SEX-SPECIFIC FORAGING PATTERNS IN THE RARE EASTERN REGAL FRITILLARY BUTTERFLY, SPEYERIA IDALIA IDALIA

A Thesis
Submitted to
the Temple University Graduate Board

In Partial Fulfillment
of the Requirements for the Degree
MASTER OF SCIENCE

by
Skyler E. Naya
December 2020

Thesis Approvals:

Dr. Rachel Spigler, Thesis Advisor, Department of Biology
Dr. Brent Sewall, Committee Member, Department of Biology

ABSTRACT

In studies of plant-pollinator interactions, much attention has been paid to interspecific variation in foraging behaviors among pollinators and their consequences for plant reproduction and the stability of plant-pollinator communities. In contrast, there has been little consideration of intraspecific variation and its consequences. Specifically, male and female pollinators may forage differently due to differences in phenology, nutritional requirements, and behavior. Here, we compare male and female foraging patterns of the rare declining eastern regal fritillary butterfly, *Speyeria idalia idalia*. Using a 21-year dataset, which monitored the abundance, sex, and foraging of *S. i.idalia* across grasslands within Fort Indiantown Gap National Guard Training Center in Pennsylvania, USA, we asked whether males and females differed with respect to the diversity of nectar plants visited and the floral compositions which they visited. To better understand the behavior of individual butterflies and draw inferences about repercussions for plant fitness, in 2019 we collected pollen loads directly from male and female *S. i.idalia* in these same grasslands and compared the diversity, composition, and size of pollen loads between the sexes after identifying pollen grains to the lowest taxonomic unit. Both datasets point to the dominant use of three nectar species by both sexes, but also show evidence of sex specific foraging pattern. Males and females differ statistically, although slightly, in the composition of plant species visited and plant species per pollen load. Females visit a significantly greater diversity of plant species when accounting for both abundance and evenness of plants visited. In contrast, pollen loads found on males contained ~3 more species on average than female pollen loads, but this difference is not supported when accounting for evenness of pollen grains/species. Finally, we find individual male butterflies carry more pollen grains than females on average. Our results contribute to understanding pollinator resource use more broadly, with implications for pollination dynamics. For conservation of this rare species our results further emphasize the importance of nectar plants critically important for male and female members of *S. i.idalia.*

ACKNOWLEDGEMENTS

I am extremely grateful to my advisor Dr. Rachel Spigler for helping me grow continuously as a student and as a scientist. Furthermore, I thank her and the DMVA graciously for the funding which made my project and largely my time at Temple possible. I thank Dr. Brent Sewall for his contributions to my project, as well as for continuing to nurture and inspire my interest in conservation. Mark Swartz and Virginia Tilden were incredible mentors to me during my time at FIG, and I will always be incredibly thankful for the opportunities they have provided me through their abundant teachings and kindness. Teagan Keszner, Safwhan Khan, and Claire Neal supported me unwaveringly in more ways than I can express, and I am indebted to them for the love and help they have provided me along this journey. I appreciate my parents deeply for always nourishing my love for science as well as natural history, and for teaching me to find the beauty and value of every creature, from flower to butterfly. Finally, I would like to thank my parents, my grandmother, and my Aunt Sandy for believing in me and encouraging me always.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

Background information

Variation in foraging behavior among pollinator species is a key factor influencing plant community composition, plant-pollinator network stability, and floral evolution (Valdovinos et al. 2016; Tepedino 1981; Kaiser-Bunbury et al. 2010; Eckhart et al. 2006). Relatively little, however, is known about variation in foraging behavior *within* pollinator species. Intraspecific variation more generally can be as ecologically important for communities as variation between species, influencing community structure and ecological processes (Roches et al. 2018; Post et al. 2008). More specifically, intraspecific variation in pollinator foraging could alter the stability of plant-pollinator networks and pollinator populations themselves as well as influence plant reproduction. Recent works have highlighted these possibilities in response to sex-specific foraging behavior of pollinators (Roswell et al. 2019; Smith et al 2019; Kishi & Kakutani 2020), but our understanding of sex differences in pollinator foraging is only just beginning.

Sex specific foraging: floral composition

Male and female pollinators may visit different suites of plant species due to divergent nutritional demands, sensory biases, and timing of life history events. In general, resource use of females is driven by higher energy demands of egg production, whereas that of males is driven by the need to search for and acquire mates. Accordingly, some studies have demonstrated male butterflies prefer nectar with higher concentrations of sucrose, whereas females prefer nectar with higher amino acid content (Rusterholz & Erhardt 2000; (Mevi-Schutz & Erhardt 2005). The phenomenon of females consuming more protein than males, likely to enhance fecundity, has been also been observed in pollinating bats (Martinoet al. 2002) as well as the females of pollen consuming butterflies in *Heliconious* (Boggs et al. 1981; Mendoza-Cuenca & Macias-Ordonez 2005). Furthermore, as eggs are composed largely of water (Kawooya & Law 1988), females could need disproportionally greater amounts of water relative to their body size than males, resulting in visits to different floral compositions (Smith et al. 2019). For example, females of the desert dwelling hawkmoth, *Manduca sexta,* spend more time than males nectaring from flowers that produce more, dilute nectar than the flowers of their own host plant (Bronstein et al 2009; Smith et al. 2018). Additionally, males and females can vary in their perception of floral cues, causing innate sensory biases. This includes sexual divergence in spectral sensitivities found in Hymenoptera and Lepidoptera (van Praagh et al. 1980; Ogawa et al. 2013; Rutowski 2000) and divergence in olfactory systems, such that males and females may differ in preference of olfactory compositions (Riffell et al. 2008; Streinzer et al 2013). Alternatively, different compositions of floral resources used by males and females might be a consequence of sexual divergence in the timing of major life cycle events. For example, males of most solitary bee species and many Lepidopteran species males emerge before females (Roswell 2019; Forsberg & Wiklund 1988; Zonneveld & Metz 1991; Wiklund & Fagerstrom 1977; Zografou et al 2017), such that males and females might simply encounter different floral buffets. Indeed, male social and solitary bees have proven to visit different nectar plant compositions than females (Roswell et al. 2019; Wolf & Moritz 2014; Ne'eman et al. 2006).

Sex specific foraging: floral visitation

Behavioral differences in male and female pollinators could additionally, or instead, result in differences in the degree of specialization with respect to floral resource use, though predictions are equivocal. In many solitary bee (Neeman et al. 2006; Roswell et al. 2019) and social bee species (Shafir et al. 2004; Wolf & Moritz 2004; Bertsch 1984) females forage both for nectar for themselves and pollen for their young, whereas males of these species forage only for their own energy gain. Combined with their greater overall nutrient requirements, females might prioritize visiting fewer floral resources that have greater nutrient returns to optimize foraging. Some evidence consistent with this hypothesis comes from higher floral constancy of females than males in solitary bee species (Ne'eman et al 2006). Indeed, floral constancy may be associated with females searching for (or avoiding) pollen or nectar with specific chemical and nutritional compositions (Keller & Imdorf 2005; Olsen et al. 1979). On the other hand, greater nutrient demands could mean females must visit a wider array of flowers to meet their nutritional needs, which could explain females foraging across more nectar in some Lepidopterans (Alarcon et al. 2008; Rusterholz & Erhardt 2000) and higher foraging rates in female bees (Roswell et al. 2019; Tepedino 1981). Other behavioral differences include "patrolling" by males of some pollinator taxa including butterflies, bees and flies, wherein males guard a small territory of valuable resources to attract mates (Ide 2004; Pascarella 2010; Luz, 2004). Flying consistently in a limited portion of their habitat could limit the diversity of nectar plants visited by males, though potentially lead to greater variation among males in nectar species resource use, depending on the heterogeneity of nectar plant composition within a habitat. Meanwhile, seasonal changes in plant diversity can also bring about differences in the diversity of nectar plants used by males and females when there are sex differences in phenology.

Sex specific foraging: fitness consequences for nectar plants

Sex specific foraging in pollinators can have direct fitness implications for the plants they visit. For example, greater floral constancy found in some female solitary bees (Ne'eman et al. 2006) could make them more efficient pollinators than males, since their fidelity would reduce the likelihood of transferring pollen between the wrong species, which can substantially reduce seed production and male fitness via pollen loss (Morales & Traveset 2008; Ashman & Arceo-Gomez 2013). In addition, foraging for both pollen and nectar could translate to females spending greater time per flower and thus picking up (and depositing) greater pollen loads than males (Ne'eman et al. 2006; Pascarella 2010). Studies in few though diverse taxa including butterflies, bees, and hawkmoths have found larger pollen loads on females (Mendoza-Cuenca & Macias-Ordonez 2005; Wolf & Moritz 2014; Pascarella 2010; Smith et al. 2019). The relative benefits of female vs. male pollinators to plant fitness could also depend on male behavior. For species with male territorial behaviors, the limited, local movements of males could increase inbreeding in plants or pollen movement between the wrong species depending on the size and plant species composition of the territory, whereas for species where males flying further distances than females (Ne'eman 2006; Smith 2019; Murawski & Gilbert 1986; Rusterholz & Erhardt 2000), males might actually increase outcrossing of plant species they visit. Due to the difficulty of determining such consequences from visitation patterns alone, studies directly examining pollen loads on male and female pollinators together with foraging observations can help better predict the potential impacts of foraging behavior on plant fitness.

Here, we evaluate patterns of sex-specific foraging in the rare eastern regal fritillary butterfly *Speyeria idalia idalia* through two complementary studies which combine the power of visitation observations and pollen load data. We use a 21-year dataset documenting floral visitation and examine the extent to which male and female regals differ in the diversity and composition of plant species visited. In a second study, we examined whether pollen loads collected from male and female regals similarly differed in diversity and composition. Finally, we infer potential consequences for plant reproduction. Given relatively little work has been conducted to evaluate sex specific foraging in Lepidoptera, our results contribute to increasing our understanding the generality of sex-specific pollinator foraging. Moreover, detailed analysis of sex-specific foraging in this rare butterfly can also inform conservation efforts aimed at restoring its habitat.

CHAPTER 2

METHODS

Study species and site

Speyeria idalia is a grassland obligate species (Moranz et al. 2014) native to USA and Canada. Its range once spanned laterally from the eastern to midwestern USA and longitudinally as far south as Georgia, USA and north as Nova Scotia, Canada (Swengel 1993). Over the last 30 years regal populations have declined as much as 95% (Caven et al. 2017), and only one eastern population remains. The rare *S. idalia* is under review for protection under the Endangered Species Act (Guertin 2015). Interestingly, the Pennsylvania population has been identified as genetically distinct from all others, including the extirpated secondary eastern population in Virginia; it has been recommended that the Pennsylvania population be recognized as a subspecies, *Speyeria idalia idalia* (Williams et al. 2002, Keyghobadi et al. 2013). The final declining eastern population is located at our study site, the Fort Indiantown Gap National Guard Training Center ('FIG', hereafter) in Annville, PA (40°26′13.15′′N, 76°34′33.8′′W) (Zografou et al. 2017). FIG has a humid continental climate and is located in the geographic region known as the Appalachian plateau (Zografou et al. 2017). FIG has a heterogeneous landscape consisting of deciduous forests and temperate grasslands; a portion of the grasslands *S. i.idalia* inhabits have been protected from military activity since 1998 (Ferster and Vulinec 2010). Being a protandrous species, females emerge approximately two weeks after the males (Zografou et al. 2017). Additionally, females undergo a reproductive diapause, which is unique to the species and aligns their reproduction with the availability of their host plant, *Viola* (Kopper 2001).

Sex differences in floral visitation

As part of the overall conservation plan for *S. i.idalia*, yearly surveys of butterfly abundance have been conducted across grasslands at FIG between June and September since 1998 using the Pollard walk method (Pollard & Yates 1993) along permanent transects (range 1557–3515 m) (Zografou et al. 2017). Observers noted all occurrences of the butterfly and any plant species on which the butterflies were nectaring. In addition, because *S. i.idalia* males and females have clear differences in color patterning on their wings, observers were also able to record the sex of each butterfly from a distance. From this large survey, we compiled a dataset consisting of all male and female identifications coinciding with nectaring events across 21 years (1998 to 2018).

To test whether male and female *S. i.idalia* differ in the diversity of plant species visited, we quantified the total number of species visited ('species richness') and the 'effective number' of species (sensu Jost et al. 2006) visited by males and females each year. We calculated effective species number, $D(H)$, for each year as $\exp(H)$, where H is Shannon's diversity index (Whittaker et al. 1972) that accounts for both the abundance and evenness of species in a given dataset. Effective species number can easily and more intuitively be interpreted in the context of our study as the number of species visited at equal frequencies (Jost et al. 2006). We used a general linear model, implemented with lmer in the *vegan* package in R (v 3.6.3; Oksanen et al. 2013), to compare differences in species richness (log-transformed to meet model assumptions) and $D(H)$ between males and females. Each year was treated as an independent replicate. We modelled 'sex' as a fixed effect and included a random 'year' effect. The year effect accounts for the paired structure of the data as well as differences in richness and *D(H)* across years that may be driven by differences in sample size; the rarity of *S. i.idalia* led to observations being as low as 40 butterflies in some years but as many as 725 observations in others. To complement these analyses and assess whether trends were consistent across years, we used a Hutcheson's t-test (Hutcheson 1970) to compare diversity, measured as H, of plants visited by males and females for each year individually. The Hutcheson's t-test compares the mean diversity of the Shannon diversities using the variance of the Shannon diversities, between males and females for each year.

We note that because our dataset is based on surveys across the entire season of regal activity, it includes periods where only male (early season) or only female (late season) *S. i.idalia* are active. Consequently, any differences detected by our analyses could reflect differences due to nectar plant phenology as well as butterfly preference. To reduce the influence of the former, we repeated our analyses after restricting our full dataset to time periods encompassing at least 25% of male and 25% of female sightings for each year (hereafter referred to as the M-F overlap dataset). This time period encompassed the end of the male life span, and the beginning portion of the female life span.

To test whether male and female *S. i.idalia* visit different compositions of nectar plant species for both the full and M-F overlap datasets, we visualized dissimilarity between male and female floral communities use nonmetric multidimensional scaling (NMDS). Points are based on species richness and weighted by abundance, with similar points being located more closely within the ordination space (Oksanen et al. 2013). We then conducted semi-parametric permutational analyses of variance (PERMANOVA), controlling for the random effect of year. This test was selected due to the test's ability to accommodate random effects and resilience to heterogeneous dispersion for balanced datasets (Anderson & Walsh 2013; Anderson 2017). We also testing for homogeneity dispersion between the sexes using PERMDISP. NMDS, PERMANOVA, and PERMDISP were completed in the *vegan* package R (v 3.6.3; Oksanen et al. 2013), all using Bray-Curtis distances as our dissimilarity measure (Bray & Curtis 1957).

Sex differences in pollen loads

In 2019, we evaluated foraging patterns of individual butterflies based on pollen loads sampled from male and female *S. i.idalia* in two grasslands of FIG, [give sizes and distance] during periods when both males and females were anticipated to be active (June 21– July 25). We captured butterflies opportunistically, whether they were actively foraging or not. Upon capture, we marked the ventral side of the hindwing with a unique four-digit identifier, recorded their sex, site of capture, and date. We gently removed pollen from each butterfly by lightly patting the legs, thorax, abdomen, and proboscis with cubes of phenol-free fuchsin gel (Beattie 1977). Any pollinia (specialized reproductive structures containing packets of pollen grains found in the genus *Asclepias*) attached to the butterflies were removed with forceps and preserved in 70% EtOH. We released the butterflies and only sampled pollen from recaptured butterflies if it had been at least 24 hours since the prior capture.

In the lab, we mounted pollen samples on standard glass microscope slide to quantify pollen loads and to identify grains to the lowest taxonomic level possible under a compound light microscope. To identify pollen grains, we used a pollen library created by collecting pollen from all known nectar species occurring in the two grasslands butterflies were captured and online resources and published pollen identification keys (e.g., paldat.org). Even with the library, however, we faced a few challenges identifying grains to species. First, there were many species found on the butterflies that were not previously known to be nectar sources and so did not occur in our library. In addition, some plant families or genera have pollen grains that are too similar to identify to species (e.g. Fabaceae, Asteraceae). We therefore grouped similar species into morphospecies when necessary, based on pollen morphology such as spine length and width and/or polar diameter. We removed any grains identified as trees, grass, spores or wind-pollinated species more broadly from our dataset. Pollen of *Asclepias* species are packaged into special structures called pollinia. We distinguished between the two species of *Asclepias* flowering during our sampling period, *A. syriaca* and *A. tuberosa* based on pollinium shape and the ratio of width to length. In order to incorporate observations of pollinia into our dataset, we converted pollinia counts to number of pollen grains based on reported averages of pollen grains per pollinium reported for the two species (88 grains/pollinium for *A.tuberosa* and 445 grains/pollinium for *A.syriaca*; Wyatt et al. 2000).

Utilizing the *lsmeans* package in R v. 3.6.3, we used ANCOVA to test for differences in species richness, effective number of species *(D(H),* and total number of grains per butterfly between the sexes. We also included Julian date of capture as a covariate to account for any seasonal trend in pollen diversity and abundance. We note that in this dataset, each butterfly represents an independent sample of a “pollen community”. Pollen load size was log transformed to meet model assumptions. Although we initially considered an interaction effect between sex and date, we elected to remove it. In only one case (pollen load) was it significant ($P<0.05$), but this was driven by the few males that were captured two weeks prior to female captures.

We then evaluated whether the composition of pollen found on males and females differed in the *vegan* package in R v 3.6.3 for the following analyses. We used an analysis of similarity (ANOSIM), using the Bray-Curtis distances, to compare species composition of pollen loads between sexes. The ANOSIM statistic R can range from -1 to 1; negative values indicating species compositions are more variable within than between groups, values with 0 indicating random grouping, and positive values indicate greater dissimilarity between groups (Oksanen et al. 2013). We chose ANOSIM rather than PERMANOVA because males have many more pollen grains per butterfly than females (see Results), which equates to greater sampling of the male “pollen communities”; the non-parametric rank-based approach of ANOSIM (Clarke 1993) is more conservative and better suited for unbalanced data using an abundance weighted dissimilarity measure, such as ours. We again visualized differences in pollen load species communities using NMDS, wherein each data point represents an individual butterflies and their positions in the ordination space are determined using Bray-Curtis distances. We then tested for differences in dispersion of the Bray-Curtis matrices between males and females by testing the assumption of homogeneity (PERMDISP) of multivariate dispersion to confirm compositional differences were not due to differences in dispersion between groups as ANOSIM can be sensitive to heterogenous dispersions among groups for unbalanced data (Anderson 2017). Finally, we calculated the similarity percentages using SIMPER to identify the top three species contributing most to the Bray-Curtis dissimilarity matrix of males and females.

CHAPTER 3

RESULTS

Sex differences in Visitation

In total, 3265 butterflies (1353 female, 1912 male) were observed foraging on 19 species, from 21 categories, across 21 years. Males were found to visit 15/19 compared to 17/19 for females. Of the 19 species, two were uniquely visited by males: *Prunella vulgaris* (Lamiaceae) and *Dianthis armeria* (Caryophyllaceae). Five species were uniquely visited by females: *Eupatorium sp.* (Asteraceae), *Vernonia sp.* (Asteraceae), *Rudbeckia hirta* (Asteraceae), *Cirsium arvense* (Asteraceae), and *Daucus carota* (Apiaceae). Although males and females visited similar total numbers of plant species per year ($F_{1,20} = 0.57$, $p = 0.46$), females visited a greater diversity, quantified as effective species richness ($F_{1,20} = 7.0$, $p = 0.016$), (Figure 1A, B). These results are supported by Hutcheson t-tests; we find females visited a greater diversity of nectar plants, measured by Shannon diversity ($H = 1.78$ vs. 1.27; $t = 12.79$, $p < 0.0001$). This translates to females visiting 2.4 more effective species than males, with effective species being evenly visited species (Jost et al. 2006).

During the narrow range of dates where males and females have the greatest overlap in foraging, 1002 butterflies (566 male, 436 female) were observed visiting 9 species across the 21 years. Three species were unique to females during this time, *Vernonia sp.*, *R. hirta*, and *Cirsium discolor*. When we restrict the dataset to this period, males and females are found to visit similar numbers of species and effective numbers of species (richness: $F_{1,20} = 1.2$, $p = 0.29$; *D(H);* $F_{1,20} = 2.0$, $p = 0.176$), though the pattern of greater female nectar plant diversity remains (Figure 1A,B)). The Hutcheson's t-test, however, reveals a significant difference in diversity, again with females visiting a greater diversity of plant species per year than males (H = 1.16 vs. 0.95, respectively; t=3.33, p = 0.0009). This translates to females visiting 0.6 more effective species than males, with effective species being evenly visited species (Jost et al. 2006).

The communities of plants visited by males and females across 21 years and representing the entire sampling period per season is visualized in Figure 2A. Based on PERMANOVA, we find a significant difference between male and female floral community compositions (F = 6.7, P = 0.0001, R^2 = 0.14). PERMDISP revealed significant heterogeneity between males and females in the dispersion of plant communities visited across years (F = 12, P=0.001); the NMDS plot (Fig2A) indicates that this is due to greater variation in the composition of nectar plant communities visited by females among years. Consequently, both differences in centroid and dispersion of plant communities visited by males and females differ. Yet, when we consider only plant species visited during periods of male and female overall, the difference in both composition (PERMANOVA: F = 0.90, R^2 = 0.02, p = 0.49) and heterogeneity of dispersion among communities (PERMDISP: F = 2.0, p = 0.17) disappear (Figure 2B).

Sex differences in pollen loads

In total across our sampling period, we made 86 captures (33 female, 53 male), with recaptures representing 22% of our samples. Sampling ability was limited in large part by low *S. i.idalia* abundances 2019 (M. Swartz, pers. comm). Across butterflies, we found a total of 9,228 individual pollen grains and 76 pollinia (representing an additional estimated 7,842 grains), which we categorized into 47 (morpho)species. Of the 17,070 pollen grains, we identified 82.47% to a single species, 4.78% to morphospecies representing 2-3 species, and 12.75% to morphospecies. Species accumulation curves (Supplemental Figure 1) were near asymptotic, indicating that we

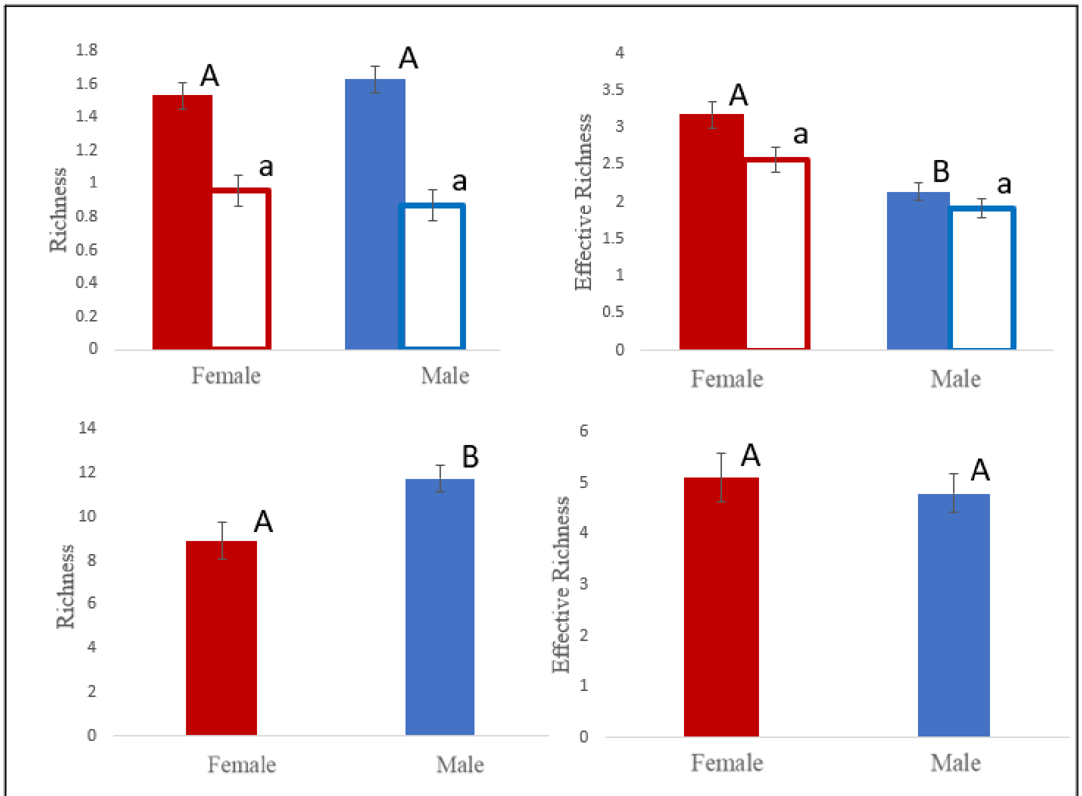


Figure 1. Sex differences in richness and effective richness across data sets

Least squares means ± standard error. Top panels represent long-term data set and richness is log transformed in the long-term data. Lower panels represent pollen data. Red bars represent females, blue bars represent males. Hollow shapes represent data restricted to Julian dates of period of overlap which accounts for differences in male and female phenology. Capitalized letters go with other capitalized letter per panel. Lower case letters go with other lower-case letter per panel. Different letters indicate a significant difference.

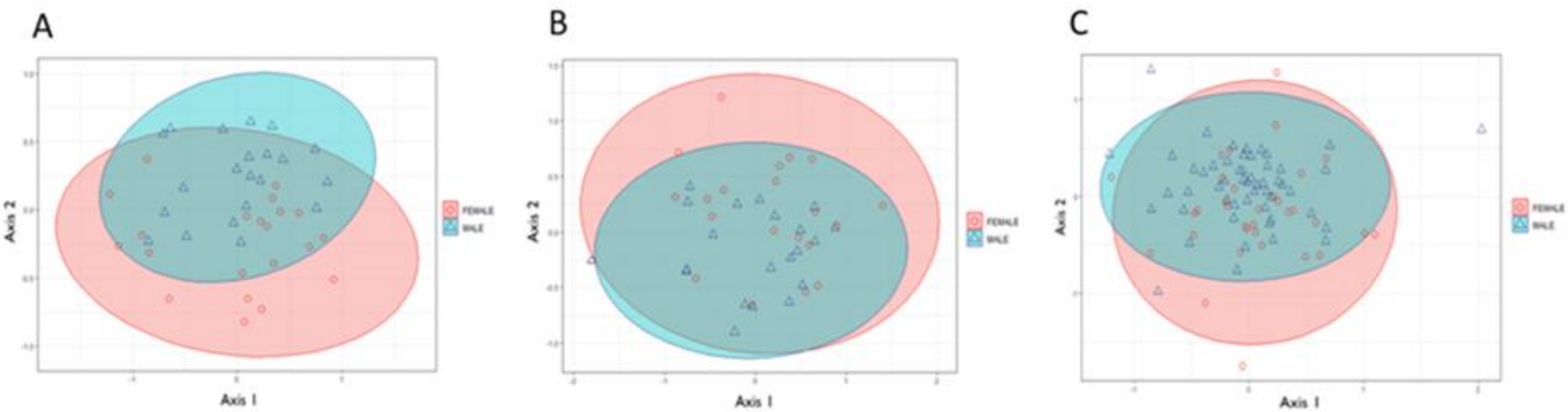


Figure 2. NMDS ordinations using Bray-Curtis distances for long term unrestricted data (A), long term data restricted to overlap period (B), and pollen data (C)

captured the majority of species visited by the sexes. Pollen grains found on males represented 45 (morpho)species, nine of which were unique to males: *Monarda fistuloda* (Asteraceae), *Apocynum cannabinum* (Apocynaceae), *Oenothera biennis* (Onagraceae), *Circisum discolor* (Asteraceae), and five unidentified morphotypes. Pollen grains found on females represented 38 (morpho)species: *Lilium superbum* (Liliaceae) and an unidentified morphotype.

Species richness of pollen loads found on male regals was significantly greater than that found on females, but there was no significant difference based on effective number of species per pollen load (Table 1; Fig. 1CD). Male butterflies carried significantly more pollen grains on average than females (Table 1). We also detected seasonal impacts; species richness and effective species richness increased over the summer, while the mean number of pollen grains found on butterflies remained consistent over time (Table 1).

Table 1.

Results for differences in richness, effective species richness, and pollen load size from pollen data

	Species richness		Effective species number		Pollen load	
Predictor	*F*	*p*	*F*	*p*	*F*	*p*
Sex	6.8	0.01	0.23	0.64	6.8	0.01
Date	4.4	0.04	5.6	0.02*	1.1	0.29

Differences in pollen load community composition between males and females are visualized in Figure 2C. ANOSIM indicated a significant difference in the composition of species found in pollen loads of male and female butterflies (R = 0.13, p = 0.001). The assumption of homogeneity of multivariate dispersion was met, indicating that differences between groups are not due to differences in dispersion (F = 0.03, p = 0.87). SIMPER identified the top three species contributing to 50.09% of the dissimilarity between males and female pollen load species composition: *A. tuberosa* (25.10%), *Cirsium pumilum* (19.12%) and *Rudbeckia hirta* (5.12%).

CHAPTER 4

DISCUSSION

This study adds to our limited understanding of sex differences in foraging by pollinators. We reveal evidence for sex-specific patterns of foraging in the regal fritillary *S. i.idalia.* Specifically, we show that male and female regals differ along multiple axes: floral species composition and diversity. Most notably, females are consistently observed to visit a greater diversity of nectar plants across years, although this greater diversity is not represented in the pollen loads that individual female butterflies carry. We consider the methodological limitations presented by both approaches, compare their results, and discuss their ecological and conservation implications.

Differences in floral community composition visited by male and female pollinators have been examined and detected in only a few studies, though across a variety of taxa including moths (Smith 2019) and social and solitary bees (Roswell et al. 2019; Wolf & Moritz 2014; Ne'eman et al. 2006). We detected significant differences in the communities of plants visited by pollinators and found in the pollen loads of male and female *S. i.idalia.* We examined visitation patterns by males and female butterflies across the entire season as well as in a narrow window of time when male and female *S. i.idalia* overlap. Notably, we only found a significant difference in nectar plant community composition for the visitation data which encompassed the entire floral season. Consequently, differences in community composition between males and females could be related to differences in phenology. Male regal fritillaries emerge two weeks before females, and females live approximately one month after males. Given plant species turnover across the season, females and males are likely to encounter different nectar resources during periods of their activity. Indeed, when we consider visitation to plants across the entire season, we find 7 out of the total 19 plant species recorded being visited by only sex. Interestingly, the majority of these species (5) are available most of the season. The exceptions to this are the early season bloomer *D. armeria*, which was visited by males, and the late season bloomer *C. arvense*, which was visited by females. *Even* for species in common to males and females, phenology seems to influence the frequency with which each sex visit them. For example, the third most visited species for females is *Cirsium discolor*, commonly known as field thistle, is a late season bloomer and accounts for 21% of female visits, but only 2% of males. Still, differences in preference could contribute to our findings, given species composition of pollen loads also vary between males and females even though sampling was completed during a period when they overlap. In this dataset, too, we find a substantial number of plant species unique to either males for females (11 out of 47 morphospecies).The majority of these were unique to males and could reflect males landing on plants less scrupulously than females due to their focus on mating (May 1985; Smith et al. 2019). We note that it is possible we were too conservative in narrowing our window of overlap when examining visitation data, thus hampering our ability to detect differences. Indeed, this window includes only 9 plant species visited by either males or females across the 21 years. Future work should follow the relative abundance of nectar plants and their composition across and within seasons to make better inferences about how the patterns we see in butterfly visitation data might relate to phenology vs. preference.

Another important driver of compositional differences in floral communities either visited by or found in pollen loads on males and female *S. i.idalia* appears to be differences in the frequency with which males and females use shared nectar plants. Males and females differed both with respect to the identity of the three most common plant species found with respect to visitation or pollen loads and the evenness with which they visited them (Supplemental Table 2). For example, *A. tuberosa* accounts for 42% of all visits by males and nearly half of the pollen grains found on males, compared to only 23% and 11% for females, respectively. In general, *A. tuberosa*, commonly known as butterfly milkweed, is the dominant nectar plant for males. In fact, based on visitation data, the second and third most visited plants by males only account for ~12% of visits combined. In contrast, females visit their top three species much more evenly, with each representing ~20% to 30% of female nectaring events observed. This emphasizes a pattern of visitation previously observed in Lepidoptera, wherein females visit a subset of their community evenly and males visited primarily one species, accompanied by occasional visits to rare species (Rusterholz & Erhardt 2000). Similar patterns are found in the pollen data. *A. syriaca* was commonly visited by females, but not males, while *R. hirta*, commonly known as black-eyed Susan, was commonly visited by males but not females. Moreover, even though both males and females commonly visit *C. pumilum*, pasture thistle, females visit it relatively more than males. All of these were identified as major contributors to compositional difference by our SIMPER analysis. Thus, while males and females share certain subsets of species, they rely on these species do different degrees, leading to differences in their community compositions. Differences such as these are important to note as conservation management officials decide which species, how many, and where to include, nectar plants during habitat restoration efforts.

Interestingly, we found that females show greater variation in the composition of plant species they visit from year to year than males, but similar levels of variation in the species found in pollen loads among butterflies. This was in contrast to what one might predict given males face fewer nutritional constraints and that the patrolling behavior of *S. i.idalia* might lead to greater stochasticity of nectar plants available depending on habitat heterogeneity. Greater variation in females across years based on visitation data could be attributed to greater variation in their emergence times across season, such that females could be emerging at different dates of each season over time, encountering different species. However, females actually show lower variation in date of first and last sighting across the 21-year dataset than males (unpublished data). Greater variation among years for females is also unlikely due to greater variation in the number of females found nectaring each year. Research at FIG indicates that the sexes fluctuate in number individuals relatively similarly over time (Zografou et al. 2017). Further research is needed to evaluate possible underlying causes including whether females may be more closely tracking fluctuations in relative abundance of plant species, in particular their preferred species, across time.

Males and females varied both quantitatively and qualitatively in the diversity of plants visited and found in their pollen loads. We show that females visit a greater diversity of plant species, coinciding with predictions on sex specific foraging patterns spanning across pollinator taxa, that females would have a higher degree of specialization due to constraints imposed by offspring provisioning (Smith et al. 2019). This greater diversity arises not because females visit a greater number of species; in fact, we see males tend to visit a greater number of species and have pollen from significantly more species on their bodies. Instead, the greater diversity arises because females visit species more evenly than males, in line with trends observed in female members of the butterfly *Lysandra bellargus* (Rusterholz & Erhardt 2000). The greater total number of species visited by and pollen grains picked up by male butterflies provides supports observations that males act as promiscuous foragers, landing on more species but many of which only rarely, even if perhaps not preferred in an attempt to refuel quickly. This unevenness is particularly acute when examining pollen loads; there were extremely low pollen counts for a greater percent of male pollen species, as 51% of species found across males were represented by less than 10 grains, compared to 38% for females. The consistency between our approaches, supports the conclusion that females rely on a greater diversity of nectar resources, which could be critical to support their fecundity.

Differences in composition of plant species found in the pollen dataset vs. the visitation dataset highlight how these approaches can complement each other. On the one hand, by sampling pollen directly from butterflies, we were able to see that they can use many more resources than indicated by visitation alone. On the other hand, pollen accumulation on butterflies does not necessarily correlate with the frequency with which they may rely on a given species. For example, we found few *M. fistulosa* pollen grains on butterflies, despite it being observed as a commonly visited species by regals in the long-term visitation data. These differences, however, can illuminate how effective *S. i.idalia* may be as a pollinator.

Sex-specific foraging patterns are predicted to have consequences for plant-pollinator interaction networks and plant fitness, but we have little evidence, in particular because these consequences are difficult to evaluate solely on observational data in isolation (Roswell et al. 2019; Kishi & Kakutani 2020; Smith et al. 2019). Studies examining pollen loads directly on pollinators have been able to shed some light onto differences between males and females. In contrast to prior studies of Lepidopteran pollinators revealing females typically carry larger pollen loads than males (Smith 2019, Appendix C ; Mendoza-Cuenca & Macías-Ordóñez 2005), we find female *S. i.idalia* carry significantly smaller loads — approximately 125 grains — nearly half as much as males. This result holds even when we remove pollen counts from the *Asclepias* pollinia, of which 5 were found on females and 71 were found on males. One explanation for this could be that females spend a large portion of their time in the grass, both to avoid harassment from undesirable males and post mating to conserve energy. Dense prairie grasses may knock more pollen off females compared to males, whose bodies are primarily surrounded by air. Another possible explanation may be that because females are the rarer sex at FIG males will repetitively attempt to mate with the smaller pool of females, removing pollen from their bodies each time they are harassed. Future work could consider how sex specific differences in mobility and behavior contribute to pollination efficiency. Whereas our visitation data further indicate females visit a greater diversity of plants and thus greater potential to transfer pollen to and from different species, greater diversity of plants visited did not translate to a greater diversity of pollen loads on females. In fact, male and female *S. i.idalia* had similar levels of diversity in their pollen loads despite males having greater species richness.

We can also make some inferences about the effectiveness of *S. i.idalia* as pollinators of some of their most preferred plants. In the long-term data for males and females combined we find that *A. tuberosa*, *C. pumilum*, and *M. fistulosa* are the top three most visited species, in that order. Specifically, *A. tuberosa* was the most visited species for males, and second most visited by females. Yet, in our pollen data, 71 of 76 pollinia were found on males, and 5 pollinia were found on females. Moreover, the frequency of pollinia found on males for females is not in proportion to differences in visitation rates between the sexes. Consequently, males may be more effective pollinators of *A. tuberosa*. Pollinia may more easily adhere to the limbs of males because they have more delicate (personal observation) limbs than females, which may be more compatible with the small flowers of *A. tuberosa*, such that the legs of males more easily insert into the stigmatic chambers of this species. Alternatively, males may have a negative impact on *A. tuberosa*'s reproduction. *A. tuberosa* tends to have a clumped distribution and *S. i.idalia* is a patrolling species. Considering that males carried the vast majority of pollinia, there may be significant consequences suffered by *A. tuberosa* due to male members of *S. i.idalia* removing large amounts of genetic material from this plant with each visit yet flying primarily in their territory. Patrolling behavior could exacerbate inbreeding if males were to visit the same plants of highly visited species such as *A. tuberosa* within a local area repetitively. Males of many members of Nymphalidae (Stylman, Penz, DeVries 2020, Mendoza-Cuenca & Macías-Ordóñez 2005, Ide 2004) exhibit this territory guarding behavior, which could lead to negative consequences for *A. tuberosa* inflicted by males across these Lepidopteran taxa. Our data also suggest *S. i.idalia* may not be a good pollinator of *M. fistulosa*, as less than 10 grains of this species were found in the pollen data compared to it being the third most visited species for males and females combined in both of the visitation datasets (Supplementary table. 2). However, we do find that males and females both appear to be effective pollinators for *C. pumilum* as this plant was highly visited by both males and females in the long term data sets and occurred in a high frequency in the pollen data.

Conclusions

Using direct pollen capture from the butterflies and a long-term dataset of regal fritillary foraging observations we identified several patterns of sexually dimorphic foraging in the diversity, pollen load size and compositions of *S. i.idalia*. Females were more diverse, but even, foragers than males, qualitatively across individuals but quantitatively across years. Females therefore may transfer more heterospecific pollen between species within a year, and cumulatively visit more species across years. In opposition to other research, pollen loads of males were larger than that of females, even in the absence of *Asclepias* pollinia. These results have implications for the proliferation and structure of local plant communities, especially for highly visited species such as *A. tuberosa* and *C. pumilum,* which may be subject to inbreeding if within a male's territory. Furthermore, these species were important to males and females, thus ensuring their conservation is vital for *S i.idalia.* Similarly to other pollinators, males and females differed significantly in floral community compositions; most notably, females varied more in their community composition from year to year than males, which is further supported by their higher diversity across years as well. Patterns detected in our research highlight that the consideration of sex specific foraging, using both visitation and pollen load data, could improve the accuracy of pollinator research, especially regarding our understanding network stability across seasons. Finally, both the consistencies in our results as well as the deficiency of relative studies demonstrates the need for ongoing research in sex specific foraging as well as intraspecific variation more broadly.

REFERENCES CITED

1. Valdovinos, F.S., Brosi, B.J., Briggs, H.M., Moisset de Espanés, P., Ramos-Jiliberto, R. and Martinez, N.D. (2016), Niche partitioning due to adaptive foraging reverses effects of nestedness and connectance on pollination network stability. Ecol Lett, 19: 1277-1286. https://doi.org/10.1111/ele.12664
2. Kaiser-Bunbury, C.N., Muff, S., Memmott, J., Müller, C.B. and Caflisch, A. (2010), The robustness of pollination networks to the loss of species and interactions: a quantitative approach incorporating pollinator behaviour. Ecology Letters, 13: 442-452. https://doi.org/10.1111/j.1461-0248.2009.01437.x
3. Tepedino, V. (1981). The Pollination Efficiency of the Squash Bee (Peponapis pruinosa) and the Honey Bee (Apis mellifera) on Summer Squash (Cucurbita pepo). *Journal of the Kansas Entomological Society, 54*(2), 359-377. Retrieved November 9, 2020, from http://www.jstor.org/stable/25084168
4. M. Eckhart, V., S. Rushing, N., M. Hart, G. and D. Hansen, J. (2006), Frequency–dependent pollinator foraging in polymorphic *Clarkia* *xantiana* ssp. *xantiana* populations: implications for flower colour evolution and pollinator interactions. Oikos, 112: 412-421. https://doi.org/10.1111/j.00301299.2006.14289.x

5. Des Roches, S., Post, D.M., Turley, N.E. *et al.* The ecological importance of intraspecific variation. *Nat Ecol Evol* **2,** 57–64 (2018). https://doi.org/10.1038/s41559-017-0402-5
6. Post, D.M., Palkovacs, E.P., Schielke, E.G. and Dodson, S.I. (2008), Intraspecific variation in a predator affects community structure and cascading trophic interactions. Ecology, 89: 2019-2032. https://doi.org/10.1890/07-1216.1
7. Roswell M, Dushoff J, Winfree R (2019) Male and female bees show large differences in floral preference. PLoS ONE 14(4): e0214909. https://doi.org/10.1371/journal.pone.0214909
8. Smith, GP, Bronstein, JL, Papaj, DR. Sex differences in pollinator behavior: Patterns across species and consequences for the mutualism. *J Anim Ecol*. 2019; 88: 971– 985. https://doi.org/10.1111/1365-2656.12988
9. Kishi S and Kakutani T (2020) Male Visitors May Decrease Modularity in Flower–Visitor Networks. Front. Ecol. Evol. 8:124. doi: 10.3389/fevo.2020.00124
10. Rusterholz, H.-P., & Erhardt, A. (2000). Can nectar properties explain sex-specific flower preferences in the Adonis Blue butterfly Lysandra bellargus? Ecological Entomology, 25, 81–90. https://doi. org/10.1046/j.1365-2311.2000.00233.x
11. Levin, E., McCue, M. D., & Davidowitz, G. (2017b). Sex differences in the utilization of essential and non-essential amino acids in Lepidoptera. Journal of Experimental Biology, 220, 2743–2747. https://doi. org/10.1242/jeb.154757

12. Mevi-Schutz, J. & Erhardt, A. (2005) Amino acids in nectar enhance butterfly fecundity: a long-awaited link. American Naturalist, 165, 411-419. https://doi.org/10.1086/429150

13. Martino, A. M. G., Aranguren, J. O., & Arends, A. (2002). Feeding habits of Leptonycteris curasoae in northern Venezuela. The Southwestern Naturalist, 47, 78–85

14. Boggs, C. L., Smiley, J. T., & Gilbert, L. E. (1981). Patterns of pollen exploitation by Heliconius butterflies. Oecologia, 48, 284–289. https:// doi.org/10.1007/BF00347978

15. Mendoza-Cuenca, L., & Macías-Ordóñez, R. (2005). Foraging polymorphism in Heliconius charitonia (Lepidoptera: Nymphalidae): Morphological constraints and behavioural compensation. Journal of Tropical Ecology, 21, 407–415. https://doi.org/10.1017/ S0266467405002385

16. Kawooya, J. K., & Law, J. H. (1988). Role of lipophorin in lipid transport to the insect egg. Journal of Biological Chemistry, 263, 8748–8753.

17. Bronstein, J. L., Huxman, T., Horvath, B., Farabee, M., & Davidowitz, G. (2009). Reproductive biology of Datura wrightii: The benefits of a herbivorous pollinator. Annals of Botany, 103, 1435–1443. https:// doi.org/10.1093/aob/mcp053

18. SMITH, G.P., JOHNSON, C.A., DAVIDOWITZ, G. and BRONSTEIN, J.L. (2018), Linkages between nectaring and oviposition preferences of *Manduca sexta* on two co-blooming *Datura* species in the Sonoran Desert. Ecol Entomol, 43: 85-92. https://doi.org/10.1111/een.12475

19. Alarcón, R., Davidowitz, G., & Bronstein, J. L. (2008). Nectar usage in a southern Arizona hawkmoth community. Ecological Entomology, 33, 503–509. https://doi.org/10.1111/j.1365-2311.2008.00996.x

20. Alarcón, R., Riffell, J. A., Davidowitz, G., Hildebrand, J. G., & Bronstein, J. L. (2010). Sex-dependent variation in the floral preferences of the hawkmoth Manduca sexta. Animal Behavior, 80, 289–296. https://doi. org/10.1016/j.anbehav.2010.05.007

21. Bertsch, A. (1984). Foraging in male bumblebees (Bomhus lucorum L.): maximizing energy or minimizing water load. Oecologia 62, 325 336

22. Wolf, S., & Moritz, R. F.A. (2014). The pollination potential offree-foraging bumblebee (Bombus spp.) males (Hymenoptera: Apidae). Apidologie, 45, 440–450. https://doi.org/10.1007/s13592-013-0259-9

23. Ne'eman, G., Shavit, O., Shaltiel, L., & Shmida, A. (2006). Foraging by male and female solitary bees with implications for pollination. Journal of Insect Behavior, 19, 383–401. https://doi.org/10.1007/ s10905-006-9030-7

24. van Praagh, J. P., Ribi, W., Wehrhahn, C., & Wittmann, D. (1980). Drone bees fixate the queen with the dorsal frontal part of their compound eyes. Journal of Comparative Physiology A, 136, 263–266. https://doi. org/10.1007/BF00657542

25. Ogawa Y., Kinoshita M., Stavenga D.G. & Arikawa K. 2013: Sex-specific retinal pigmentation results in sexually dimorphic long-wavelength-sensitive photoreceptors in the eastern pale clouded yellow butterfly, Colias erate. - J. Exp. Biol. 216: 1916-1923 doi: 10.1242/jeb.083485

26. Rutowski, R.L. (2000), Variation of eye size in butterflies: inter- and intraspecific patterns. Journal of Zoology, 252: 187-195. https://doi.org/10.1111/j.14697998.2000.tb00614.x

27. Riffell JA, Alarcon R, Abrell L, Davidowitz G, Bronstein JL, Hildebrand JG. 2008. Behavioral consequences of innate preferences and olfactory learning in hawkmoth-flower interactions. Proc. Natl. Acad. Sci. USA 105:3404–9

28. Streinzer, M., Kelber, C., Pfabigan, S., Kleineidam, C. J., & Spaethe, J. (2013). Sexual divergence in the olfactory system of a solitary and a eusocial bee species. Journal of Comparative Neurology, 521, 2742– 2755. https://doi.org/10.1002/cne.23312

29. Forsberg, J., & Wiklund, C. (1988). Protandry in the Green-Veined White Butterfly, Pieris napi L. (Lepidoptera; Pieridae). *Functional Ecology, 2*(1), 81-88. doi:10.2307/2389464

30. Wiklund, Christer. (1977). Wiklund C, Fagerström T. Why do males emerge before females? A hypothesis to explain the incidence of protandry in butterflies. Oecologia. Oecologia. 31. 153-158.

31. Zonneveld, C, & Metz, J.A. (1991). Models on butterfly protandry: virgin females are at risk to die. Theoretical Population Biology 40, 308–321.

32. WildEarth Guardians. (2013), Petition to List the Regal Fritillary (Speyeria Idalia) Under the Endangered Species Act.

33. Shafir, S., Menda, G., & Smith, B. H. (2005). Caste-specific differences in risk sensitivity in honeybees, Apis mellifera. Animal Behavior, 69, 859–868. https://doi.org/10.1016/j.anbehav.2004.07.011

34. Irene Keller, Peter Fluri & Anton Imdorf (2005) Pollen nutrition and colony development in honey bees: part 1, Bee World, 86:1, 3-10, DOI: 10.1080/0005772X.2005.11099641

35. Larry G. Olsen, Roger Hoopingarner & E. C. Martin (1979) Pollen Preferences of Honeybees Sited on four Cultivated Crops, Journal of Apicultural Research, 18:3, 196-200, DOI: 10.1080/00218839.1979.11099967

36. Ide, J. Y. (2004). Diurnal and seasonal changes in the mate-locating behavior of the satyrine butterfly Lethe diana. Ecological Research, 19, 189–196. https://doi.org/10.1111/j.1440-1703.2003.00623.x

37. Pascarella, J. B. (2010). Pollination biology of Gelsemium sempervirens L. (Ait.) (Gelsemiaceae): Do male and female Habropoda laboriosa F. (Hymenoptera, Apidae) differ in pollination efficiency? Journal of Apicultural Research, 49, 170–176. https://doi.org/10.3896/ IBRA.1.49.2.05

38. Luz, J. R. P. (2004). On the association of Philopota sp. (Diptera, Acroceridae) with flower of gervão-azul, Stachytarphetta cayenensis (Verbenaceae) in Marambaia Island, Rio de Janeiro, Brazil. Entomology and Vectors, 11, 681–687. https://doi.org/10.1590/ S0328-03812004000400010

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

40. 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, 1061–1070. https://doi.org/10.3732/ajb.1200496

41. Smith, G. P. (2019). Sex-associated variation in the foraging behavior of hawkmoths (Doctoral dissertation). University of Arizona.
42. Murawski,D.A&Gilbert,L.E(1986). Pollen flow in Psiguriawarscewiczii – a comparison of Heliconius butterflies and hum-mingbirds. Oecologia 68, 161–167.
43. Dunlap-Pianka, H., Boggs, C., & Gilbert, L. (1977). Ovarian Dynamics in Heliconiine Butterflies: Programmed Senescence versus Eternal Youth. *Science, 197*(4302), 487-490. Retrieved November 9, 2020, from http://www.jstor.org/stable/1744989
44. Moranz,R.A., Fuhlendorf, S.D., Engle, D.M., 2014. Making sense of a prairie butterflyparadox: the effects of grazing, time sincefire, and sampling period on regal fritillaryabundance. Biol. Conserv. 173, 32–41. https://doi.org/10.1016/j.biocon.2014.03.003
45. Swengel, A. (1993). Regal fritillary: prairie royalty. Amer. Butterflies, 1(1), 4-9.
46. Caven, A.J., King, K.C., Wiese, J.D. *et al.* A descriptive analysis of Regal Fritillary (*Speyeria idalia*) habitat utilizing biological monitoring data along the big bend of the Platte River, NE. *J Insect Conserv* **21,** 183–205 (2017). https://doi.org/10.1007/s10841-017-9968-0
47. Selby, G. (2007) Regal Fritillary (Speyeria Idalia Drury): ATechnical Conservation Assessment. USDA Forest Service,Rocky Mountain Region. <http://www.fs.fed.us/r2/projects/scp/assessments/regalfritillary.pdf> 4th June 2011

48. Williams, B.L. (2002) Conservation genetics, extinction, and taxo-nomic status: a case history of the regal fritillary. ConservationBiology, 16, 148–157.

49. Keyghobadi, N., Koscinski, D., Weintraub, J.D. and Fonseca, D.M. (2013), Historical specimens reveal past relationships and current conservation status of populations in a declining species: the regal fritillary butterfly. Insect Conservation and Diversity, 6: 234-242. https://doi.org/10.1111/j.1752-4598.2012.00208.x

50. Zografou,K.,Swartz,M.T.,Tilden,V.P.,McKinney,E.N.,Eckenrode,J.A.,Sewall,B.J.,2017.Severedeclineandpartialrecoveryofararebutterflyonanactivemilitarytrainingarea.Biol.Conserv.216,43–50.https://doi.org/10.1016/j.biocon.2017.09.026.S.R. Svenningsen, et al.Land Use Policy 84 (2019) 114–126126

51. Ferster, B., Vulinec, K. Population size and conservation of the last eastern remnants of the regal fritillary, *Speyeria idalia* (Drury) [Lepidoptera, Nymphalidae]; implications for temperate grassland restoration. *J Insect Conserv* **14,** 31–42 (2010). https://doi.org/10.1007/s10841-009-9222-5

52. Brian J. Kopper, Shengqiang Shu, Ralph E. Charlton, Sonny B. Ramaswamy, Evidence for Reproductive Diapause in the Fritillary *Speyeria idalia* (Lepidoptera: Nymphalidae), *Annals of the Entomological Society of America*, Volume 94, Issue 3, 1 May 2001, Pages 427–432, https://doi.org/10.1603/0013-8746(2001)094[0427:EFRDIT]2.0.CO;2

53. Jost, L. (2006), Entropy and diversity. Oikos, 113: 363-375. https://doi.org/10.1111/j.2006.0030-1299.14714.x

54. Pollard, E & Yates, T.J. (1993). Monitoring Butterflies for Ecology andConservation; The British Butterfly Monitoring Scheme. Chapman &Hall, London.

55. Whittaker, R.H. (1972) Evolution and measurement of species diversity. Taxon, 21, 213–251. https://doi.org/10.2307/1218190

56. Hutcheson, I. 1970. A test for comparing diversities based on the Shannon formula. J. Theor. Biol., 29:151-154

57. Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'Hara, R.B. et al. (2013) Vegan: Community Ecology Package. R package version 2.0-6. https://cran.r-project.org/web/packages/vegan/index.html

58. Bray, J., & Curtis, J. (1957). An Ordination of the Upland Forest Communities of Southern Wisconsin. Ecological Monographs, 27(4), 326-349. doi:10.2307/1942268

59. Anderson, M.J. and Walsh, D.C.I. (2013) What null hypothesis are you testing? PERMANOVA, ANOSIM and the Mantel test in the face of heterogeneous dispersions. Ecol. Monogr., 83, 557–574.

60. Anderson, M.J. (2017). Permutational Multivariate Analysis of Variance (PERMANOVA). In Wiley StatsRef: Statistics Reference Online (eds N. Balakrishnan, T. Colton, B. Everitt, W. Piegorsch, F. Ruggeri and J.L. Teugels). https://doi.org/10.1002/9781118445112.stat07841

61. CLARKE, K.R. (1993), Non-parametric multivariate analyses of changes in community structure. Australian Journal of Ecology, 18: 117-143. https://doi.org/10.1111/j.1442-9993.1993.tb00438.x

62. Wickham H (2009). ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag, New York.

63. Robert Wyatt, Steven B. Broyles, Sara R. Lipow "Pollen-ovule Ratios in Milkweeds (Asclepiadaceae): an Exception that Probes the Rule," Systematic Botany, 25(2), 171-180, (1 April 2000)

64. Beattie, A. J. 1971. A technique for the study of insect-borne pollen. - Pan-Pacific Ent. 47: 82.

65. Thomas, R., & Schultz, C. (2016). Resource Selection in an Endangered Butterfly: Females Select Native Nectar Species. The Journal of Wildlife Management, 80(1), 171-180. Retrieved November 9, 2020, from http://www.jstor.org/stable/24764940

66. Ken R. Helms (1994). Sexual Size Divergence and Sex Ratios in Bees and Wasps The American Naturalist 143:3, 418-434

67. Guertin, S. (2015, September 9). *Endangered and Threatened Wildlife and Plants; 90-Day Findings on 25 Petitions.* U.S. Fish & Wildlife Service. 201523315.pdf(govinfo.gov)

68. PalDat – a palynological database (2000 onwards, www.paldat.org)

APPENDIX A.
SUPPLEMENTARY TABLES

Supplemental table 1.

Nectar species with highest frequency across data sets

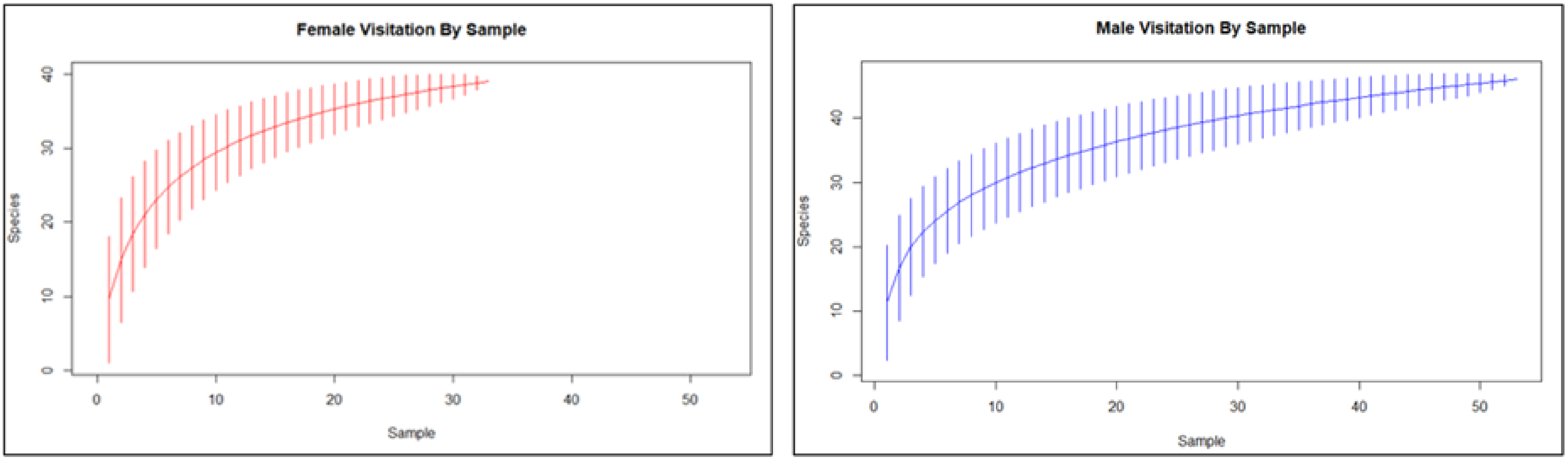
Top 3 most frequent species	Long term data	Long term (M-F overlap)	Pollen data
1	51% *A. tuberosa*	54% *A. tuberosa*	35% *A. tuberosa*
2	12% *M. fistulosa*	23% *M. fistulosa*	30% C. pumilum
3	10% *C. pumilum*	18% *C. discolor*	5% *A. syriaca*

Supplemental table 2.

Nectar species with highest frequency across data sets by sex

Top 3 most frequent species	Long term data		Long term (M-F overlap)		Pollen data	
Sex	Female	Male	Female	Male	Female	Male
1	32% *C. pumilum*	42 % *A. tuberosa*	42% *A. tuberosa*	64% *A. tuberosa*	39% *C. pumilum*	48% *A. tuberosa*
2	23% *A. tuberosa*	5.6 % *C. pumilum*	26% *M. fistulosa*	20% *M. fistulosa*	13% *A. syriaca*	28% *C. pumilum*
3	21% *C. discolor*	5.6 % *M. fistulosa*	20% *C. pumilum*	14% *C. pumilum*	11% *A. tuberosa*	4.6% *R. hirta*

APPENDIX B.
SUPPLEMENTARY FIGURES



Supplemental figure 1. Species accumulation curves for pollen data.

Species accumulation curves for pollen data generated in "*vegan*", using "exact" method. 86 butterflies (33 female, 53 male). Males visited 45/47 morphospecies. Females visited 38/47 morphospecies. Two species unique to females, nine species unique to males.