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Congruence and Temporal Variation of Floral Visitation and Pollen Transport Networks in  
Southern Appalachia

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A thesis

presented to

the faculty of the Department of Biological Sciences

East Tennessee State University

In partial fulfillment

of the requirements for the degree

Master of Science in Biology

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by

Daniel Barker

August 2020

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Dr. Darrell Moore

Keywords: Floral Visitation, Pollen Transport, Ecology, Plant-Pollinator Interactions

## ABSTRACT

### Congruence and Temporal Variation of Floral Visitation and Pollen Transport Networks in Southern Appalachia

by

Daniel A. Barker

Observation of floral visitation is an accepted method to describe plant-pollinator interactions despite potential biases. Collecting pollen from pollinators offers new insights on the structure and function of plant-pollinator communities. Furthermore, the strength and frequency of plant-pollinator interactions can vary across temporal scales. However, within-season and within-day (morning vs. evening) variation in plant-pollinator networks has been little studied. By evaluating variation in network structure across these biologically relevant time scales, we will gain a better understanding of the factors that shape plant-pollinator communities. The objectives of this study are to 1) Compare the structure of plant-pollinator networks built on floral visitation and pollen transport data, 2) Evaluate intra-annual variation in plant-pollinator network structure and 3) Evaluate variation in plant-pollinator structure within a single day (i.e. morning vs. evening).

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## CHAPTER 1. INTRODUCTION

### *Introduction*

Up to 96% of flowering plants world-wide are dependent on animal pollinators for successful reproduction (Ollerton et al. 2011). Such dependency on animal pollinators has been a key force in generating, maintaining, and organizing plant diversity in nature, specifically among angiosperm species that depend heavily on interactions with pollinators. (Bronstein et al. 2006; Thébault and Fontaine 2010; Valverde et al. 2018). For instance, these mutualistic interactions can be important in mediating floral trait evolution, plant distribution, plant species establishment and reproductive success, as well as in mediating plant coexistence (Bronstein et al. 2006; Fenster et al. 2004). However, our understanding of the ecological and evolutionary consequences of plant-pollinator interactions largely relies on the study of interactions between a single plant and pollinator species (Olesen et al. 2011; Trøjelsgaard and Olesen 2016), even though these interactions take place within larger plant and pollinator communities where multiple species can interact directly and/or indirectly (Jordán et al. 2008). Thus, improving our understanding of the importance of community-wide plant-pollinator interactions and their role in community assembly, plant evolution and reproductive success is crucial for predicting community-wide responses to changes in entire ecosystems as a result of human disturbances (e.g. extinction and introduction of species, habitat loss) (Memmott et al. 2004; Katariya et al. 2017; Pérez-Méndez et al. 2018).

*Ecological Network Theory.* The complexity of species interactions at the community level has recently been revealed by the use of network theory and analysis (Popic et al. 2013; Nielsen and Totland 2014). Mutualistic interactions are represented in a network framework with top and bottom nodes representing plant and pollinator species with links representing the

interactions between them (Figure 1; Palla et al. 2005; Mason and Verwoerd 2006; Pocock et al. 2016).

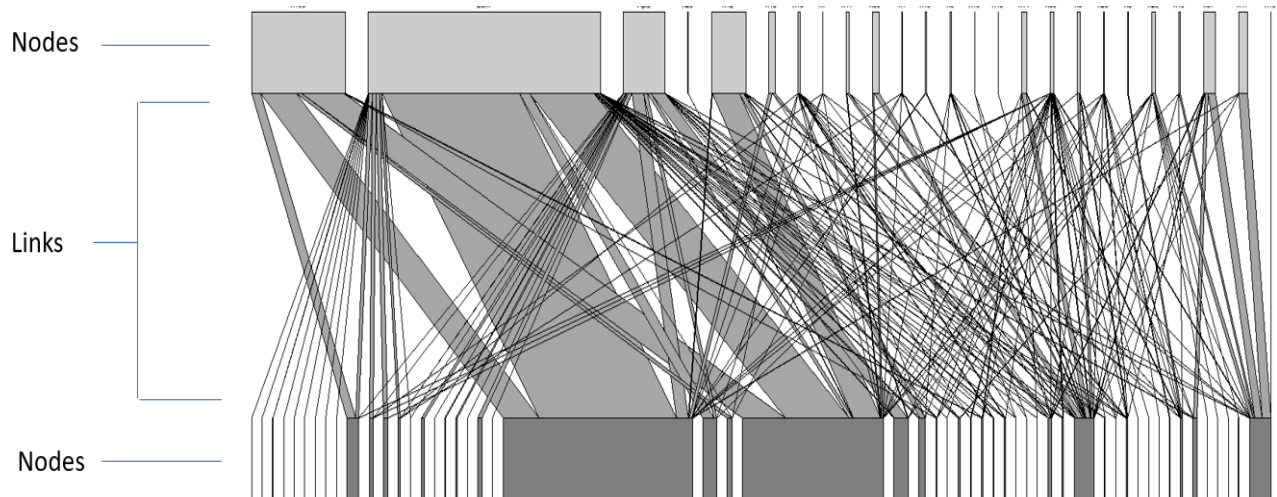


Figure 1: *Bipartite Network Layout*. Example of bipartite network with floral visitors represented by the top row of rectangles and flowering plant species represented by the rectangles on the bottom row. Each rectangle or node represents a species and the lines between the rows indicate the links or interactions between species.

Utilizing network theory to study mutualistic interactions also helps integrate information on the identity, diversity and strength (i.e. frequency) of interactions to more fully assess their importance. Thus far, evaluating mutualistic interactions within a community has helped uncover fundamental characteristics in the structure of these interactions (Pocock et al. 2016). For example, network studies have shown that plant-pollinator interactions are often more generalized (interactions among multiple species in a community) than specialized (one to one interactions) (Ferry-Graham 2002; Ollerton et al. 2006).

Plant-pollinator networks also have been shown to have specialist species interacting with generalist species (i.e. nestedness), a pattern with implications for the function and stability of communities (Olesen et al. 2007; Jędrzejewska-Szmek and Zych 2013). For instance, highly

nested communities have been shown to be more robust, having a greater ability to withstand disturbances, as the redundancy of interactions may help communities buffer against species loss (Nielsen and Totland 2014). Thus, evaluating the structure of community-level plant-pollinator interactions can help advance our understanding of the impacts of human-mediated disturbances on plant and pollinator communities.

*Floral Visitation and Pollen Transport Networks.* Previous studies on plant-pollinator network structure have been based on information about floral visitation patterns by animal pollinators (Alarcón 2010). However, floral visitation may not accurately characterize the realized diversity and strength of plant-pollinator interactions that are present in a community (Vanbergen et al. 2014). For, instance, visitation by a pollinator to a flower may not result in pollen transport (King et al. 2013). Thus, floral visitors that move across flowers without picking up pollen (and thus not transporting pollen), may have an inflated level of importance and lead to a biased representation of interaction network structure within a community (King et al. 2013). This, in turn, may lead to an inaccurate assessment of community stability and tolerance to disturbances.

By using information on the identity and amount of pollen grains found on the bodies of floral visitors (i.e. pollen transport), instead of floral visitation patterns, we may be able to gain better insights into the structure of pollination networks (Tur et al. 2014). For instance, the use of pollen data from floral visitors may reveal the existence of plant-pollinator interactions that may be hard to detect through pollinator observations alone, i.e. rare interactions. (Jędrzejewska-Szmek and Zych 2013). Furthermore, the resulting pollen transport networks may provide a more functionally relevant depiction of plant-pollinator interactions as these will represent actual pollen pick up and transport by floral visitors (i.e. the quantity and identity of pollen that is

carried by a pollinator), and not just flower visitation events (Devoto et al. 2011; Jędrzejewska-Szmek and Zych 2013; Tur et al. 2014). Thus, the limitations of floral visitation networks may be overcome by the use of pollen transport networks hence advancing our understanding of the structure of plant pollinator communities.

*Temporal Variation in Pollination Networks.* As species composition changes, the structure of plant-pollinator interaction networks is also subject to change (Burkle and Alarcón 2011). For example, pollination networks have been shown to vary temporally with up to 70% species turnover in a four-year period (Petanidou et al. 2008). However, plant-pollinator interactions also have the potential to change within a single flowering season, as pollinator preferences change with addition or loss of floral resources throughout the season (CaraDonna et al. 2017). Even though among-year variation in network structure is well documented, within-annual variability in network structure has largely been overlooked (Olesen et al. 2008; CaraDonna et al. 2017). Thus, how pollination network structure changes over short periods of time (i.e. the Summer season) is largely unknown (CaraDonna et al. 2017). Variation in the identity and frequency of interactions for any given plant or pollinator species may also occur over the course of a single day. As a result, sampling plant-pollinator communities at a specific time of day, as opposed to sampling throughout the day, can result in the exclusion of interactions that may be exclusive to certain times of days (i.e. morning to afternoon). For instance, certain plant species are known to produce floral rewards only during the early morning and are, thus, unavailable to floral visitors active later in the day (Schlising 2015). Thus, by evaluating the structure of plant-pollinator interactions at more biologically relevant time scales, i.e. those that take into account variation in species phenologies, a more ecologically relevant

understanding of plant-pollinator network structure and function can be gain (Burkle and Alarcón 2011).

In order to address these shortcomings in our current understanding of plant-pollinator interactions, the objectives of this study are to 1) Compare the congruence of the structure of plant-pollinator networks built on floral visitation and pollen transport data, 2) Evaluate intra-annual variation in plant-pollinator network structure (i.e. over the course of a single season), and 3) Evaluate variation in plant-pollinator structure within a single day (i.e. morning vs. evening).

## CHAPTER 2. CONGRUENCE AND TEMPORAL VARIATION OF FLORAL VISITATION AND POLLEN TRANSPORT NETWORKS IN SOUTHERN APPALACHIA

### *Introduction*

Ecological interactions do not occur in isolation, rather they take place within large communities where multiple species can interact directly and/or indirectly (Jordán et al. 2008; Burkle and Alarcón 2011; Carstensen et al. 2014; Grover et al. 2017). The makeup of these interactions can ultimately help shape the structure and stability of the community. For example, the direction and strength of interactions between plants and their animal pollinators can alter plant reproductive success, influence the evolution of floral traits and mediate plant community composition via pollinator preference for certain floral characteristics (Fenster et al. 2004; Bronstein et al. 2006). Therefore, a thorough understanding of the role of plant-pollinator interactions in shaping plant communities is essential when predicting community-level responses to human disturbances (e.g. extinction and introduction of species, habitat loss) (Memmott et al. 2004; Katariya et al. 2017; Pérez-Méndez et al. 2018).

The complexity of actual community interactions has placed analytical approaches to community analysis out of reach. Although, network theory has been successfully applied to the study of plant-animal interactions (Palla et al. 2005; Mason and Verwoerd 2006; Popic et al. 2013; Pocock et al. 2016). The use of network theory to the study of plant-animal interactions has revealed key generalities in the assembly of plant-pollinator communities and how these may impact the function of those assemblages (Ollerton et al. 2006; Jędrzejewska-Szmek and Zych 2013; Pocock et al. 2016). For example, highly connected plant-animal communities have been shown to possess higher levels of robustness when faced with disturbance (e.g. species invasion, extinction) as opposed to communities that are not stressed by disturbance, mainly due to rewiring, i.e. the presence of redundant interactions (Nielsen and Totland 2014). Evaluation of

plant-pollinator communities using network theory, thus allows for the simplification of complex sets of interactions such that the structure and potential implications of those interactions can be evaluated.

Observance of floral visitation has been used as a means to identify interactions between plants and their animal floral visitors in a network context (i.e. links) (Dupont et al. 2003; Alarcón 2010; Popic et al. 2013). However, floral observation alone may overlook potential differences in the quality of floral visits (i.e. transport or deposition of pollen) and can result in the inclusion of floral visitors that are not actively participating in the transfer of pollen (King et al. 2013). Furthermore, the accuracy of networks built from floral observation is highly dependent on the amount of sampling, as some pollination events may be difficult to observe. As a result, rare interactions have greater potential for exclusion when relying on floral observations (Jędrzejewska-Szmek and Zych 2013). Thus, networks derived from floral observations can be incomplete and lead to mischaracterization of plant-pollinator community structure (Alarcón 2010; Jędrzejewska-Szmek and Zych 2013; Popic et al. 2013).

Alternatively, sampling the surface pollen loads of floral visitors (i.e. pollen transport) and identifying pollen grains to assess patterns of pollinator visitation (i.e. determine which plants have been visited) can overcome some of the potential biases produced by the use of floral visitation data alone (Tur et al. 2014). Furthermore, pollen transport data reveals non-pollinating individuals (i.e. do not vary pollen) that may have been considered an active pollinator when only relying on floral observations. In addition, pollen transport networks can provide information on the quality of plant-pollinator interactions by identifying the types and quantities of pollen grains picked up by floral visitors, thereby, providing more functionally relevant information (Jacobs et al. 2010). Finally, quantification of pollen transport by insects may reduce

the chance of losing rare interactions that are harder to observe (Jędrzejewska-Szmek and Zych 2013). Overall, the use of pollen transport networks, may help achieve a more accurate characterization of network properties and thus of the function and stability of plant-pollinator communities.

Perceived plant-pollinator network structure and function may also be affected by the timing of sampling. Pollination networks have traditionally been examined over large spans of time, including one (i.e. whole summer) or multiple flowering seasons (Basilio et al. 2006; Olesen et al. 2008, 2011). Plant-pollinator interactions however, have been shown to vary between flowering seasons with as much as 25% turnover within plant and pollinator communities (Petanidou et al. 2008). Temporal variability in pollination networks not only occurs among seasons but may also occur within the same flowering season. Within-season variability may occur due to differences in flowering phenology among plant species as well as variation in the timing of activity of pollinators during the flowering season (Olesen et al. 2008; CaraDonna et al. 2017). In this sense, plant-pollinator interactions can be expected to change as pollinator preferences for floral resources change with changes in the availability of floral resources and as new pollinators emerge (Fowler et al. 2016; CaraDonna et al. 2017). Evaluating changes in network structure within a single season may thus avoid biases that result from the inclusion of links that are ecologically impossible due to differences in species phenology (i.e. forbidden links; Olesen et al. 2010). However, studies that evaluate within season variation in network structure are scarce. It is also known that pollination network structure varies between diurnal and nocturnal periods, due to changes in pollinator composition (Devoto et al. 2011). In this sense, it is also possible that plant-pollinator interactions may vary across the scale of a day (i.e. morning vs afternoon). Certain plant species have been shown to produce floral rewards at



certain times of day, making them unavailable to pollinators whose activity does not coincide with the availability of those resources (Masierowska 2012; Schlising 2015). Therefore, studying plant-pollinator interactions at and how these may change within a single season and within a single day may help advance our understanding of plant-pollinator interaction structure and its importance in natural communities.

Here, I intend to compare pollination network structure based on floral visitation and pollen transport and evaluate within-season as well as within-day variation in plant-pollinator network structure.

### *Methods*

*Study Site.* This study was conducted in Hampton Creek Cove State Natural Area (N 36°08.843', W 82°02.794', Elevation: 971m). The study site is an approximately 1.87 ha abandoned agricultural field undergoing secondary succession with mix of annual and perennial, as well as native and non-native plants inhabiting the site (Table 1). The plant community consisted mainly of animal-pollinated species with some grass species intermixed. The study site is bordered by a mixed deciduous forest to the north and agricultural fields to the south. Flowering commenced in late April and early May and continued into late August and early September (Daniels and Arceo-Gómez 2019). Temperature at the study site ranged from 22°C-34°C during the day. There were several federally recognized endangered and threatened plant species located in the study site including: Blue Ridge Goldenrod (*Solidago spithamea*), Roan Mountain Bluet (*Houstonia montana*), and Spreading Avens (*Geum radiatum*) and thus understanding the structure of plant-pollinator interactions in this area is of important conservation concern.

*Pollinator Collection.* To sample the pollinator community, four 1x40 m transects were set up. Each transect was walked at a slow to moderate pace twice per week until a maximum of 60 floral visitors were caught between 8:00 AM and 3:00 PM for each day. To capture within-day temporal change, the transects were also walked once per week after 3:00 PM until a maximum of 30 floral visitors were captured for that day (97 total hours of sampling). Insects were collected with butterfly nets when they were observed visiting the flower's reproductive structures (anthers and stigma). Upon collection, the insect was then placed in a 1.5 mL Eppendorf tube and then placed in a cooler with ice packs. The Eppendorf tube was marked with the transect number, date of capture, the time of capture, and the plant species that the pollinator was captured on.

*Pollen Load Sampling.* Insect pollen loads were collected by swabbing the bodies of the floral visitors collected with fuchsin jelly cubes (Beattie 1971; Kearns and Inouye 1993). The fuchsin jelly was made by mixing 175 ml of distilled water to 150 ml of glycerol and 50 g of gelatin which was then mixed with basic fuchsin crystals (Beattie 1971). For swabbing the insects, the jelly was cut into approximately 3x3x1 mm cubes and then applied to the top and bottom of the thorax and abdomen, the head and mouth parts, antennae if present, and to the legs of each insect. However, the corbiculae of bee species were avoided as the pollen located within it, is not available for deposition on receptive flowers (Johnson and Ashman 2019). Each appendage was swabbed three times to standardize sampling. Fuchsin jelly swabs with pollen samples were then placed on microscope slides and melted over a hot plate before being sealed under a glass cover slip with fingernail polish (Figure 2). The pollen loads of 917 insects were sampled and are represented in this study.

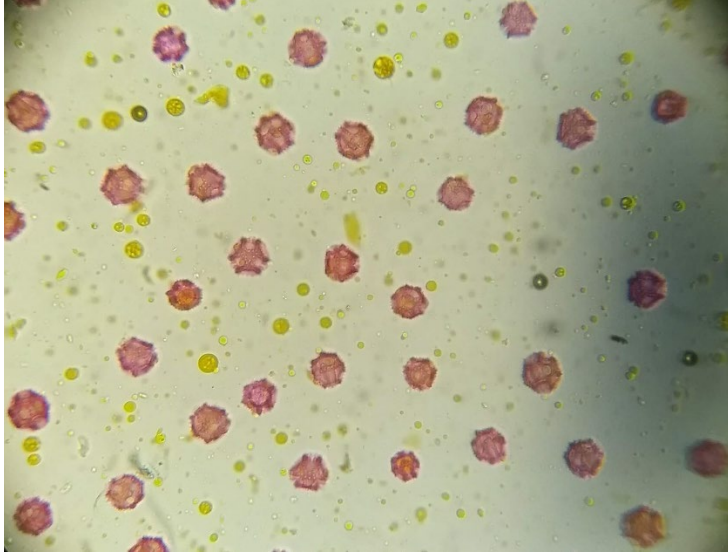


Figure 2: *Fuchsin Stained Pollen*. *Cryptotaenia canadensis* pollen sampled from pollinator in fuchsin jelly.

*Pollen Load Quantification.* After pollen samples had been mounted on microscope slides, each sample was observed under a microscope and all pollen in the sample was identified and counted. Identification of pollen grains was done by reference to an established pollen reference library of all plant species at the study site (Daniels and Arceo-Gómez 2019). Identities of pollen grains was established by the collection of anthers from each plant species at the site. Pollen morphology for each plant species was then catalogued for quick identification of pollen grains. Quantification and identification of pollen grains was done with a compound light microscope at 400x magnification. If identification of pollen could not be confirmed by the pollen reference library, they were marked as unknown. Plant species with similar pollen morphology as determined by the pollen reference library were combined into one group (four species in two groups). In total, 214,346 pollen grains were found and identified to 48 species of plants.

*Insect Identification.* The identities of 1000 insect specimens were confirmed to the lowest taxonomic group possible using several published insect identification guides (Field Guide to Insects of North America, Peterson Field Guides Insects, Field Guide to Insects and Spiders of North America etc.). Where species identity could not be confirmed, individuals were divided into morphogroups. In total 103 morphospecies/groups were found at the study site (Table 2).

### *Analysis*

*Floral Visitation and Pollen Transport Networks.* Pollen load data and floral visitation data were organized into matrices with plant species as columns and insect species as rows. Within each matrix (e.g. pollen transport, floral visitation) the position where each plant species intersects with each insect species indicates the observed number of visits (or pollen grains found on a pollinator species). If no visit/pollen was observed between certain plants and insects, a zero was recorded. For the pollen transport network, the average number of pollen grains per plant species found on each insect group were used in place of floral visits as described above. However, it has been reported that pollinators can pick up pollen during ‘accidental’ visits to flowers that they would not normally visit (i.e. incidental pollination), or pick up multiple pollen grain types on a single flower, and this could overestimate the relevant number of interactions (Ne’Eman et al. 2010). To account for this ‘incidental’ pollination, we only considered as functional plant-pollinator interactions those where five or more pollen grains of a specific plant species were found on a floral visitor (less than five grains total is considered incidental; Johnson and Ashman 2019). We further improved reliability of the network by applying a “5% rule” to the five pollen grain threshold so that pollinator species that have normally carry small pollen loads are not eliminated. Thus, only interactions that total less than five pollen grains and

represented less than 5% of the total pollen load were removed from the matrix. As a result, 455 interactions were removed from the pollen transport network and included interactions from all groups (Hymenoptera, Coleoptera, Lepidoptera, etc.). Bipartite networks and network metrics were then generated using R version 1.143 and the bipartite package version 2.11.

*Temporal Variation in Pollination Networks.* Temporal variability in pollination networks was assessed by partitioning the pollen transport data into 3 four-week intervals: Early, Mid, and Late Summer to coincide with turnover events observed in the plant community (D. Barker pers. obs.). For instance, during previous seasons *Jacobaea vulgaris*, *Glechoma hederacea*, and *Achillea millefolium* were observed in their flowering peak during these three distinct periods in the flowering season (one species in each time period). Matrices for each time period were thus constructed as described above. Temporal variation within a single day was assessed by separating pollen transport data into “morning” and “afternoon”, where morning is considered 8:00 AM-12:59 PM and afternoon is considered 1:00 PM-6:00 PM. These time frames were chosen due to observed phenology of certain plant and animal species at the study site. For instance, *Silene latifolia* flowers were observed to be receptive during the morning hours between 8:00AM and approximately 1:00PM, after which they would close.

*Statistical Analysis.* To determine differences between the generated plant-pollinator networks, a one-way ANOVA was used in R (v3.5.2). For overall comparison of floral visitation and pollen transport networks, 13 weekly matrices for each network were constructed and used as replicates of network type (i.e. floral visitation or pollen transport). These interactions all belong to the same plant and pollinator communities (abundance and diversity), however, they change week to week. Thus, may be considered as distinct plant-pollinator communities. Plant network type (i.e. floral visitation or pollen transport) was then considered as a fixed effect in the

analyses. We evaluated differences in network metrics including weighted connectance, links per species, weighted nestedness, linkage density, and modularity. All these metrics were generated using the “bipartite” package in R (Table 3). Each metric is indicative of network properties that are heavily dependent on the participants and the frequency of their interactions, which may vary temporally and methodologically and are frequently used to describe plant-pollinator network structure (Ponisio et al. 2017; Valido et al. 2019).

Table 1: *Networks Metrics*. List of metrics used in analysis of plant-pollinator networks and their descriptions.

<i>Metric</i>	<i>Definition</i>
<i>Weighted Connectance</i>	Interaction diversity of each network participant weighted by the total number of participants
<i>Weighted Nestedness</i>	Proportion of generalists interacting with specialists weighted by interaction frequency
<i>Modularity</i>	Compartmentalization of networks into a series of interacting nodes
<i>Links per Species</i>	Average number of links per species
<i>Linkage Density</i>	Interaction diversity per network participant

To evaluate within-season variation in pollen transport network structure, the pollen transport data were divided into three segments (early, middle, and late) consisting of four-week intervals. For this model, each segment of the growing season was used as fixed effects with the same metrics described above as response variables. Finally, morning and afternoon comparison (within-day variation) was done by making weekly matrices for morning and afternoon networks which only included days where sampling took place in both morning and afternoon (ten weeks per network). Morning and afternoon were used as fixed effects with the aforementioned metrics as response variables.

In addition to performing the above ANOVAs, a Procrustes analysis was conducted to compare each set of networks (i.e. floral visitation vs pollen transport, early, mid, late growing season, and morning vs afternoon) (Alarcón 2010; Johnson and Ashman 2019). Procrustes analysis determines the differences between shapes and has been used extensively in the assessment of anatomical characters in animals before being adapted to the analysis of mutualistic interactions (Wang et al. 2010; Demayo et al. 2011; Piazzon et al. 2011; Dehling et al. 2016). During a Procrustes analysis, corresponding key points or landmarks are established as part of the test within the two networks being compared. Landmarks in each network are then matched together while attempting to reduce the distance (i.e. the least sum of squares) between them by rotating, inverting, enlarging, or reducing the networks (Alarcón et al. 2008).

In contrast to traditional network metrics (e.g. nestedness, modularity), Procrustes analysis takes into consideration the identity of network nodes (species) and their interactions (links) to determine similarities in network structure. Therefore, Procrustes analysis is a good complement to an ANOVA analysis when determining differences in network structure as it takes into account network species composition and individual species position within a network. On the other hand, ANOVA is useful in determining overall differences in network structure regardless of the position of individual nodes (species) within a network.

### *Results*

In total, 1,447 floral visitors were collected at the study site. Rarefaction analysis showed that my sampling captured 72% of the pollinator community (Figure 3). Of the total number of captured insects, 917 individuals were identified to major taxonomic insect orders (Lepidoptera, Hymenoptera, Hemiptera, Coleoptera, and Diptera) and subsequently processed for pollen loads. In total, 214,346 pollen grains were obtained from the fuchsin jelly swabs. Of the total sampled

pollen grains, 5,448 (4.5%) could not be matched to the pollen reference library and were subsequently classified as “unknown” in 42 groups but were not included in the analysis. The remaining pollen grains (95.5%) were identified to 48 species of plants found at the study site.

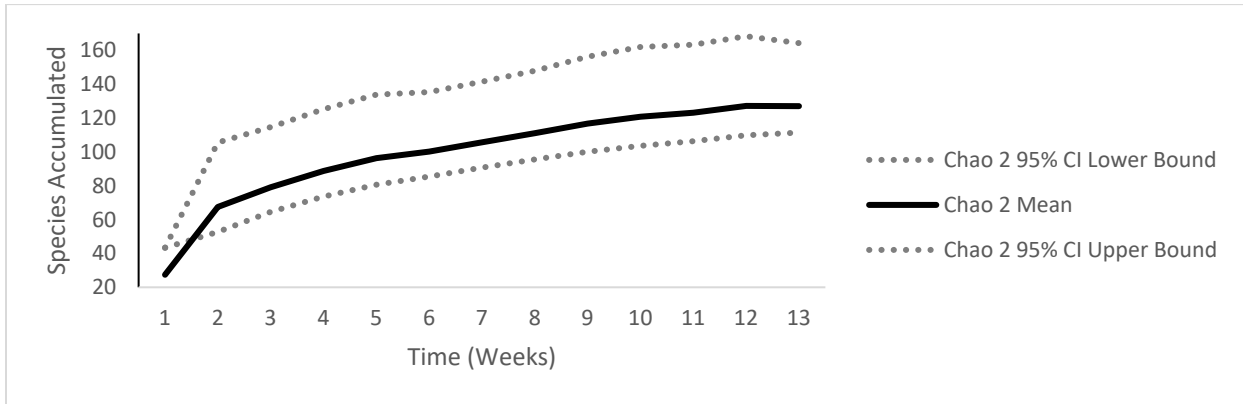


Figure 3: *Sampling Effort*. Species accumulation curve for the study site. Over 13 weeks, 103 insect species were collected, accounting for 72% of the pollinator community when using the Chao 2 estimator.

*Congruence between Floral Visitation and Pollen Transport Networks.* Constructed pollen transport (PT) and floral visitation (FV) networks contained 554 and 375 unique interactions, respectively (Figure 4). Furthermore, there were 95 (PT) and 103 (FV) floral visitors interacting with 43 and 39 plant species, respectively (Figure 4). The networks differed significantly in weighted connectance ( $P=0.02$ ), links per species ( $P=0.0001$ ), and weighted nestedness ( $P=0.006$ ) (Table 4). However, there was no significant difference in Specialization (H2) ( $P=0.06$ ) between the networks (Table 4). Furthermore, Procrustes analysis showed that the pollen transport and floral visitation networks were not significantly different ( $P=0.2$ ).

*Intra-Annual Variation.* Early (ES), Middle (MS), and Late (LS) Summer Networks contained 260, 229, 333 interactions, respectively (Figure 5). Each network contained 51, 45, and 68 active floral visitors and 29, 29, and 34 species of plants, respectively. There was no significant difference between any of the three networks in the number of links per species



(ES:3.25, MS:3.14, LS:3.30;  $P>0.98$ ), weighted nestedness (0.60, 0.66, 0.69,  $P>0.05$ ), linkage density (5.74, 8.41, 9.10;  $P>0.05$ ), weighted connectance (0.07, 0.12, 0.09;  $P>0.05$ ), Specialization ( $H_2$ ) (0.57, 0.55, 0.48;  $P>0.05$ ) (Table 5). Even though there was no significant difference between the network metrics, Procrustes analysis indicated that the ES, MS, and LS networks were all significantly different from each other (ES-MS:  $P=0.028$ ; ES-LS:  $P=0.029$ ; MS-LS:  $P=0.029$ ).

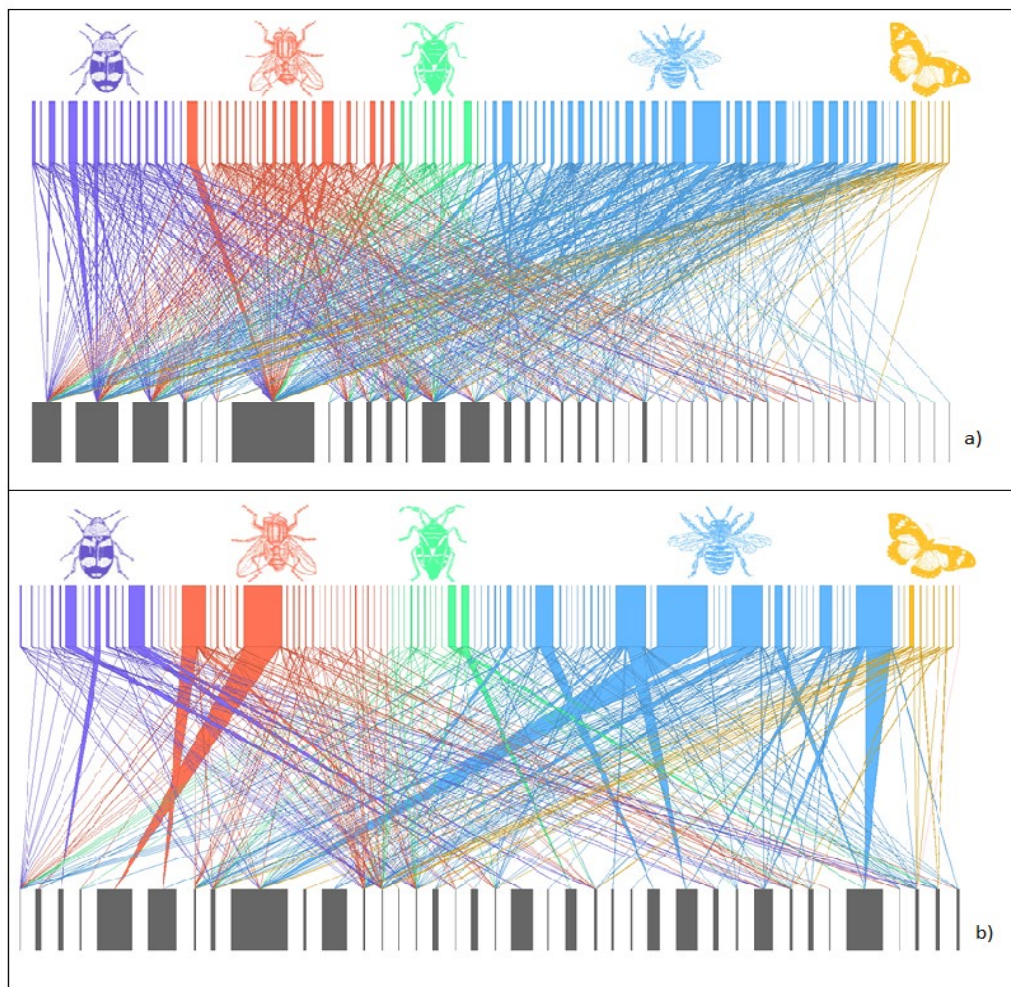


Figure 4: *Floral Observation and Pollen Transport Bipartite Networks*. a) Bipartite Network of the pollen transport data and b) floral visitation data. Insect morphospecies are represented by nodes on the top (purple= Coleoptera, red= Diptera, green= Hemiptera, blue= Hymenoptera, orange= Lepidoptera) and plants on the bottom. Interactions and their intensity are represented by the lines between each row of nodes. The pollen transport network shows many more interactions than the floral observation network.

Table 2: *Pollen Transport and Floral Observation Network Metrics*- Network metrics generated in the bipartite package of R. The pollen transport network contained twice as many links per species than the floral visitation network. All metrics were determined to be different from random chance by null model analysis with 1,000 permutations. Asterisks indicate significance.

	<i>Weighted Nestedness</i>	<i>Modularity</i>	<i>Weighted Connectance</i>	<i>Links per Species</i>	<i>Specialization(H2)</i>
<i>Floral Visitation</i>	0.46	0.72	0.10	2.7	0.76
<i>Pollen Transport</i>	0.68	0.44	0.16	4.65	0.50
<i>ANOVA</i>	P=0.01*	P=0.99	P=0.00*	P=0.00*	P=0.06
<i>Null Model</i>	P<0.05*	P<0.05*	P<0.05*	P<0.05*	P<0.05*

*Daily Variation.* The morning network contained 179 interactions between 70 floral visitors and 30 plant species, while the afternoon network consisted of 352 interactions between 66 pollinator species and 39 plant species (Figure 6). Average links per species (P=0.72), nestedness (P=0.61), Specialization (P=0.61), link density (P=0.58) and weighted connectance (P=0.09) were not significantly different between the morning and afternoon networks (Table 6). However, Procrustes analysis indicated that the differences between morning and afternoon networks were marginally significant (P=0.07).

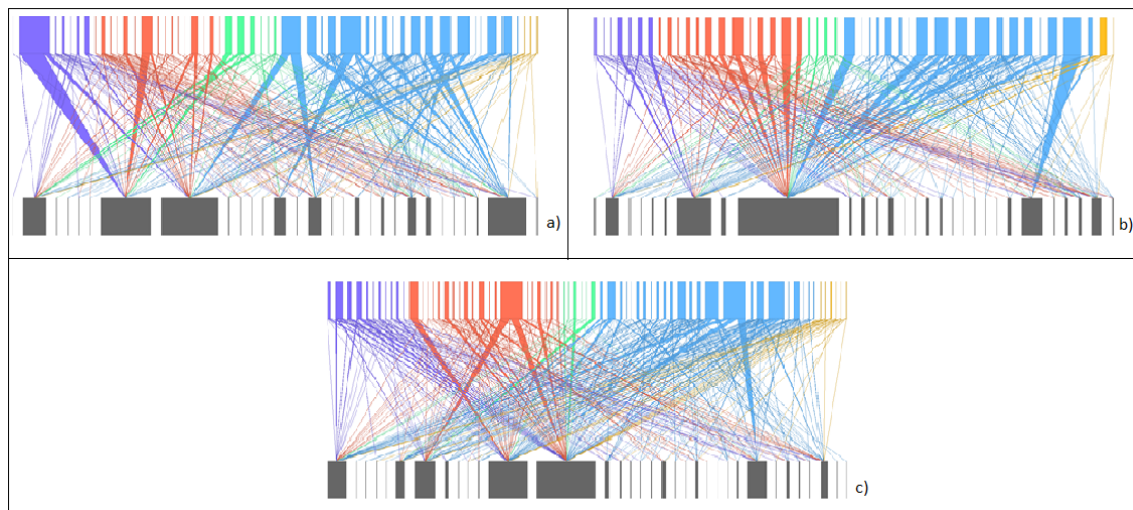


Figure 5- a) ES b) MS and c) LS pollen transport networks. Each network consists of four-week intervals for which there was no statistical difference in network metrics. However, the structure of each network was determined to be statistically different by Procrustes analysis.

Table 3: *Early, Middle, and Late Season Network Metrics*. Metrics generated for the ES, MS, and LS periods of the growing season. None of the selected metrics were shown to be significantly different. All metrics were shown to be significantly different from random chance by null model analysis.

<i>Time Period</i>	<i>Links per Species</i>	<i>Weighted Nestedness</i>	<i>Weighted Connectance</i>	<i>Modularity</i>	<i>Specialization (H2)</i>
<i>Early</i>	3.25	0.599	0.072	0.552	0.57
<i>Middle</i>	3.147	0.656	0.115	0.528	0.55
<i>Late</i>	3.297	0.689	0.090	0.455	0.48
<i>ANOVA</i>	P=0.10	P=0.54	P=0.46	P=0.79	P=0.85
<i>Null Model</i>	P<0.05*	P<0.05*	P<0.05*	P<0.05*	P<0.05*

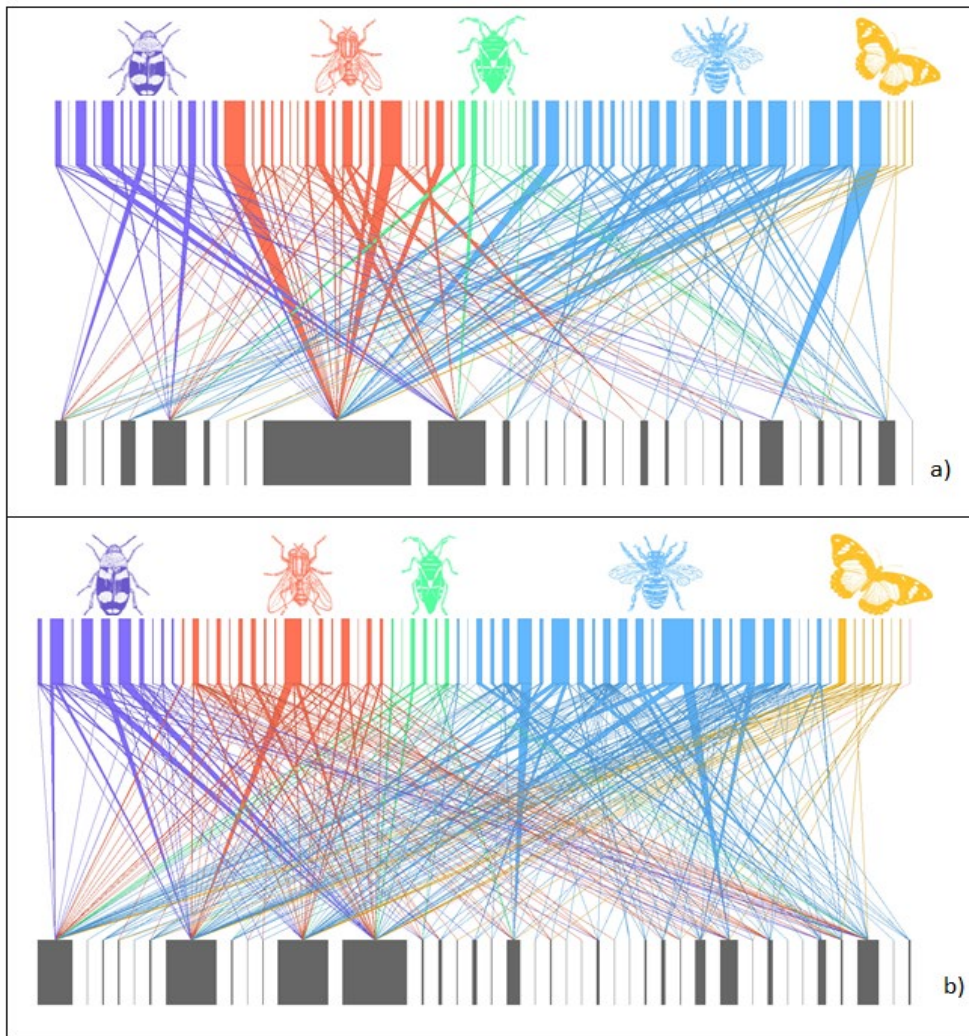


Figure 6- *Morning and Afternoon Bipartite Networks*. a) Morning and b) Afternoon pollen transport networks. Both networks were determined to be similar by Procrustes analysis. However, the afternoon network contains more interactions than the morning network.

Table 4: *Morning and Afternoon Network Metrics*. Metrics generated for morning and afternoon networks are not significantly different from each other. However, weighted connectance was nearing significance.

<i>Time Period</i>	<i>Weighted Nestedness</i>	<i>Modularity</i>	<i>Weighted Connectance</i>	<i>Links per Species</i>	<i>Link Density</i>	<i>Specialization(H2)</i>
<i>Morning</i>	0.56	0.58	0.65	1.8	6.53	0.73
<i>Afternoon</i>	0.62	0.51	0.07	3.35	7.70	0.56
<i>ANOVA</i>	P=0.61	P=0.99	P=0.09	P=0.72	P=0.58	P=0.61
<i>Null Model</i>	P<0.05*	P<0.05*	P<0.05*	P<0.05*	P<0.05*	P<0.05*

### *Discussion*

Observing floral visitation patterns has been considered a standard when analyzing plant-pollinator network structure (Dupont et al. 2003; Alarcón 2010; Popic et al. 2013). The data presented in this study indicates that current interpretations of network structure and function based on floral visitation may be incomplete. Comparisons of the pollen transport network to the floral visitation network show that as many as 38% of interactions are unaccounted for using observation of floral visitation alone. Furthermore, the pollen transport network identified approximately twice as many links per species as the floral visitation network emphasizing their value in capturing a larger range of interactions some of which may be hard to observe. Moreover, while the floral visitation network contained 103 floral visitors interacting with 39 plant species, the pollen transport network contained 93 floral visitors interacting with 43 plant species. This indicates that 6 insect species ‘dropped out’ of the network when pollen transport was analyzed, and four additional plant species were included. These differences are likely due to the exclusion of insects that were observed “visiting” flowers but not carrying pollen (Table 7) and the inclusion of rare interactions with four plant species.

Table 5: *Removed Insect Morphospecies*. Pollinator morphogroups removed from pollination network. Species listed here were found not be carrying pollen and are considered non-pollinating insects.

<i>Order</i>	<i>Family</i>	<i>Genus</i>	<i>Pollinator Morphogroup</i>
<i>Lepidoptera</i>	Hesperiidae	Hylephila	Hylephila phyleus
<i>Coleoptera</i>	Curculionidae	Hypera	Hypera postica
<i>Hymenoptera</i>	Sphecidae	Amnophila	Amnophila sp. 1
<i>Hemiptera</i>	Cicadellidae	Draeculacephal	Draeculacephala sp.89
<i>Hymenoptera</i>	Braconidae	Eubazus	Eubazus sp. 31
<i>Orthoptera</i>	Tettigoniidae	Scudderia	Scudderia sp.87

Our study shows the two data collection methods provide different network structures. Specifically, the pollen transport network was significantly more nested and contained higher levels of connectance when compared to the floral visitation network. Previous studies have used network metrics as a means to indicate community functions (Memmott and Waser 2002; Bastolla et al. 2009; Thébault and Fontaine 2010; Nielsen and Totland 2014). For example, networks that have higher levels of connectance possess an increased tolerance to disturbance due to redundancy in interactions (Dunne et al. 2002). Furthermore, higher levels of nestedness have been associated with increased resistance to species loss (Burgos et al. 2007). Therefore, strictly relying on floral visitation to analyze a community, in this case, would have resulted in an underestimation of network resilience and ability to withstand species loss. Overall, we would have concluded that the study community was less tolerant to disturbance than what the pollen transport network indicates.

As stated previously, the plant-pollinator networks established by both methods were not significantly different from each other in terms of network structure and participants. The identity of network participants (i.e. plants and their insect mutualists) and their interactions were not significantly different, as indicated by Procrustes analysis ( $P > 0.05$ ). Therefore, realistic representations of plant-pollinator interactions at the community level that account for a greater

portion of the interactions present and that allow for better interpretations of network structure can be achieved by using pollen transport. Therefore, floral visitation is better utilized as a tool for establishing generalized interaction networks with a focus on the most active species in the community (Alarcón 2010).

Furthermore, my results show that plant-pollinator network structure can be considerably stable throughout the flowering season. That is, none of the network metrics were shown to differ significantly at any of the selected time periods (early, middle, and late). The network stability described above is especially interesting, given that the identity of the participants and their interactions within the early, middle, and late summer networks were significantly different from each other. For example, *Jacobaea vulgaris* was observed as the dominant plant species, growing between May 15<sup>th</sup> and June 7<sup>th</sup> but was overtaken by the growth of *Crepis capillaris* which emerged in large numbers around June 12<sup>th</sup>. Despite changing species population and interactions (i.e. species turnover), metrics generated for each time period remained statistically the same. Each segment of the growing season maintained 3.2 links per species and maintained a steady level of nestedness of  $\sim 0.65$ . This implies that there is an overarching ‘blueprint’ for the structure of these communities regardless of species composition during the flowering season (Alarcón et al. 2008). A similar trend can be observed at a larger scale in communities that are highly nested, where network structure remains the same between years despite vast changes in participant species (Alarcón et al. 2008). Such an overarching ‘blueprint’ may help guide the establishment of species interactions as new plants and insects emerge or as species turnover across seasons.

This same temporal stability appears to exist within single days as well. None of the network metrics were shown to be significantly different between morning and afternoon

networks. Despite not reaching significance, the afternoon networks contained twice the average number of links per species as the morning network. Also, several species were identified to be active in the morning but not in the afternoon. Specifically, the wasp *Leucospis affinis* was found to not carry pollen in the morning hours but carried pollen from five different plant species in the afternoon. Furthermore, pollinator preference for plant mutualists was shown to switch between morning and afternoon. *Epargyreus clarus* visited four plant species in the morning hours (CEBR, CLVU, SIAT, TRPE), however, visited three different plant species in the afternoon (CHLE, DACA, TRPR/TRRE) (Table 1). Therefore, plant-pollinator interactions exhibit daily temporal variation generated in part by the activity and preferences of the pollinating insects at different times of the day. Overall, morning networks contained almost 50% less interactions compared to the afternoon network (179 and 352 interactions respectively), likely due to temperature requirements of insect pollinators such as bees (Herrera 1990). These changes in within-day pollinator activity and preference, however, did not seem to affect overall network structure.

### *Conclusion*

The results of this study indicate that plant-pollinator interactions are more complex than previously thought. Specifically, plant-pollinator communities are much more interaction dense than previously shown. The use of floral visitation to describe plant-pollinator interactions are limited and many interactions are left out when compared to networks based on pollen transport networks. With this in mind, caution should be applied to communities described by floral observation. Furthermore, plant-pollinator interactions are variable at relatively small scales (i.e. morning and afternoon). This study shows that active pollinators and plants can be different depending on the time of the day. Interaction preference is also subject to change as each day

progresses, more research will be needed to determine if this is due to time sensitive floral rewards or the whims of pollinators. Also, the establishment of plant-pollinator interactions follows a set pattern/blueprint to the turnover of interactions as the growing season progresses. As such, new importance is given to understanding the role that each pollinator and plant species performs in community persistence.



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APPENDIX: PLANTS AND INSECTS AT STUDY SITE

List of plant species found at the study site in Hampton Creek Cove State Natural Area

	<i>Code</i>	<i>Family</i>	<i>Genus</i>	<i>Species</i>
1	RUAR	Rosaceae	<i>Rubus</i>	<i>argutus</i>
2	TRPR	Fabaceae	<i>Trifolium</i>	<i>pratense</i>
3	TRRE	Fabaceae	<i>Trifolium</i>	<i>repens</i>
4	RABU	Ranunculaceae	<i>Ranunculus</i>	<i>bulbosa</i>
5	GLHE	Lamiaceae	<i>Glechoma</i>	<i>hederacea</i>
6	ACMI	Asteraceae	<i>Achillea</i>	<i>millefolium</i>
7	ERAN	Asteraceae	<i>Erigeron</i>	<i>annuus</i>
8	ROMU	Rosaceae	<i>Rosa</i>	<i>multiflora</i>
9	GAMO	Rubiaceae	<i>Gallium</i>	<i>mollugo</i>
10	POSI	Rosaceae	<i>Potentilla</i>	<i>simplex</i>
11	JAVU	Asteraceae	<i>Jacobaea</i>	<i>vulgaris</i>
12	TAOF	Asteraceae	<i>Taraxacum</i>	<i>officinale</i>
13	CIAR	Asteraceae	<i>Cirsium</i>	<i>arvense</i>
17	DACA	Umbellifers	<i>Daucus</i>	<i>carota</i>
19	DUIN	Rosaceae	<i>Duchesnia</i>	<i>indica</i>
20	FRVE	Rosaceae	<i>Fragaria</i>	<i>vesca</i>
21	BAVU	Brassicaceae	<i>Barbarea</i>	<i>vulgaris</i>
22	CRCA	Asteraceae	<i>Crepis</i>	<i>capillarus</i>
23	CEBR	Caryophyllaceae	<i>Cerastium</i>	<i>brachypetalum</i>
24	GECA	Geraniaceae	<i>Geranium</i>	<i>carolinianum</i>
25	VISA	Fabaceae	<i>Vicia</i>	<i>sativa</i>
26	TRCA	Fabaceae	<i>Trifolium</i>	<i>campestre</i>
28	STGR	Caryophyllaceae	<i>Stellaria</i>	<i>graminea</i>
29	OXST	Oxalidaceae	<i>Oxalis</i>	<i>stricta</i>
31	DIAR	Caryophyllaceae	<i>Dianthus</i>	<i>armeria</i>
32	SIAT	Iridaceae	<i>Sisyrinchium</i>	<i>atlanticum</i>
33	SILA	Caryophyllaceae	<i>Silene</i>	<i>latifolia</i>
34	CHLE	Asteraceae	<i>Chrysanthemum</i>	<i>leucanthemum</i>
35	n/a	NO ID	NO ID	NO ID
36	MEOF	Fabaceae	<i>Melilotus</i>	<i>officinalus</i>
38	MYAQ	Caryophyllaceae	<i>Myosoton</i>	<i>aquaticum</i>
39	PORE	Rosaceae	<i>Potentilla</i>	<i>recta</i>
41	TRPE	Campanulaceae	<i>Triodanis</i>	<i>perfoliata</i>
42	SACA	Adoxaceae	<i>Sambuca</i>	<i>canadensis</i>
43	PRVU	Lamiaceae	<i>Prunella</i>	<i>vulgaris</i>
44	ASSY	Apocynaceae	<i>Asclepias</i>	<i>syriaca</i>

45	ANVI	Ranunculaceae	<i>Anemone</i>	<i>virginiana</i>
47	GECA2	Rosaceae	<i>Geum</i>	<i>canadense</i>
49	CRCA2	unknown	<i>Cryptotaenia</i>	<i>canadensis</i>
50	PELO	Polygonaceae	<i>Persicaria</i>	<i>longiseta</i>
51	VETH	Scrophulariaceae	<i>Verbascum</i>	<i>thapsus</i>
52	HYPV	Hypericaceae	<i>Hypericum</i>	<i>punctatum</i>
53	SOCA	Solanaceae	<i>Solanum</i>	<i>carolinense</i>
54	AGPU	Rosaceae	<i>Agrimonia</i>	<i>pubescens</i>
55	CLVU	Lamiaceae	<i>Clinopodium</i>	<i>vulgare</i>
56	LYCI	Primulaceae	<i>Lysimachia</i>	<i>ciliata</i>
58	OEFR	Onagraceae	<i>Oenothera</i>	<i>fruticosa</i>
61	CASE	Convolvulaceae	<i>Calystagia</i>	<i>sepium</i>
62	RUHI	Asteraceae	<i>Rudbeckia</i>	<i>hirta</i>
63	VEUR	Verbenaceae	<i>Verbena</i>	<i>urticifolia</i>
64	LEVI	Brassicaceae	<i>Lepidium</i>	<i>virginicum</i>
65	GAPI	Rubiaceae	<i>Galium</i>	<i>pilosum</i>
66	CLVI	Ranunculaceae	<i>Clematis</i>	<i>virginiana</i>
67	CIIN	Asteraceae	<i>Cichorium</i>	<i>intybus</i>
68	GAPI	Rubiaceae	<i>Galium</i>	<i>pilosum</i>
69	SOAS	Asteraceae	<i>Sonchus</i>	<i>asper</i>
70	VEOC	Asteraceae	<i>Verbiscina</i>	<i>occidentalis</i>
70(2)	n/a	NO ID	NO ID	NO ID
71	APCA	Apocynaceae	<i>Apocynum</i>	<i>cannabinum</i>
72	COBE	Asteraceae	<i>Xanthium</i>	sp. 1

List of pollinating insects collected at Hampton Creek Cove. Identified to the lowest taxonomic level possible

<i>Family</i>	<i>Genus/Species</i>
Megachilidae	<i>Megachile pugnata</i>
Megachilidae	<i>Megachile sp.9</i>
Braconidae	<i>Spathius elegans</i>
Adrenidae	<i>Adrena sp.15</i>
Halictidae	<i>Agapostemon virescens</i>
Agromyzidae	<i>Agromyza sp.55</i>
Chrysomelidae	<i>Altica bimarginata</i>
Sphecidae	<i>Ammophila sp. 1</i>
Crabronidae	<i>Anacrabro ocellatus</i>
Coreidae	<i>Anasa tristis</i>
Andrenidae	<i>Andrena sp. 1</i>
Andrenidae	<i>Andrena vicina</i>

Pompilidae	<i>Anoplius sp.42</i>
Formicidae	<i>Ant sp.</i>
Megachilidae	<i>Anthidium illustre</i>
Apidae	<i>Apis mellifera</i>
Halictidae	<i>Augochlora pura</i>
Apidae	<i>Bombus sp.5</i>
Bombyliidae	<i>Bombylius major</i>
Apidae	<i>Ceratina sp. 4</i>
Crabronidae	<i>Cerceris sp.6</i>
Cantharidae	<i>Chauliognathus marginatus</i>
Cantharidae	<i>Chauliognathus pennsylvanicus</i>
Chrysomelidae	<i>Chrysolina quadrigemina</i>
Apidae	<i>Clisodon sp.32</i>
Nymphalidae	<i>Clossiana bellona</i>
Megachilidae	<i>Coelioxys octodenata</i>
Miridae	<i>Collaria oculata</i>
Colletidae	<i>Colletes compactus</i>
Colletidae	<i>Colletes sp.33</i>
Thyreocoridae	<i>Corimelaena obscura</i>
Pentatomidae	<i>Cosmopepla lintneriana</i>
Tachinidae	<i>Cylindromyia sp.47</i>
Lonchaeidae	<i>Dasiops sp.54</i>
Chrysomelidae	<i>Donacia sp.66</i>
Cicadellidae	<i>Draeculacephala sp.89</i>
Hesperiidae	<i>Epargyreus clarus</i>
Sphecidae	<i>Eremnophila aureonotata</i>
Syrphidae	<i>Eristalis arbustorum</i>
Syrphidae	<i>Eristalis tenax</i>
Tephritidae	<i>Euaresta bella</i>
Braconidae	<i>Eubazus sp. 31</i>
Braconidae	<i>Eubazus sp. 65</i>
Braconidae	<i>Eubazus sp. 80</i>
Vespidae	<i>Euodyeris hidalgo</i>
Hesperiidae	<i>Euphyes dukesi</i>
Coreidae	<i>Euthochtha galeator</i>
Nymphalidae	<i>Everes comyntas</i>
Tachinidae	<i>Gymnosoma sp.49</i>
Muscidae	<i>Haematobia irritans</i>
Miridae	<i>Halticus apterus</i>
Rhopalidae	<i>Harmostes reflexulus</i>

Asilidae	<i>Holcocephala sp.53</i>
Megachilidae	<i>Hoplitis sp.100</i>
Colletidae	<i>Hylaeus modestus</i>
Hesperiidae	<i>Hylephila phyleus</i>
Curculionidae	<i>Hypera postica</i>
Curculionidae	<i>Hypera sp.72</i>
Noctuidae	<i>Idia sp.20</i>
Tachinidae	<i>Juriniopsis floridensis</i>
Halictidae	<i>Lasioglossum sp.30</i>
Cecidomyiidae	<i>Lasioptera sp.34</i>
Leucospidae	<i>Leucospis affinis</i>
Miridae	<i>Lopidea sp.90</i>
Lygaeidae	<i>Lygaeus turcicus</i>
Miridae	<i>Lygus lineolaris</i>
Megachilidae	<i>Megachile gemula</i>
Crabronidae	<i>Mimesa sp.99</i>
Mordellidae	<i>Mordella atrata</i>
Mordellidae	<i>Mordella marginata</i>
Muscidae	<i>Musca domestica</i>
Ichneumonidae	<i>Neorhacodes sp.79</i>
Curculionidae	<i>Odontocorynus sp.73</i>
Phalacridae	<i>Olibrus sp.27</i>
Pelecniidae	<i>Pelecinius polyturator</i>
Andrenidae	<i>Perdita sp.98</i>
Nymphalidae	<i>Phyciodes tharos</i>
Reduviidae	<i>Phymata americana</i>
Cantharidae	<i>Podabrus sp.26</i>
Vespidae	<i>Polistes dominula</i>
Scarabaeidae	<i>Popillia japonica</i>
Oecophoridae	<i>Pyramidobela sp.21</i>
Mydidae	<i>Rhaphiomidas sp.94</i>
Syrphidae	<i>Rhingia sp.69</i>
Sarcophagidae	<i>Sarcophaga pernix</i>
Tettigoniidae	<i>Scudderia sp.87</i>
Curculionidae	<i>Sitophilus oryzae</i>
Syrphidae	<i>Sphaerophoria contigua</i>
Syrphidae	<i>Syritta pipiens</i>
Syrphidae	<i>Syrphus ribesii</i>
Cerambycidae	<i>Tetraopes tetropthalmus</i>
Myopinae	<i>Thecophora propinqua</i>
Tiphiidae	<i>Tiphia sp.</i>



Syrphidae	<i>Toxomerus marginatus</i>
Tachinidae	<i>Trichopoda pennipes</i>
Chrysomelidae	<i>Trirhabda sp.84</i>
Vespidae	<i>Vespula malculifrons</i>
Bombyliidae	<i>Villa lateralis</i>
Apidae	<i>Xylocopa virginica</i>

VITA

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- Education: M.S. Biology, East Tennessee State University, Johnson City, Tennessee, 2020  
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