactions. *Trends in Ecology & Evolution*, *22*(8), 432–439. https://doi.org/10.1016/j.tree.2007.05.006

Fang, Q., Gao, J., Armbruster, W. S., & Huang, S. (2019). Multi-year stigmatic pollen-load sampling reveals temporal stability in interspecific pollination of flowers in a subalpine meadow. *Oikos*, *128*(12), 1739–1747. https://doi.org/10.1111/oik.06447

Fang, Q., & Huang, S.-Q. (2013). A directed network analysis of heterospecific pollen transfer in a biodiverse community. *Ecology*, *94*(5), 1176–1185. https://doi.org/10.1890/12-1634.1

Fang, Q., & Huang, S. Q. (2012). Relative stability of core groups in pollination networks in a biodiversity hotspot over four years. *PLoS ONE*, *7*(3). https://doi.org/10.1371/journal.pone.0032663

Feinsinger, P., Tiebout, H. M., & Young, B. E. (1991). Do tropical bird-pollinated plants exhibit density-dependent interactions? Field experiments. *Ecology*, *72*(6), 1953–1963. https://doi.org/10.2307/1941550

Flanagan, R. J., Mitchell, R. J., Knutowski, D., & Karron, J. D. (2009). Interspecific pollinator movements reduce pollen deposition and seed production in *mimulus ringens* (Phrymaceae). *American Journal of Botany*, *96*(4), 809–815. https://doi.org/10.3732/ajb.0800317

Fort, H., Vázquez, D. P., & Lan, B. L. (2016). Abundance and generalisation in mutualistic networks: Solving the chicken-and-egg dilemma. *Ecology Letters*, *19*(1), 4–11. https://doi.org/10.1111/ele.12535

Fründ, J., Dormann, C. F., Holzschuh, A., & Tscharntke, T. (2013). Bee diversity effects on pollination depend on functional complementarity and niche shifts. *Ecology*, *94*(9), 2042–2054. https://doi.org/10.1890/12-1620.1

Galen, C., & Gregory, T. (1989). Interspecific pollen transfer as a mechanism of competition: Consequences of foreign pollen contamination for seed set in the alpine wildflower, *Polemonium viscosum*. *Oecologia*, *81*(1), 120–123. https://doi.org/10.1007/BF00377020

Ghazoul, J. (2005). Pollen and seed dispersal among dispersed plants. *Biological Reviews*, *80*(3), 413–443.

Gómez, J. M., & Perfectti, F. (2012). Fitness consequences of centrality in mutualistic individual-based networks. In *Proceedings of the Royal Society B: Biological Sciences* (Vol. 279, Issue 1734, pp. 1754–1760). https://doi.org/10.1098/rspb.2011.2244

Gorenflo, A., Kleunen, M. Van, Wolters, V., & Jauker, F. (2017). Contrasting pollination efficiency and effectiveness among flower visitors of *Malva sylvestris*, *Borago officianalis* and *Onobrychis viciifolia*. *Journal of Pollination Ecology*, *21*(1), 62–70.

Goulson, D. (1994). A model to predict the influence of insect flower constancy on interspecific competition between insect pollinated plants. *Journal of Theoretical Biology*, *168*(3), 309–314. https://doi.org/10.1006/jtbi.1994.1111

Goulson, D. (2003). *Bumblebees: their behaviour and ecology*. Oxford University Press.

Gross, C. L. (1992). Floral traits and pollinator constancy: Foraging by native bees among three sympatric legumes. *Australian Journal of Ecology*, *17*(1), 67–74. https://doi.org/10.1111/j.1442-9993.1992.tb00781.x

Ha, M. K., & Ivey, C. T. (2017). Pollinator-mediated interactions in experimental arrays vary with neighbor identity. *American Journal of Botany*, *104*(2), 252–260. https://doi.org/10.3732/ajb.1600293

Hallmann, C. A., Sorg, M., Jongejans, E., Siepel, H., Hofland, N., Schwan, H., Stenmans, W., Müller, A., Sumser, H., Hörren, T., Goulson, D., & De Kroon, H. (2017). More than 75 percent decline over 27 years in total flying insect biomass in protected areas. *PLoS ONE*. https://doi.org/10.1371/journal.pone.0185809

Heinrich, B. (1979). Resource heterogeneity and patterns of movement in foraging bumblebees. *Oecologia*, *40*(3), 235–245. https://doi.org/10.1007/BF00345321

Herrera, C. M. (1989). Pollinator abundance, morphology, and flower visitation rate: analysis of the “quantity” component in a plant-pollinator system. *Oecologia*, *80*, 241–248.

Hervías-Parejo, S., Tur, C., Heleno, R., Nogales, M., Timóteo, S., & Traveset, A. (2020). Species functional traits and abundance as drivers of multiplex ecological networks: First empirical quantification of inter-layer edge weights: Pollination-seed dispersal networks. *Proceedings of the Royal Society B: Biological Sciences*, *287*(1939). https://doi.org/10.1098/rspb.2020.2127rspb20202127

Inouye, D. W. (1977). Resource partitioning in bumblebees. *Journal of the New York Entomological Society*, *85*(4), 253–254. http://agris.fao.org/agris-search/search/display.do?f=1978/US/US78051.xml;US7884273

Johnson, A. L., & Ashman, T. L. (2019). Consequences of invasion for pollen transfer and pollination revealed in a tropical island ecosystem. *New Phytologist*, *221*(1), 142–154. https://doi.org/10.1111/nph.15366

Jordano, P. (1987). Patterns of mutualistic interactions in pollination and seed dispersal - connectance, dependence asymmetries, and coevolution. *American Naturalist, 129*(5), 657–677. https://doi.org/10.1086/284665

Jordano, P., Bascompte, J., & Olesen, J. M. (2003). Invariant properties in coevolutionary networks of plant-animal interactions. *Ecology Letters*, *6*(1), 69–81. https://doi.org/10.1046/j.1461-0248.2003.00403.x

Junker, R. R., Blüthgen, N., Brehm, T., Binkenstein, J., Paulus, J., Martin Schaefer, H., & Stang, M. (2013). Specialization on traits as basis for the niche-breadth of flower visitors and as structuring mechanism of ecological networks. *Functional Ecology*, *27*(2), 329–341. https://doi.org/10.1111/1365-2435.12005

Kaiser-Bunbury, C. N., Muff, S., Memmott, J., Müller, C. B., & Caflisch, A. (2010). The robustness of pollination networks to the loss of species and interactions: A quantitative approach incorporating pollinator behaviour. *Ecology Letters*, *13*(4), 442–452. https://doi.org/10.1111/j.1461-0248.2009.01437.x

Kaiser-Bunbury, C. N., Valentin, T., Mougal, J., Matatiken, D., & Ghazoul, J. (2011).

The tolerance of island plant-pollinator networks to alien plants. *Journal of Ecology*, *99*(1), 202–213. https://doi.org/10.1111/j.1365-2745.2010.01732.x

Kaiser-Bunbury, C. N., Vázquez, D. P., Stang, M., & Ghazoul, J. (2014). *Determinants of the microstructure of plant—pollinator networks*. *95*(12), 3314–3324.

Kantsa, A., Raguso, R. A., Dyer, A. G., Olesen, J. M., Tscheulin, T., & Petanidou, T. (2018). Disentangling the role of floral sensory stimuli in pollination networks. *Nature Communications*, *9*(1), 1041. https://doi.org/10.1038/s41467-018-03448-w

Kearns, C. A., & Inouye, D. W. (1993). Techniques for pollination biologists. University press of Colorado. *University Press of Colorado.*

King, C., Ballantyne, G., & Willmer, P. G. (2013). Why flower visitation is a poor proxy for pollination: measuring single-visit pollen deposition, with implications for pollination networks and conservation. *Methods in Ecology and Evolution*, *4*(9), 811–818. https://doi.org/10.1111/2041-210X.12074

Kitamoto, N., Ueno, S., Takenaka, A., Tsumura, Y., Washitani, I., & Ohsawa, R. (2006). Effect of flowering phenology on pollen flow distance and the consequences for spatial genetic structure within a population of *Primula sieboldii* (Primulaceae). *American Journal of Botany*, *93*(2), 226–233. https://doi.org/10.3732/ajb.93.2.226

Knight, T. M., Ashman, T. L., Bennett, J. M., Burns, J. H., Passonneau, S., & Steets, J. A. (2018). Reflections on, and visions for, the changing field of pollination ecology. *Ecology Letters*, *21*(8), 1282–1295. https://doi.org/10.1111/ele.13094

Konzmann, S., Koethe, S., & Lunau, K. (2019). Pollen grain morphology is not exclusively responsible for pollen collectability in bumble bees. *Scientific Reports*, *9*(1), 4705. https://doi.org/10.1038/s41598-019-41262-6

Koski, M. H., Ison, J. L., Padilla, A., Pham, A. Q., & Galloway, L. F. (2018). Linking pollinator efficiency to patterns of pollen limitation: small bees exploit the plant–pollinator mutualism. *Proceedings of the Royal Society B*, *285*(1880), 20180635. https://doi.org/10.1098/rspb.2018.0635

Koski, M. H., Meindl, G. A., Arceo-Gómez, G., Wolowski, M., LeCroy, K. A., & Ashman, T. L. (2015). Plant–flower visitor networks in a serpentine metacommunity: assessing traits associated with keystone plant species. *Arthropod-Plant Interactions*, *9*(1), 9–21. https://doi.org/10.1007/s11829-014-9353-9

Krakos, K. N., & Austin, M. W. (2021). Testing pollination syndromes in *Oenothera* (Onagraceae). *Journal of Pollination Ecology*, *26*(7), 52–66.

Kunin, W., & Iwasa, Y. (1996). Pollinator foraging strategies in mixed floral arrays: density effects and floral constancy. *Theoretical Population Biology*, *49*(2), 232–263. https://doi.org/10.1006/tpbi.1996.0013

Kuppler, J., Höfers, M. K., Wiesmann, L., & Junker, R. R. (2016). Time-invariant differences between plant individuals in interactions with arthropods correlate with intraspecific variation in plant phenology, morphology and floral scent. *New Phytologist*, *210*(4), 1357–1368. https://doi.org/10.1111/nph.13858

Landi, P., Minoarivelo, H. O., Brännström, Å., Hui, C., & Dieckmann, U. (2018).

Complexity and stability of ecological networks: a review of the theory. *Population Ecology*, *60*(4), 319–345. https://doi.org/10.1007/s10144-018-0628-3

Lázaro, A., Gómez-Martínez, C., Alomar, D., González-Estévez, M. A., & Traveset, A. (2020). Linking species-level network metrics to flower traits and plant fitness. *Journal of Ecology*, *108*(4), 1287–1298. https://doi.org/10.1111/1365-2745.13334

Legendre, P. (2014). Interpreting the replacement and richness difference components of beta diversity. *Global Ecology and Biogeography*, *23*(11), 1324–1334. https://doi.org/10.1111/geb.12207

Liao, K., Gituru, R. W., Guo, Y. H., & Wang, Q. F. (2011). The presence of co-flowering species facilitates reproductive success of *Pedicularis monbeigiana* (Orobanchaceae) through variation in bumble-bee foraging behaviour. *Annals of Botany*, *108*(5), 877–884. https://doi.org/10.1093/aob/mcr216

Lopezaraiza-Mikel, M. E., Hayes, R. B., Whalley, M. R., & Memmott, J. (2007). The impact of an alien plant on a native plant-pollinator network: An experimental approach. *Ecology Letters*, *10*(7), 539–550. https://doi.org/10.1111/j.1461-0248.2007.01055.x

Loy, X., & Brosi, B. J. (2022). The effects of pollinator diversity on pollination function. *Ecology*, *October 2021*, 1–14. https://doi.org/10.1002/ecy.3631

Lundberg, J., & Moberg, F. (2003). Mobile link organisms and ecosystem functioning: Implications for ecosystem resilience and management. *Ecosystems*. https://doi.org/10.1007/s10021-002-0150-4

MacLeod, M., Genung, M. A., Ascher, J. S., & Winfree, R. (2016). Measuring partner choice in plant-pollinator networks: Using null models to separate rewiring and fidelity from chance. *Ecology*, *97*(11), 2925–2931. https://doi.org/10.1002/ecy.1574

Maglianesi, M. A., BlüThgen, N., BöHning-Gaese, K., & Schleuning, M. (2014). Morphological traits determine specialization and resource use in plant-hummingbird networks in the neotropics. *Ecology*, *95*(12), 3325–3334. https://doi.org/10.1890/13-2261.1

McLernon, S. M., Murphy, S. D., & Aarssen, L. W. (1996). Heterospecific pollen transfer between sympatric species in a midsuccessional old-field community. *American Journal of Botany*, *83*(9), 1168–1174. https://doi.org/10.2307/2446200

Méndez, M., & Díaz, A. (2001). Flowering dynamics in Arum italicum (Araceae): Relative role of inflorescence traits, flowering synchrony, and pollination context on fruit initiation. *American Journal of Botany*, *88*(1–12), 1774–1780. https://doi.org/10.2307/3558352

Miele, V., Ramos-Jiliberto, R., & Vázquez, D. P. (2020). Core–periphery dynamics in a plant–pollinator network. *Journal of Animal Ecology*, *89*(7), 1670–1677. https://doi.org/10.1111/1365-2656.13217

Minnaar, C., Anderson, B., De Jager, M. L., & Karron, J. D. (2019). Plant-pollinator interactions along the pathway to paternity. *Annals of Botany*, *123*(2), 225–245. https://doi.org/10.1093/aob/mcy167

Mitchell, R. J., Flanagan, R. J., Brown, B. J., Waser, N. M., & Karron, J. D. (2009). New frontiers in competition for pollination. *Annals of Botany*, *103*(9), 1403–1413. https://doi.org/10.1093/aob/mcp062

Montgomery, B. R., & Rathcke, B. J. (2012). Effects of floral restrictiveness and stigma size on heterospecific pollen receipt in a prairie community. *Oecologia*, *168*(2), 449–458. https://doi.org/10.1007/s00442-011-2094-x

Mora, B. B., Shin, E., CaraDonna, P. J., & Stouffer, D. B. (2020). Untangling the seasonal dynamics of plant-pollinator communities. *Nature Communications*, *11*(1). https://doi.org/10.1038/s41467-020-17894-y

Morales, C. L., & Traveset, A. (2008). Interspecific pollen transfer: magnitude, prevalence and consequences for plant fitness. *Critical Reviews in Plant Sciences*, *27*(4), 221–238. https://doi.org/10.1080/07352680802205631

Morales, C. L., & Traveset, A. (2009). A meta-analysis of impacts of alien vs. native plants on pollinator visitation and reproductive success of co-flowering native plants. *Ecology Letters*, *12*(7), 716–728. https://doi.org/10.1111/j.1461-0248.2009.01319.x

Moreira-Hernández, J. I., & Muchhala, N. (2019). Importance of pollinator-mediated interspecific pollen transfer for angiosperm evolution. *Annual Review of Ecology, Evolution, and Systematics*, *50*(1), 191–217. https://doi.org/10.1146/annurev-ecolsys-110218-024804

Mosquin, T. (1971). Competition for pollinators as a stimulus for the evolution of flowering time as a stimulus for for pollinators competition of flowering time the evolution. *Oikos*, *22*, 398–402.

Mothershead, K., & Marquis, R. J. (2000). Fitness impacts of herbivory through indirect effects on plant-pollinator interactions in *Oenothera macrocarpa*. *Ecology*, *81*(1), 30–40. https://doi.org/10.1890/0012-9658(2000)081[0030:fiohti]2.0.co;2

Muchhala, N., & Thomson, J. D. (2012). Interspecific competition in pollination systems: costs to male fitness via pollen misplacement. *Functional Ecology*, *26*(2), 476–482. https://doi.org/10.1111/j.1365-2435.2011.01950.x

Murphy, S. D., & Aarssen, L. W. (1995). Allelopathic pollen extract from Phleum pratense L. (Poaceae) reduces germination, in vitro, of pollen of sympatric species. *International Journal of Plant Sciences*, *156*(4), 425–434. https://doi.org/10.1086/297264

Nesom, G. L. (1989). The Solidago canadensis (Asteraceae: Astereae) complex in Texas with a new species from Texas and Mexico. *Phytologi*, *67*(6), 441–450.

Newcomb, L. (1977). *Newcomb's wildflower guide* (1st ed.). Little, Brown and Company.

Noreika, N., Bartomeus, I., Winsa, M., Bommarco, R., & Öckinger, E. (2019). Pollinator foraging flexibility mediates rapid plant-pollinator network restoration in semi-natural grasslands. *Scientific Reports*, *9*(1), 1–11. https://doi.org/10.1038/s41598-019-51912-4

Olesen, J. M., Bascompte, J., Dupont, Y. L., & Jordano, P. (2007). The modularity of pollination networks. *Proceedings of the National Academy of Sciences*, *104*(50), 19891–19896. https://doi.org/10.1073/pnas.0706375104

Olesen, J. M., Bascompte, J., Elberling, H., & Jordano, P. (2008). Temporal dynamics in a pollination network. *Ecology*, *89*(6), 1573–1582. https://doi.org/10.1890/07-0451.1

Olito, C., & Fox, J. W. (2015). Species traits and abundances predict metrics of plant-pollinator network structure, but not pairwise interactions. *Oikos*, *124*(4), 428–436. https://doi.org/10.1111/oik.01439

Oliver, T. H., Heard, M. S., Isaac, N. J. B., Roy, D. B., Procter, D., Eigenbrod, F., Freckleton, R., Hector, A., Orme, C. D. L., Petchey, O. L., Proença, V., Raffaelli, D., Suttle, K. B., Mace, G. M., Martín-López, B., Woodcock, B. A., & Bullock, J. M. (2015). Biodiversity and Resilience of Ecosystem Functions. *Trends in Ecology and Evolution*, *30*(11), 673–684. https://doi.org/10.1016/j.tree.2015.08.009

Ollerton, J., Winfree, R., & Tarrant, S. (2011). How many flowering plants are pollinated by animals? *Oikos*, *120*(3), 321–326. https://doi.org/10.1111/j.1600-0706.2010.18644.x

Page, M. L., Ison, J. L., Bewley, A. L., Holsinger, K. M., Kaul, A. D., Koch, K. E., Kolis, K. M., & Wagenius, S. (2019). Pollinator effectiveness in a composite: a specialist bee pollinates more florets but does not move pollen farther than other visitors. *American Journal of Botany*, *106*(11), 1487–1498. https://doi.org/10.1002/ajb2.1383

Parachnowitsch, A. L., Raguso, R. A., & Kessler, A. (2012). Phenotypic selection to increase floral scent emission, but not flower size or colour in bee-pollinated *Penstemon digitalis*. *New Phytologist*, *195*(3), 667–675. https://doi.org/10.1111/j.1469-8137.2012.04188.x

Parra-Tabla, V., Alonso, C., Ashman, T. L., Raguso, R. A., Albor, C., Sosenski, P., Carmona, D., & Arceo-Gómez, G. (2020). Pollen transfer networks reveal alien species as main heterospecific pollen donors with fitness consequences for natives. *Journal of Ecology*, *108*, 1–13. https://doi.org/10.1111/1365-2745.13520

Petanidou, T., Kallimanis, A. S., Tzanopoulos, J., Sgardelis, S. P., & Pantis, J. D. (2008). Long-term observation of a pollination network: Fluctuation in species and interactions, relative invariance of network structure and implications for estimates of specialization. *Ecology Letters*, *11*(6), 564–575. https://doi.org/10.1111/j.1461-0248.2008.01170.x

Pleasants, J. M. (1980). Competition for bumblebee pollinators in Rocky Mountain plant communities. *Ecology*, *61*(6), 1446–1459. https://doi.org/10.2307/1939053

Poisot, T., Canard, E., Mouillot, D., Mouquet, N., & Gravel, D. (2012). The dissimilarity of species interaction networks. *Ecology Letters*, *15*(12), 1353–1361. https://doi.org/10.1111/ele.12002

Poisot, T., Stouffer, D. B., & Gravel, D. (2015). Beyond species: Why ecological interaction networks vary through space and time. *Oikos*, *124*(3), 243–251. https://doi.org/10.1111/oik.01719

Potts, S. G., Imperatriz-Fonseca, V., Ngo, H. T., Aizen, M. A., Biesmeijer, J. C., Breeze,

T. D., Dicks, L. V., Garibaldi, L. A., Hill, R., Settele, J., & Vanbergen, A. J. (2016). Safeguarding pollinators and their values to human well-being. *Nature*, *540*(7632), 220–229. https://doi.org/10.1038/nature20588

Primack, R. B., & Silander, J. A. (1975). Measuring the relative importance of different pollinators to plants. *Nature, 255*(5504), 143–144. https://doi.org/10.1038/255143a0

Pyke, G. H. (1978). Optimal foraging: Movement patterns of bumblebees between inflorescences. *Theoretical Population Biology*, *13*(1), 72–98. https://doi.org/10.1016/0040-5809(78)90036-9

R Core Team. (2022). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing. https://www.r-project.org/

Randall, J. L., & Hilu, K. W. (1990). Interference through improper pollen transfer in mixed stands of *Impatiens capensis* and *I. pallida* (Balsaminaceae). *American Journal of Botany*, *77*(7), 939–944. https://doi.org/10.2307/2444509

Randle, A. M., Spigler, R. B., & Kalisz, S. (2018). Shifts to earlier selfing in sympatry may reduce costs of pollinator sharing. *Evolution*, *72*(8), 1587–1599. https://doi.org/10.1111/evo.13522

Rasmussen, C., Dupont, Y. L., Mosbacher, J. B., Trojelsgaard, K., & Olesen, J. M. (2013). Strong Impact of Temporal Resolution on the Structure of an Ecological Network. *Plos One*, *8*(12). https://doi.org/10.1371/journal.pone.0081694

Rathcke, B. J. (1983). Competition and facilitation among plants for pollination. In

Pollination Biology (pp. 205–329). Academic Press.

Rhodes, M. K., Fant, J. B., & Skogen, K. A. (2017). Pollinator identity and spatial isolation influence multiple paternity in an annual plant. *Molecular Ecology*, *26*(16), 4296–4308. https://doi.org/10.1111/mec.14115

Roll, J., Mitchell, R. J., Cabin, R. J., & Marshall, D. L. (1997). Reproductive success increases with local density of conspecifics in a desert mustard. *Conservation Biology*, *11*(3), 738–746. https://doi.org/10.1046/j.1523-1739.1997.96013.x

Sahli, H. F., & Conner, J. K. (2006). Characterizing ecological generalization in plant-pollination systems. *Oecologia*, *148*(3), 365–372. https://doi.org/10.1007/s00442-006-0396-1

Sahli, H. F., & Conner, J. K. (2007). Visitation, effectiveness, and efficiency of 15 genera of visitors to wild radish, *Raphanus raphanistrum* (Brassicaceae). *American Journal of Botany*, *94*(2), 203–209. https://doi.org/10.3732/ajb.94.2.203

Silander, J., & Primack, R. (1978). Pollination intensity and seed set in the evening primrose (*Oenothera fruticosa*). *The American Midland Naturalist*, *100*(1), 213–216.

Simanonok, M. P., & Burkle, L. A. (2014). Partitioning interaction turnover among alpine pollination networks: Spatial, temporal, and environmental patterns. *Ecosphere*, *5*(11), 1–17. https://doi.org/10.1890/ES14-00323.1

Smith, G. P., Bronstein, J. L., & Papaj, D. R. (2019). Sex differences in pollinator behavior: Patterns across species and consequences for the mutualism. *Journal of Animal Ecology*, *88*(7), 971–985. https://doi.org/10.1111/1365-2656.12988

Smith, G. X., Swartz, M. T., & Spigler, R. B. (2021). Causes and consequences of variation in heterospecific pollen receipt in Oenothera fruticosa. *American Journal of Botany*, *108*(9), 1612–1624. https://doi.org/10.1002/ajb2.1720

Smithson, A. (2001). Pollinator preference, frequency dependence, and floral evolution. In *Cognitive Ecology of Pollination* (pp. 237–258). Cambridge University Press. https://doi.org/10.1017/CBO9780511542268.013

Souza, C. S., Maruyama, P. K., Santos, K. C. B. S., Varassin, I. G., Gross, C. L., & Araujo, A. C. (2021). Plant-centred sampling estimates higher beta diversity of interactions than pollinator-based sampling across habitats. *New Phytologist*, 2501–2512. https://doi.org/10.1111/nph.17334

Spigler, R. B., & Chang, S. M. (2008). Effects of plant abundance on reproductive success in the biennial *Sabatia angularis* (Gentianaceae): spatial scale matters. *Journal of Ecology*, *96*(2), 323–333. https://doi.org/10.1111/j.1365-2745.2007.01335.x

Stone, G. N., Willmer, P., & Alexandra Rowe, J. (1998). Partitioning of pollinators during flowering in an African *Acacia* community. *Ecology*, *79*(8), 2808–2827. https://doi.org/10.1890/0012-9658(1998)079[2808:popdfi]2.0.co;2

Stout, J. C., Allen, J. A., & Goulson, D. (1998). The influence of relative plant density and floral morphological complexity on the behaviour of bumblebees. *Oecologia*, *117*(4), 543–550. https://doi.org/10.1007/s004420050691

Stout, J. C., & Tiedeken, E. J. (2017). Direct interactions between invasive plants and native pollinators: evidence, impacts and approaches. *Functional Ecology*, *31*(1), 38–46. https://doi.org/10.1111/1365-2435.12751

Suárez-Mariño, A., Arceo-Gómez, G., Sosenski, P., & Parra-Tabla, V. (2019). Patterns and effects of heterospecific pollen transfer between an invasive and two native plant species: the importance of pollen arrival time to the stigma. *American Journal of Botany*, *106*(10), 1308–1315. https://doi.org/10.1002/ajb2.1361

Szigeti, V., Kőrösi, Á., Harnos, A., & Kis, J. (2019). Lifelong foraging and individual specialisation are influenced by temporal changes of resource availability. *Oikos*, *128*(5), 649–658. https://doi.org/10.1111/oik.05400

Thebault, E., & Fontaine, C. (2010). Stability of ecological communities and the architecture of mutualistic and trophic networks. *Science*, *329*(5993), 853–856. https://doi.org/10.1126/science.1188321

Thompson, J. N. (1999). The evolution of species interactions. In *Science*. https://doi.org/10.1126/science.284.5423.2116

Thomson, J. D., Andrews, B. J., & Plowright, R. C. (1982). The effect of a foreign pollen on ovule development in Diervilla lonicera (Caprifoliaceae). *New Phytologist*, *90*(4), 777–783. https://doi.org/10.1111/j.1469-8137.1982.tb03286.x

Thomson, J. D., Fung, H. F., & Ogilvie, J. E. (2019). Effects of spatial patterning of co-flowering plant species on pollination quantity and purity. *Annals of Botany*, *123*(2), 303–310. https://doi.org/10.1093/aob/mcy120

Tong, Z. Y., & Huang, S. Q. (2016). Pre- and post-pollination interaction between six co-flowering Pedicularis species via heterospecific pollen transfer. *The New Phytologist*, *211*(4), 1452–1461. https://doi.org/10.1111/nph.14005

Trøjelsgaard, K., & Olesen, J. M. (2016). Ecological networks in motion: micro- and macroscopic variability across scales. *Functional Ecology*, *30*(12), 1926–1935. https://doi.org/10.1111/1365-2435.12710

Tur, C., Sáez, A., Traveset, A., & Aizen, M. A. (2016). Evaluating the effects of pollinator-mediated interactions using pollen transfer networks: evidence of widespread facilitation in south Andean plant communities. *Ecology Letters*, *19*(5), 576–586. https://doi.org/10.1111/ele.12594

Tur, C., Vigalondo, B., Trøjelsgaard, K., Olesen, J. M., & Traveset, A. (2014). Downscaling pollen-transport networks to the level of individuals. *Journal of Animal Ecology*, *83*(1), 306–317. https://doi.org/10.1111/1365-2656.12130

Tylianakis, J. M., Laliberté, E., Nielsen, A., & Bascompte, J. (2010). Conservation of species interaction networks. *Biological Conservation*, *143*(10), 2270–2279. https://doi.org/10.1016/j.biocon.2009.12.004

Valdovinos, F. S. (2019). Mutualistic networks: moving closer to a predictive theory. *Ecology Letters*, *22*(9), 1517–1534. https://doi.org/10.1111/ele.13279

Valdovinos, F. S., Brosi, B. J., Briggs, H. M., Moisset de Espanés, P., Ramos-Jiliberto, R., & Martinez, N. D. (2016). Niche partitioning due to adaptive foraging reverses effects of nestedness and connectance on pollination network stability. *Ecology Letters*, *19*(10), 1277–1286. https://doi.org/10.1111/ele.12664

Valdovinos, F. S., Moisset de Espanés, P., Flores, J. D., & Ramos-Jiliberto, R. (2013). Adaptive foraging allows the maintenance of biodiversity of pollination networks. *Oikos*, *122*(6), 907–917. https://doi.org/10.1111/j.1600-0706.2012.20830.x

Valiente-Banuet, A., Aizen, M. A., Alcántara, J. M., Arroyo, J., Cocucci, A., Galetti, M., García, M. B., García, D., Gómez, J. M., Jordano, P., Medel, R., Navarro, L., Obeso, J. R., Oviedo, R., Ramírez, N., Rey, P. J., Traveset, A., Verdú, M., & Zamora, R. (2015). Beyond species loss: The extinction of ecological interactions in a changing world. *Functional Ecology*. https://doi.org/10.1111/1365-2435.12356

Vazquez, D. P., & Aizen, M. A. (2004). Asymmetric specialization: a pervasive feature of plant-pollinator interactions. *Ecology*, *85*(5), 1251–1257. https://doi.org/10.1890/03-3112

Vázquez, D. P., & Aizen, M. A. (2004). Asymmetric specialization: a pervasive feature of plant-pollinator interactions. *Ecology*, *85*(5), 1251–1257. https://doi.org/10.1890/03-3112

Vázquez, D. P., Bluthgen, N., Cagnolo, L., & Chacoff, N. P. (2009). Uniting pattern and process in plant-animal mutualistic networks: A review. *Annals of Botany*, *103*(9), 1445–1457. https://doi.org/10.1093/aob/mcp057

Vázquez, D. P., Lomascolo, S. B., Belen Maldonado, M., Chacoff, N. P., Dorado, J., Stevani, E. L., & Vitale, N. L. (2012). The strength of plant-pollinator interactions. *Ecology*, *93*(4), 719–725. https://doi.org/10.1890/11-1356.1

Vázquez, D. P., Morris, W. F., & Jordano, P. (2005). Interaction frequency as a surrogate for the total effect of animal mutualists on plants. *Ecology Letters*, *8*(10), 1088–1094. https://doi.org/10.1111/j.1461-0248.2005.00810.x

Waser, N. M. (1978). Competition for hummingbird pollination and sequential flowering in two colorado wildflowers. *Ecology*, *59*(5), 934–944. https://doi.org/10.2307/1938545

Waser, N. M. (1986). Flower constancy: definition, cause, and measurement. *The American Naturalist*, *127*(5), 593–603.

Waser, N. M., Chittka, L., Price, M. V, Williams, N. M., & Ollertons, J. (1996). *Emphasizing new ideas to stimulate research in ecology: Generalization in pollination systems, and why it matters*. *77*(4), 1043–1060.

Weinstein, B. G. (2015). MotionMeerkat: integrating motion video detection and ecological monitoring. *Methods in Ecology and Evolution*, *6*(3), 357–362. https://doi.org/10.1111/2041-210x.12320

Weinstein, B. G., & Graham, C. H. (2017). Persistent bill and corolla matching despite shifting temporal resources in tropical hummingbird-plant interactions. *Ecology Letters*, *20*(3), 326–335. https://doi.org/10.1111/ele.12730

Winfree, R., Fox, J. W., Williams, N. M., Reilly, J. R., & Cariveau, D. P. (2015). Abundance of common species, not species richness, drives delivery of a real-world ecosystem service. *Ecology Letters*, *18*(7), 626–635. https://doi.org/10.1111/ele.12424

APPENDIX A
List of Pollinator Morphospecies

Allograpta obliqua
Anatrytone logan
Ancyloxypha numitor
Andrena spp.
Anoplius spp.
Anthidiellum notatum notatum
Anthidium spp.
Anthomyiidae spp.
Anthophora spp.
Apis mellifera
Archytas apicifer
Archytas aterrimus
Archytas metallicus
Atalopedes campestris
Augochlora pura
Augochlorella aurata
Augochloropsis spp.
Belvosia unifasciata
Boloria bellona
Bombus bimaculatus
Bombus fervidus
Bombus griseocollis
Bombus impatiens
Bombus perplexus
Bombus vagans
Calliphoridae spp.
Calopteran spp.
Calpodes ethlius
Ceratina spp.
Chaetogaedia spp.
Chalcosyrphus piger
Chauliognathus marginatus
Chauliognathus pensylvanicus
Chimarra spp.
Chrysididae spp.
Chrysotoxum spp.
Coelioxys spp.
Coleomegilla maculata
Coleoptera 1
Coleoptera 2
Coleoptera 3
Coleoptera 4
Coleoptera 5

Appendix A (continued)

Coleoptera 6
Colias eurytheme
Colias philodice
Condylostylus spp.
Conopidae spp.
Conura spp.
Crabroninae spp.
Cylindromyia spp.
Danaus plexippus
Diabrotica undecimpunctata howardi
Didea fuscipes
Diptera 1
Dolichovespula arenaria
Elateridae spp.
Empididae spp.
Enoclerus spp.
Entypus spp.
Epargyreus clarus
Epistrophe grossulariae
Eristalis arbustorum
Eristalis stipator
Eristalis tenax
Eristalis transversa
Ernestiini spp.
Eryciini spp.
Euaresta spp.
Eumenes fraternus
Eumenidae spp.
Eupeodes americanus/pomus
Euphyes vestris
Genea pavonacea
Gnadochaeta spp.
Goniini spp.
Gymnoclytia spp.
Gymnosoma spp.
Halictus confusus
Halictus ligatus
Halictus rubicundus
Harmonia spp.
Heliophilus faciatus
Heliophilus latifrons
Hemaris diffinis
Hemaris gracilis

Appendix A (continued)

Hemaris thysbe
Hesperia leonardus
Hoplitis spp.
Hylaeus spp.
Hymenoptera 1
Ichneumonidae spp.
Isodontia apicalis
Junonia coenia
Juriniopsis spp.
Lampyridae spp.
Lasioglossum spp.
Lebia spp.
Lucilia spp.
Mallota bautias
Megachile spp.
Melissodes spp.
Meloidae spp.
Milesia virginiensis
Mordellidae spp.
Muscidae spp.
Myrmecothea spp.
Nastra Iherminier
Noctuidae spp.
Nomada spp.
Bomyliidae spp.
Ocyptamus spp.
Orthonevra nitida
Osmia spp.
Palpada vinetorum
Papilio glaucus
Papilio troilus
Paragus spp.
Paranthidium/Anthidium spp.
Parhelophilus spp.
Peleteria haemorrhoa
Pepsis menechma
Phasia spp.
Phyciodes cocyta
Phyciodes tharos
Pieris rapae
Pipizini spp.
Plagiomima spp.
Poanes zabulon

Appendix A (continued)

Polistes spp.
Polites origenes
Polites peckius
Polites themistocles
Pollenia spp.
Pompeius verna
Popillia japonica
Propylea quatuordecimpuncta
Pseudoanthidium nanum
Pterophoridae spp.
Pyrausta spp.
Rhagoletis spp.
Sarcophagid spp.
Scolia bicincta
Scythrididae spp.
Siphona spp.
Speyeria aphrodite
Speyeria cybele
Speyeria idalia
Sphaerophoria novaeangliae/contigua/pyrrhina
Sphaerophoria philanthus/asymmetrica/abbreviata
Sphex ichneumoneus
Spilomyia alcimus
Spilomyia longicornis
Strongygaster spp.
Syritta pipiens
Syrphus spp.
Tetraopes tetrophthalmus
Thyris maculata
Toxomerus geminatus
Toxomerus marginatus
Toxomerus politus
Trichopoda pennipes
Tropidia albistylum
Tropidia quadrata
Typocerus velutinus
Unknown small moth
Vanessa virginiensis
Vespula spp.
Villa spp.
Wallengrenia egeremet
Xylocopa virginica
Zodion spp.