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The Role of Continuous Flowering Phenology for in Neotropical Plant-Pollinator Interactions for Use in Conservation

Chelsea Renee Hinton
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THE ROLE OF CONTINUOUS FLOWERING PHENOLOGY IN NEOTROPICAL
PLANT-POLLINATOR INTERACTTIONS FOR USE IN CONSERVATION

BY

CHELSEA HINTON

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THE ROLE OF CONTINUOUS FLOWERING PHENOLOGY IN NEOTROPICAL
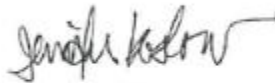
PLANT-POLLINATOR INTERACTIONS FOR USE IN CONSERVATION

BY

CHELSEA HINTON

Submitted to the Faculty of the Graduate School of
Eastern Kentucky University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

2019

DEDICATION

I would like to dedicate this manuscript to my family for their unconditional support

and Dr. Valerie Peters for her invaluable guidance and for making it all possible.

ACKNOWLEDGEMENTS

CHAPTER I: ABSTRACT

The diversity of mutualistic interactions in the Neotropics exceeds that of all other

tropical regions and is posited to result from a unique assemblage of plant species that

produce the highest spatio-temporal predictability of food resources. A rare component of

the Neotropical flora that contributes largely to the spatio-temporal predictability of food

resources is found in understory shrub or treelet species with a continuous reproductive

phenology (i.e. produce fruit and flowers daily during all months of the year). Plant-

animal interaction science suggests that plant species with a longer duration of

reproductive phenology will accumulate more mutualistic partners over time and

therefore play a more central role in the network. Here we focus on plant and insect

pollinator interactions within the lowland understory shrub community to ask: (a) Do

shrub species with the continuous flowering phenology share the same role in networks

and (b) Does network structure or shrub species role in the network change between

seasons or interannually. Plant-pollinator interactions and plant species role in the

network were quantified using bipartite network analysis, Chao similarity index and

modularity analysis. Modularity analyses indicate focal shrubs do not share similar roles

as peripherals (*z-score* < 2.5, *c-score* < 0.62), two species scored above the among

module connectivity threshold (*c-score* > 0.62) and were thus assigned connector roles.

These results differ from theoretical studies relating flowering duration to plant network

role. Floral abundance has a significant impact on pollinator richness ($X^2 = 11.43$, p <

0.001) and abundance ($X^2 = 7.75$, p = 0.005). Focal shrubs differed significantly in

pollinator richness ($X^2 = 17.85$, p = 0.001) and abundance ($X^2 = 31.81$, p < 0.001).

Caesalpinia pulcherrima exceeded all other focal shrubs in pollinator richness ($\bar{x} =$

3.83±0.35) and abundance ($\bar{x} = 11.81±1.50$). Pollinator community composition and interaction composition varied between years, but the core set of interactions remained consistent for focal shrub species. Not all plant species with the continuous flowering phenology may accumulate more partners over time as observed with more intermediate flowering phenologies, owing to the low abundance of flowers produced daily or the higher degree of interaction specialization.

I.   CHAPTER II: ABSTRACT

The neotropics hosts a higher diversity of plant-animal mutualisms, even compared to other tropical regions. Shrub species with a relatively rare reproductive phenology can be found in the understory layer of Neotropical wet forests that likely contributes to the predictability of food resources. These shrub species produce fruit and flowers during all months of the year and therefore are ideal candidates for testing the mutualistic network theory that plants with a longer reproductive phenophase will host a more diverse foraging assemblage due to the accumulation of more partners over time. Two shrub species with very distinct floral traits, *Hamelia patens* and *Stachytarpheta frantzii*, are the only naturally occurring plant species with the continuous reproductive phenology found along the Pacific slopes of Northern Costa Rica. The proportion of the pollinator community using floral resources of the two plant species was quantified in order to understand whether these species accumulate more partners over time or if the plants provide resources to a smaller and fixed, predictable subset of all possible pollinator consumers. In addition, the pollinator community (i.e. all Apoidea and Lepidoptera) of the two plant species were compared across different elevations to understand how warming temperatures might affect pollinator communities and their interactions. We conducted timed observations at *H. patens* and *S. franzii* flowers and compared pollinator assemblages visiting these species to other shorter duration flowering plant species in the study area, and to pollinator species assemblages collected using other active and passive capture methods. Consumer specificity for the two plant species was 23.4 percent and 27.2 percent, for *H. patens* and *S. frantzii*, respectively, showing a very diverse visitor assemblage for both plant species. but exemplified

differences in pollinator foraging preferences. Results of a Chao similarity index indicate

H. patens supported a predictable visiting assemblage (Chao June v. July = 0.78), but *S.*

frantzii demonstrated very low overlap in community composition (Chao June v. July = 0.36)

suggesting a more opportunistic role. Ordination revealed elevation as an important factor

in structuring the bee assemblage visiting the two continuous flowering species ($F_{1,169}$ =

1.97, p = 0.001). Rainfall patterns between the two years indicated that intra-annual

variation in rainfall and less predictable rainfall patterns in the future may increase the

importance of the continuous flowering shrub species for the conservation of bee

communities.

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Introduction

The diversity of mutualistic plant-animal interactions in the Neotropics exceeds that of all other terrestrial regions (Fleming and Kress 2013). Animal-mediated seed dispersal and pollination are key ecological processes that maintain global biodiversity patterns (Balvanera et al. 2005, Burkle and Alarcon 2011, Hoiss et al. 2015, Howe 2016). Thus, successful conservation and restoration efforts of Neotropical biodiversity requires a better understanding of the ecological underpinnings of seed dispersal and pollination, as well as how changes in land-use and climate will affect the species participating in these mutualisms and their interactions (Biesmeijer et al. 2006, Tylianakis et al. 2008; Hegland et al. 2009; Garibaldi et al. 2011).

Intact Neotropical forests harbor an assemblage of plant species that, together, produce the highest spatial and temporal predictability of fruit and nectar resources when compared to all other regions (Fleming and Kress 2013). It has been posited that the higher diversity of mutualistic plant-animal interactions in the Neotropics is a result of the increased predictability of fruit and nectar resources, and that these abundant resources can be attributed to the unique evolutionary history of the Neotropical flora, which includes (a) an Andean-centered radiation of epiphytes, understory shrubs and palmetto-like monocots and (b) an Amazonian-centered radiation of canopy tree species and lianas (Fleming et al. 1987; Fleming and Muchhala 2008). Owing to the longer duration of their reproductive phenology, certain plant species, or genera within these

two plant groups, contribute substantially more to reducing resource patchiness in the

Neotropics, implying that these plant species may play a disproportionally greater role

in supporting biodiversity (Peters et al. 2016). However, this idea has not yet been

empirically tested. Plant-animal network theory provides additional evidence that plant

species with a longer duration of reproductive phenology may support greater numbers

of species, as this plant trait has been hypothesized to be associated with hub species in

the network (Carlo et al. 2007). Mutualistic network theory holds that plant species with

a longer phenophase will accumulate more species over time, and because of this, they

would be considered network hubs (Yang et al 2013; Olsen et al. 2008; Burkle and

Alarcon 2011). Hubs are defined as those plant species with a disproportionately large

number of connections compared with that of other species in the same network, and

they are expected to play key organizational and structural roles in mutualistic

communities, such that their loss from the network is expected to have the greatest

overall effect on the risk of secondary extinctions (Bascompte and Jordano 2014; Mello

et al. 2015). While mutualistic network studies continue to find that most networks

include hub species, i.e. that most empirically studied networks include the presence of

some plant species capturing a disproportionately higher number of interactions in the

network (Bascompte and Jordano 2007; Bascompte 2009), little attention has been paid

to empirically testing which attributes or traits of plant species are shared across hub

species. Furthermore, there is an urgent need to identify if hub species share traits and

to empirically test whether plant species with those shared traits can be used in

conservation and restoration applications for biodiversity protection. Given that only a

subset of trees establish during unassisted restoration and that much variability is

observed in the process, new research needs to focus on developing active restoration strategies that can better and more consistently restore ecosystem services found within – or leading to – mature forest systems (Chazdon 2008; Cardinale et al. 2012).

While most plant species have temporally well-defined phenology patterns, a few Neotropical shrub and treelet species show steady-state reproductive phenology strategies in which they produce resources daily over extended periods that span up to entire years (Gentry 1974; Newstrom et al. 1994). In Costa Rican lowland wet forests, approximately 7% of shrub and treelet species exhibit a steady-state or continuous reproductive phenology (Opler et al. 1980, Bawa et al. 2003). This rare phenology pattern in the shrub community provides a unique opportunity to evaluate the role of resource duration in ecological networks. If a longer phenophase allows plant species to accumulate more partners, then mutualistic networks constructed of tropical shrub communities should find that those shrub species with a continuous reproductive phenology are the most well-connected species of the network. While some evidence provides support that shrub species with a continuous flowering phenology can be intercropped in agricultural lands for pollinator conservation (Peters 2014), it remains unclear if all species with this reproductive phenology can be equally beneficial for biodiversity and whether the suite of shrub species sharing this phenology will hold the same role in mutualistic networks.

Here, we focus on plant-pollinator interactions within the lowland shrub community of the Neotropics to address the following questions:

(a) Do shrub species with the longest possible duration of flowering phenology (i.e. produce new flowers daily and during all months of the year) share the same

role in plant-pollinator networks (i.e. are all peripherals, connectors, module hubs or network hubs)?

(b) Does floral abundance regardless of plant identity affect the microstructure mutualistic networks?

(c) Does network structure or shrub species role in the network change between seasons or interannually for the tropical shrub community?

(d) Does floral abundance in neotropical shrub species with continuous flowering phenology affect the species richness or abundance of flower visitors?

(e) Owing to the daily production of a predictable floral resource, do shrub species with a continuous flowering phenology support only a subset of the pollinator community but predictably over time, or is there a high degree of pollinator species overlap amongst the shrub community

Methods

Study Site

Our study took place in the southern Pacific lowlands of the Puntarenas Province of Costa Rica in the Osa Peninsula (OP; 8˚N, -83˚W). Since the 1970's, the OP has been a priority interest in conservation as a melting pot of biodiversity between the Americas, containing 2.5% of the world's biodiversity. This interest has led to the development of the Corcovado National Park, which takes up the majority of region (Fig. 1). This ecoregion has a drier period from November-December and a rainier season beginning April-May, although the seasonal climates are much less extreme

compared to the Northern Pacific slope of Costa Rica (Grub and Whitmore 1966).

Annual rainfall throughout this region fluctuates between 3000 and 7000 mm and mean
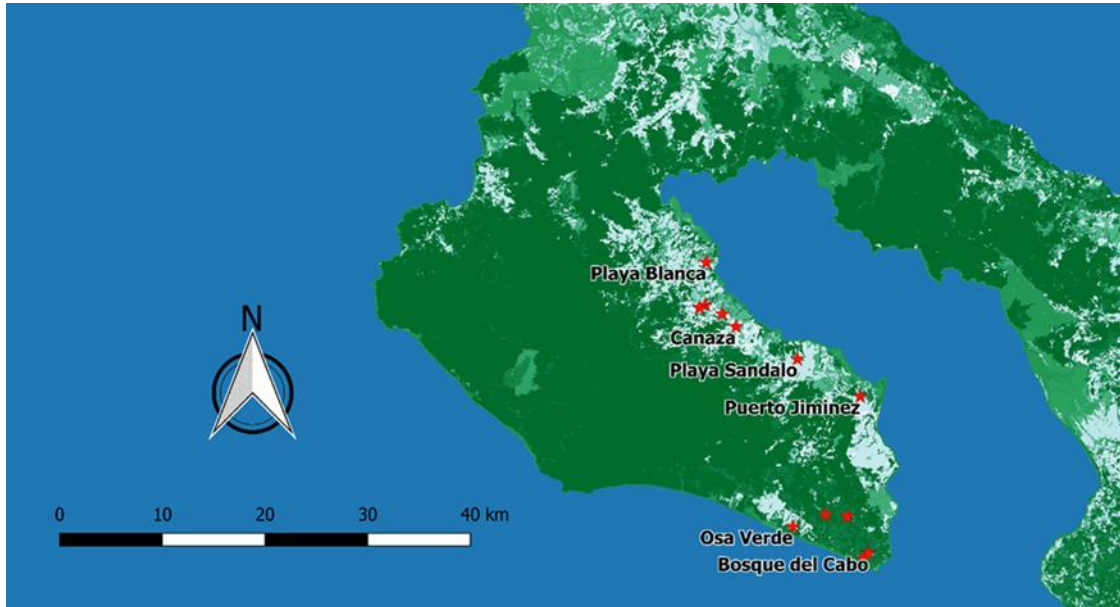
Figure 1. Map of Study Sites

temperatures range from 24-26.5˚C (Taylor et al. 2015). Within the OP, we sampled

plant species from several sites. Finca Kobo (8˚36'23" N, -83˚26'38" W) is a rich

agroforest producing many fruits for local communities and tourists, such as starfruit,

chocolate, noni fruit, a variety of bananas, pineapple and many others. The

heterogenous matrix of plant diveristy, canopy gaps and forest edge supported the

growth of shrub species that display a continuous reproductive phenology and many

other flowering plants. Palo Seco (8˚36'14" N, -83˚26'57" W) is a rural village adjacent

to Finca Kobo and houses a handful of residents who operate small farms and pastures

for their families. This countryside matrix brushes right up against the Corcovado

National Park and hosts a wealth of native ornamentals and forest edge habitat which

appropriated an ideal location for sampling accessible flowering plants. In transit between sites, we located roadside habitat near Playa Sandalo (8˚33' N, -83˚21' W), Rio Barrigones (8˚35' N, -83˚25' W), Cañaza (8˚35' N, -83˚24' W), and Playa Blanca (8˚38' N, -83˚26) that also offered naturally occurring shrubs that display a continuous reproductive phenology as well as other flowering plants to sample the network. Puerto Jimenez is urban town which intercepts the most human traffic in the localized area and contains the majority of the local businesses. The town is lined by the beaches of Golfo Dulce. Other sites, Bosque del Cabo (8˚23'21" N, -83˚17'54" W) and El Romanso (8˚23'05" N, -83˚18'11" W), were secluded resorts nestled within forested areas and contained a suite of ornamental flowering plants from which we sampled species flowering at the time of our study.

Osa Verde (8°24'44" N, -83°21'54" W) is a 10-hectare farm site adjacent to an old landing strip and is owned and operated by Osa Conservation. Osa Verde was previously deforested to raise cattle and provide graze, however, since 2003, has now become a site dominated by grasses and shrubs often associated with agricultural disturbance. Osa Piro (8˚24'11" N, -83˚20'14" W) is also owned and operated by Osa conservation but is surrounded by secondary growth forest. This site is populated with planted, and naturally occurring native shrubs within the matrix and around the forest edge. All of these sites were selected for their locality, providing a diversity of habitat types from which plants were accessible and frequently occurring, and plant composition, containing a combination of shrub species with a continuous reproductive phenology and those exhibiting a shorter reproductive duration.

Plant Selection

Throughout the study area, we located naturally occurring and planted individuals of five native shrub species that have been documented to produce fruit and flowers during all months of the year in the region. These continuous flowering shrubs were *Caesalpinia pulcherrima* (L.) Sw. (Fabaceae), *Conostegia subcrustulata* (Beurl.) Triana (Melastomataceae), *Hamelia patens* Jacq. (Rubiaceae), *Lantana camara* L. (Verbenaceae) and *Stachytarpheta frantzii* Pol. (Verbenaceae; Table 1). Individuals of these five focal shrub species were abundant throughout the countryside (i.e. agricultural edges and villages) of the region. We focused on the tropical countryside rather than intact natural systems because (a) pollinators are more readily sampled from open, sunny areas and (b) naturally occurring individuals displaying a continuous flowering phenology would only occur in forest edges and treefall gaps, making sampling these areas more logistically challenging. Shrub individuals selected for sampling were located in a variety of habitat types including agroforests, secondary growth forests, old cattle pastures, and semi-urban and rural roadsides. In order to construct a network of all flowering shrub species in the study area during the time of the study, we also sampled other flowering shrubs and herbaceous plants found occurring in the tropical countryside, however, as the continuous flowering phenology is only displayed within the shrub community we did not include flowering tree species in the network.

Table 1. Plant Species Descriptions

Focal Species	Family	Flowering	Description	Citation
Caesalpinia pulcherrima (L.) Sw.	Fabaceae	Continual	Shrub or tree; flowers are yellow or orange-red with wrinkled petal margins, styles and stamens protrude roughly 8 cm out from the flowers	Zuchowski 2007
Conostegia subcrustulata (Beurl.) Triana	Melastomataceae	Continual	Shrub; flowers are radially symmetrical and pale pink	Kriebel 2016
Hamelia patens Jacq.	Rubiaceae	Continual	Shrub or tree; Orange-red tubular shaped flowers growing in racemes	Bawa 2003, Opler et al 1980; Bawa and Beach 1983

Table 1. (continued)

Focal Species	Family	Flowering	Description	Citation
Lantana camara L. *(sensu lato)*	Verbenaceae	Continual	Shrub; dome shaped flower clusters, with very small four-lobed flowers, yellow in color for newer blooms transitioning to orange-red for older flowers.	Schemske 1976
Stachytarpheta frantzii Pol.	Verbenaceae	Continual	Shrub, Pale purple flowers with five lobes seated in the rachis	Bawa 2003, Woodsen et al. 1973
Senna alata (L.) Roxb.	Fabaceae	Late rainy to early dry season	Shrub or tree; Soft yellow flowers with five petals	Zuchowski 2007

Table 1. (continued)

Focal Species	Family	Flowering	Description	Citation
Turnera subulata J. E. Smith	Tuneraceae	Peak flowering is February to November, but can be seen flowering beyond either extreme.	Sub-shrub; cream colored, funnel shaped flowers with a deep-purple or black center; flowers are ephemeral, open only a portion of the day but varies daily	Arbo 2007
Cornutia pyramidata L.	Lamiaceae	Early rainy season	Shrub or tree; Inflorescences with purple flowers arranged in a terminal panicle.	

Table 1. (continued)

Focal Species	Family	Flowering	Description	Citation
Ochroma pyramidale (Cav. ex Lam.) Urb.	Bombaceae	Typical flowering occurs in the dry season	Tree; Flowers are bell-shaped and have five white petals folded under.	Zuchowski 2007
Morinda citrifolia	Rubiaceae	-	Small shrub or tree; small white flowers with five petals	Zuchowski 2007
Arachis pintoi Krapov. & W. C. Greg.	Fabaceae	Flowering onset initiated by rainfall, but can sometimes be seen flowers throughout the year	Small, herbaceous, trailing plant; yellow pea-like flowers	Zuchowski 2007

Table 1. (continued)

Focal Species	Family	Flowering	Description	Citation
Musa sp.	Musaceae	Asynchronous flowering, continual at the population level	Tall herb; large inflorescence with purple bracht from which pale purple and yellow flower clusters are concealed; very fragrant	Zuchowski 2007
Melastomataceae sp.	Melastomataceae	-	-	
Isertia haenkeana	Rubiaceae	Peak flowering occurs in the early rainy season	Shrub or tree; Inflorescences have tubular, yellow flowers with 3-3.5 cm long corolla. Inflorescences arranged in terminal, stout panicles.	Boom 1984

Table 1. (continued)

Focal Species	Family	Flowering	Description	Citation
Zinnia peruviana (L.) L.	Asteraceae	April-October	Herbaceous plant; Flowers are red or yellow in a ray, with linear or spatulate petals.	Torres 1963
Cocos nucifera L.	Arecaeae	-	Tree; Branched inflorescence, or spadix, with several hundred small flowers, mostly male.	Regi and Josephrajkumar 2013, Zuchowski 2007
Yellow *Herbaceous sp.*	-	-	-	-

Pollinator Sampling

This study was conducted in June—July 2017 and 2018, and December 2017. Pollinator sampling was conducted daily from 0800 –1500 hours. All insect visitors to open flowers of selected shrub individuals were collected during a 30-minute sampling period and euthanized for later identification. For each individual shrub observed, we estimated floral abundance by counting open flowers on 5-10 branches and then

multiplying this number by the number of branches on the plant. We also noted the degree of sun exposure during the observation period.

Although all insect visitors to flowers were collected, we primarily captured insects from the superfamily Apoidea: bees (Hymenoptera: Apoidae) and butterflies (Lepidoptera), and therefore our network is constructed from only these two pollinator groups. Collected Lepidoptera were taken to the UGA field station in NW Costa Rica and were identified to species or genus by J. Montero. Bees were preserved in 70% ethanol and were exported to Eastern Kentucky University where they were identified to species or genus using several keys: Michener (2000), Mawdsley (2017), Aguiar and Filella (2011), and Roubik and Hanson (2004).

Data Analysis

To evaluate our overall sampling effort, we composed individual-based species accumulation curves (Fig. 2). To account for variation in sampling effort, we first constructed individual-based rarefaction curves to assess differences in pollinator richness between plant species (Fig. 3). Next, to quantify the role of our 5 focal continuous flowering shrub species in the network, we generated a plant-pollinator quantitative interaction matrix representing a weighted bipartite network (Fig. 4) using all recorded observations. This interaction matrix was then used to evaluate modularity roles for the lower trophic level (i.e. all plant species). Modularity means that there are groups of pollinators that strongly interact with a set of plant species in the same module (Olesen et al. 2007). Networks with high modularity are thought to be more resilient to disturbance and to protect communities from species loss (Ramos-Robles et

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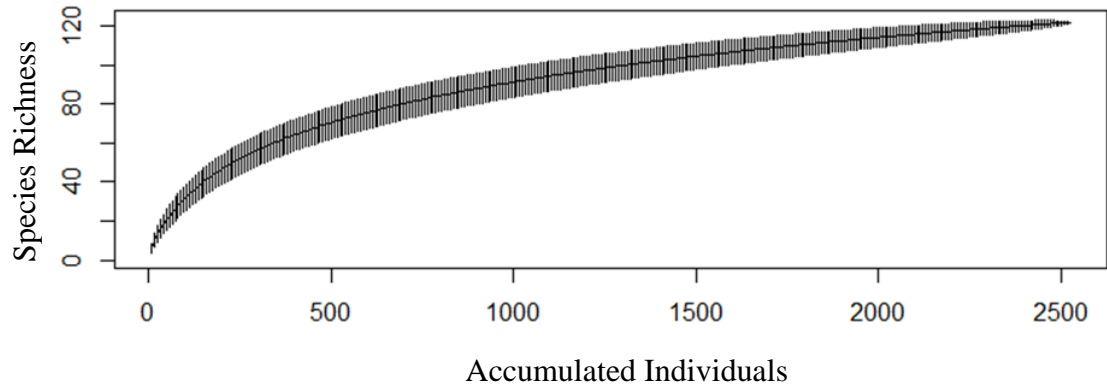
Figure 2. Species Accumulation Curve
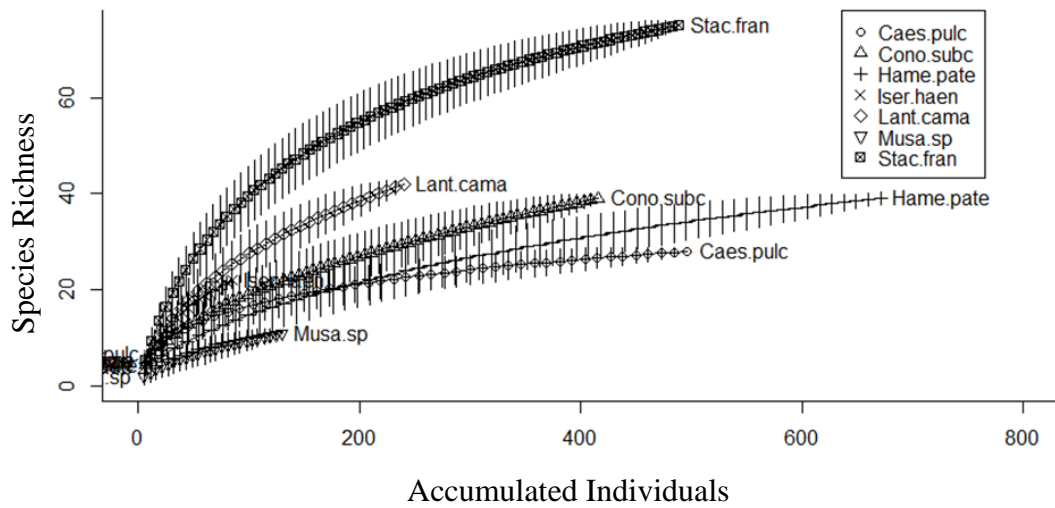
Figure 3. Species Richness Curves

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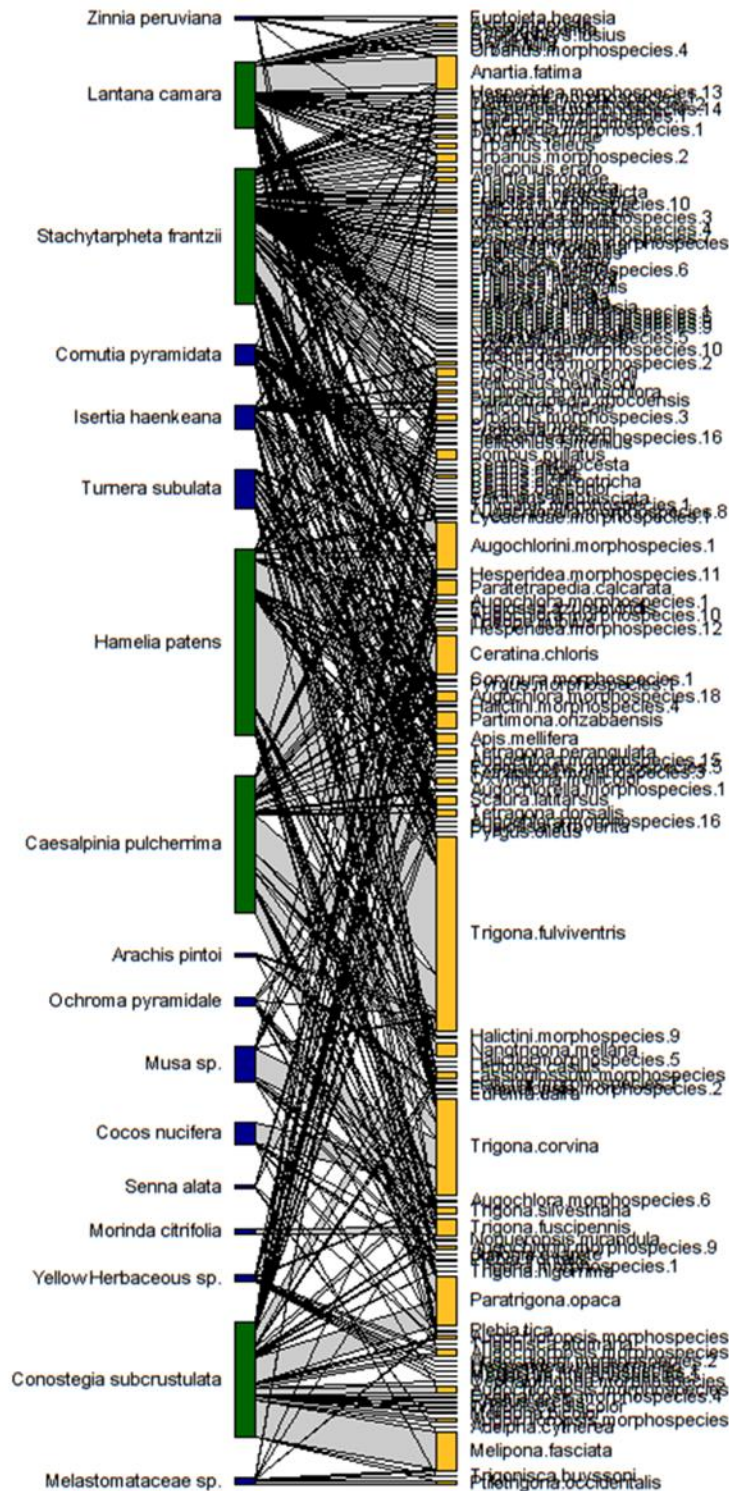
Figure 4. Bipartite Network Diagram Representing the Observed Interaction Matrix

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al. 2018). Modularity analyses can identify the role of a given species within the network by analyzing its position in relation to other species. Modularity algorithms achieve this through repeated partitioning of the network into sub-networks, or modules, to optimize modularity (Q). From that point, the analysis examines interactions of a particular species within their respective module and interactions occurring among other modules (Beckett 2016, Dormann and Strauss 2014, Olesen et al. 2007). The resulting output gives two values, *c-score* and *z-score*, which quantify between module connectivity and within module connectivity, respectively. Using thresholds assigned by Olesen et al. (2007), there are four network roles that can be assigned to species: peripherals, connectors, module hubs and network hubs. Peripherals are species that have less frequent interactions with other species in the network compared to other species (*c-score* < 0.62 and *z-score* < 2.5). Species that connect several modules to each other are considered connectors (*c-score* < 0.62 and *z-score* ≥ 2.5). Module hubs are species that have the highest number of interactions but only within the module that they occur. Network hubs are species that provide disproportionately more support for partners within the module, as well as for partners among other modules (*c-score* ≥ 0.62 and *z-score* ≥ 2.5, respectively, Olesen et al., 2007). Species with the roles of connectors and network hubs play key roles in mutualist systems linking the network together with a disproportionately higher number of interactions (hubs) and binding modules (connectors) for network stability (Bascompte and Jordano 2007, Peters et al. 2016; Mello et al. 2015).

Quantitative networks were analyzed for modularity and species roles using the DIRTLPAwb+ algorithm modification using R 3.5.3 'bipartite' package (Beckett 2016,

Dormann et al. 2009 with 2019 revisions). DIRTLPAwb+, a quantitative form of

modularity testing, assesses interactions different from binary data, which weights the

importance of strong interactions similar to rare interactions, and instead tends to form

modules around stronger interactions (Beckett 2016). Most modularity algorithms have

been crafted to represent binary networks, such as the one developed by Guimera and

Amaral (2005). However, new modifications of algorithms have been developed for

testing quantitative, or weighted, ecological networks (Beckett 2016, Dormann and

Strauss 2014). Both methods have value in analyzing network data, but for this study

we used quantitative data because we wanted to accurately account for the higher

number of interactions that would be observed for the shrub species with continuous

flowering since they were flowering during all sampling periods. Analyzing weighted

networks can also provide more insight into the ecological underpinnings of pollination

and seed dispersal (Beckett 2016, Gilarranz et al. 2012, Jordano 1987, Veen et al.

2006). We obtained the maximum modularity value from 100 repeated runs. The *c-* and

*z-score*s were extracted from the output and critical values were used to delineate plant

species roles using species strength (Dormann et al. 2018 Bipartite R revision, Guimera

and Amaral 2005, Olesen et al. 2007).

      To determine the effect of floral abundance on network roles we also generated

a floral abundance network retaining the quantitative interaction matrix. Floral

abundance was binned across all plants to simulate nodes in the lower trophic level. For

this analysis we combined all data collected throughout our study and followed the

same modularity testing procedures.

In recent decades, studies have found temporal variation in plant-pollinator network interaction composition while still retaining broad-scale structural properties (Alarcon et al., 2008, Burkle and Alarcon 2011; Chacoff et al. 2018; Olesen et al. 2008). To examine temporal differences in network roles using modularity algorithms, we performed modularity analysis on rainy season and dry season interactions separately. Data collected from 2017 were separated into two groups, June-July (rainy season) and December (dry season), to evaluate seasonal variation in network roles. For interannual variation we excluded data collected in December of 2017 since no dry season data had been collected the following year, therefore interannual modularity analyses only used data from June—July 2017 and 2018. To obtain network roles for each group, we implemented the same modularity procedures as described above from analysis on the overall network. We compared the calculated network role of rainy season 2017 to the dry season of 2017 for seasonal variation. Rainy season modularity was compared between 2017 and 2018 to evaluate interannual changes in network roles.

To assess temporal variation in interaction composition we used a Whittaker's beta diversity index and multiple Chao similarity indices. Whittaker's beta diversity index was calculated for species turnover between years (June—July 2017 and 2018) for focal shrub species. We excluded samples from December 2017 since dry season data for the following year had not been collected. Chao similarity matrix was used to quantify overlap in pollinator species compositions interannually (2017-2018) and seasonally (rainy and dry) for each focal shrub species. The dry season was only sampled during December 2017, so we constrained the intra-annual matrix to only data

collected within that year. Chao method is best suited for study systems that are species rich, inherent for the tropics, and for variation in sample sizes (Chao et al. 2004).

To evaluate the roles of floral abundance and shrub species identity on the number of pollinators and pollinator species visiting the focal shrub species, a combination of generalized linear mixed models (GLMM) and liner mixed models (LMM) were used within the lme4 R package (Bates et al. 2015). GLMMs were constructed to evaluate the effect of each factor on pollinator richness using a negative binomial distribution. We used a goodness of fit test to ensure negative binomial was the appropriate fit. LMMs were used to model pollinator abundance against each factor, with pollinator abundance being log-transformed for normality. Each model was then tested against a null model to obtain significance values. We conducted post-hoc multiple comparisons tests using a 'single-step' adjustment following both models that assessed shrub species identity to delineate directional differences between shrub species in relation to pollinator abundance and richness.

Results

A total of 2949 plant-pollinator interactions from 377 samples were observed during our study. The higher trophic level contained 138 pollinator species, of which 87 were bee species and 51 were Lepidopteran species. The lower trophic level included 17 plant species (Fig. 4). Modularity partitioning of the overall network from 100 repeated runs resulted in a maximum modularity score (Q) of 0.488 (Fig. 5). Among module connectivity and within module connectivity values extracted from the modularity analysis scored three of the five focal shrubs below the critical values *c-score* < 0.62,
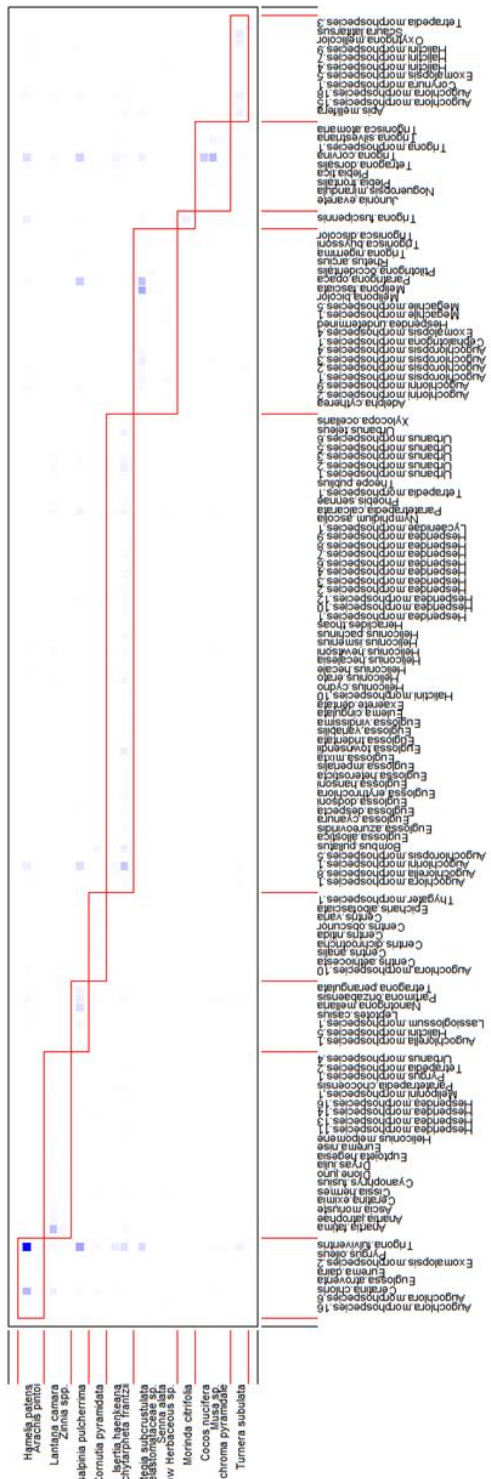
Figure 5. Modular Structure of Overall Network

z-score < 2.5, and therefore these three focal shrub species were assigned peripheral

roles in the overall network (Fig. 6a). Two shrub species with the continuous flowering

phenology, however, *H. patens* and *C. pulcherrima*, were connector species within the

network, *H. patens* with a *c-score* of 0.73 and *z-score* of 0.71, and *C. pulcherrima* with

a *c-score* 0.65 and did not receive a z-score because it was the only plant within the

module therefore it can't be calculated (Fig. 6a). Modularity testing and subsequent

module connectivity calculations did not score any plants with a *z-score* above the

critical value of 2.5, therefore the overall network did not contain module hubs or

network hubs.

The rainy season network of 2017 comprised of 762 interactions, 7 plant

species, 37 butterfly species and 38 bee species (Table 2), received a modularity value

of Q = 0.467. All shrub species with the continuous flowering phenology, as well as all

other plant species sampled were designated peripherals, *c-score*s < 0.62 and *z-score* <

2.5 (Fig. 6b). The dry season network of 2017 comprised of 327 interactions, 8 plant

species, 38 bee species and no butterfly species, also didn't have any plant species

exceeding the critical values for between module and within module connectivity

(Fig.6c). The rainy season network of 2018 comprised of 1860 interactions, 15 plant

species, 36 butterfly species and 73 bee species, had a modularity value of 0.549 (Fig.

6d). Three plant species were connector species in this network. Two plant species with

continuous flowering phenology, *H. patens, C. pulcherrima,* were connectors, with *c-*

scores of 0.73 and 0.65 and *z-scores* of 1.13 and 0.86, respectively. All three seasonal

networks also didn't show any plants with a *z-score* of 2.5 or greater and therefore did

not contain any module hubs or network hubs.
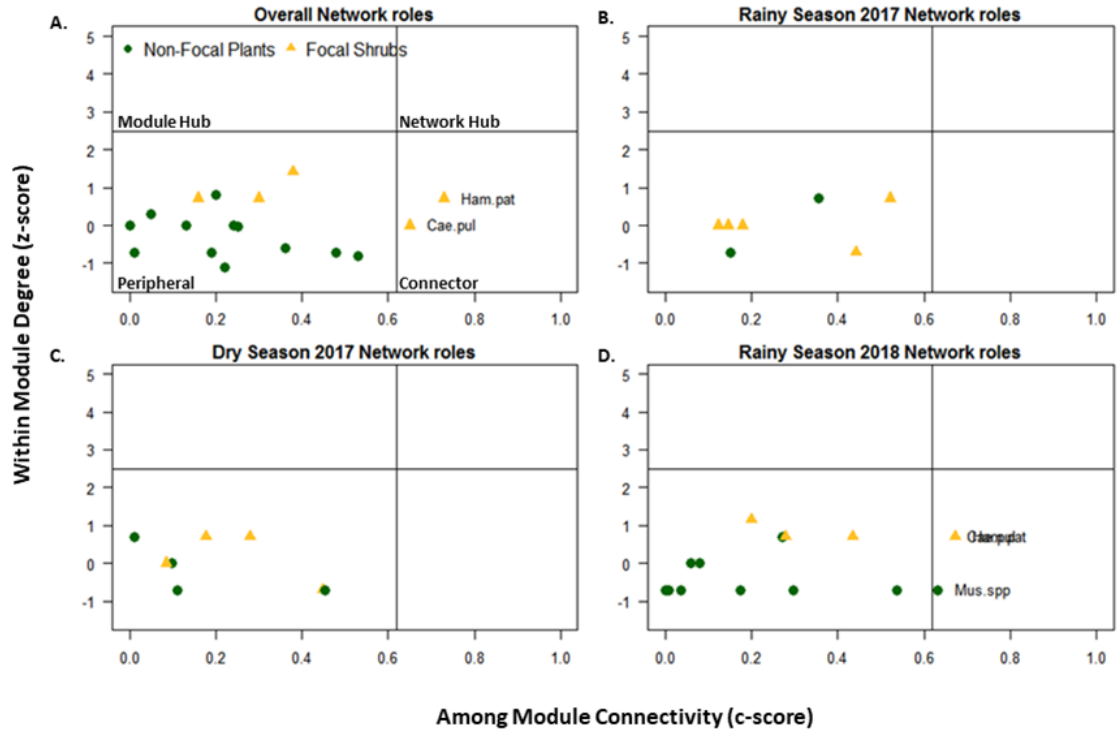
Figure 6. Plant Network Roles of the Overall Network and within each Temporal Grouping

Table 2. Interactions and Degree of Modularity for all Temporal Groupings

| | 2017 | | 2018 |
	Rainy Season	Dry Season	Rainy Season
Observed Interactions	762	327	1860
Number of plant species	7	8	15
Modularity	0.467	0.477	0.549

The floral abundance network was comprised of 2949 interactions, 22 binned abundance classes, 87 bee species and 51 butterfly species. Modularity for the network was $Q = 0.30$. Only one floral abundance class exceeded the set within module critical value. Samples obtained from plants having 1000-1099 flowers are a module hub (*c-score* = 0.08, *z-score* = 2.55, Fig. 7).
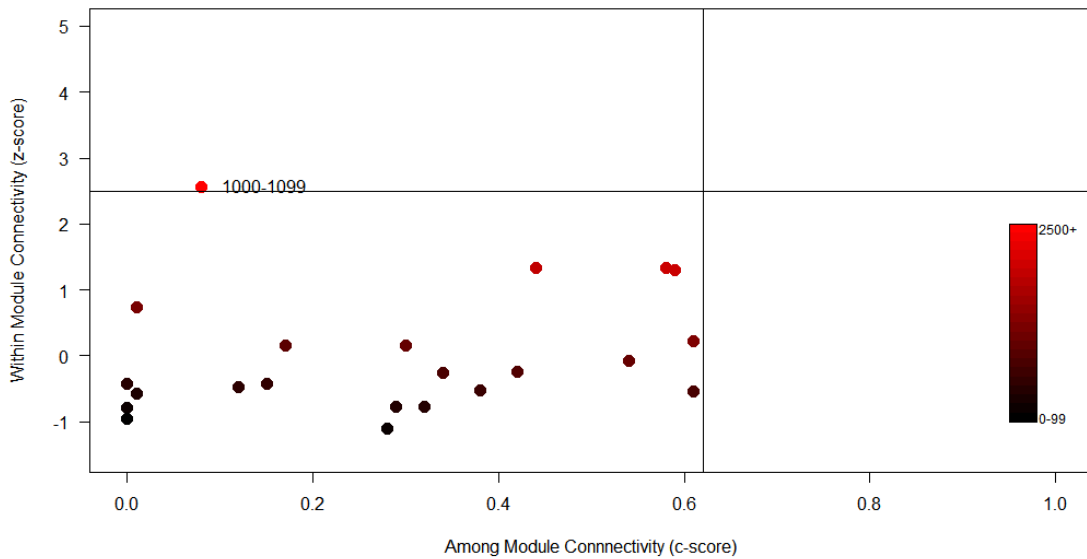
Figure 7. Floral Abundance and Network Roles

Shrub species differed significantly in pollinator richness ($X^2 = 17.85$, p = 0.001). *Stachytarpheta frantzii* had the highest projected species richness based on the rarefaction curves scaled by individuals (Fig. 3), however, *Caesalpinia pulcherrima* had significantly more species rich samples ($\bar{x} = 3.83 \pm 0.35$), while *S. frantzii* did not significantly differ from other shrub species ($\bar{x} = 3.72 \pm 0.30$) based on post-hoc comparisons (Fig. 8a). Pollinator abundance differed significantly between shrub species ($X^2 = 31.81$, p < 0.001) with *C. pulcherrima* having more pollinators visiting per sample ($\bar{x} = 11.81 \pm 1.50$). *Hamelia patens* ($\bar{x} = 10.48 \pm 1.37$) had significantly more interactions than *Lantana camara* ($\bar{x} = 6.15 \pm 1.22$) but did not greatly differ from other focal shrubs (Fig. 8c). Floral abundance had a significantly positive effect for pollinator richness ($X^2 = 11.43$, p < 0.001) and abundance ($X^2 = 7.75$, p = 0.005). Plants with

more flowers were visited by more species and more frequently per sample (Fig. 8b & d).

Species turnover between years varied based on focal plant species identity (Table 3). *C. pulcherrima* had the lowest turnover between years (w = 1.33) and *S. frantzii* had the highest turnover (w = 1.62). *C pulcherrima* had the highest compositional overlap between years (chao = 0.97). *H. patens* also had a relatively high pollinator community composition overlap between years (chao = 0.96). *S. frantzii* had the least similar pollinator community composition from year to year (0.64). All other focal species ranked high in compositional similarity (Table 3).
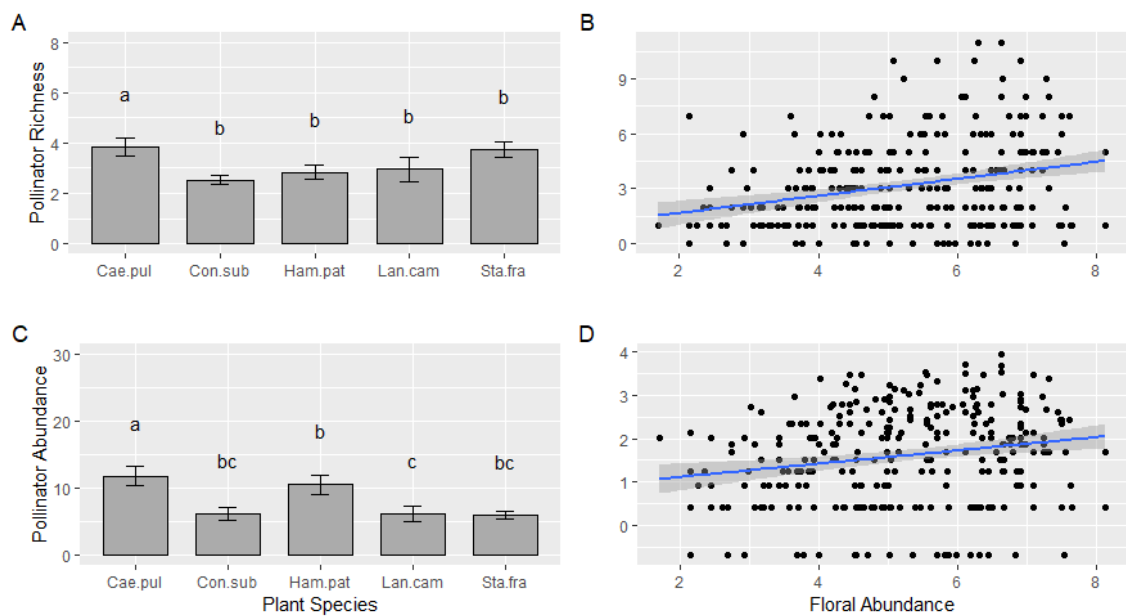
Figure 8. Effects of Floral Abundance and Plant Identity on Pollinator Assemblage Visiting Focal Shrubs

Table 3. Temporal Variation in Pollinator Assemblage of Focal Shrubs

2017	2018				
	Cae.pul	*Con.sub*	*Ham.pat*	*Lan.cam*	*Sta.fra*
Cae.pul	0.967	--	--	--	--
Con.sub	--	0.812	--	--	--
Ham.pat	--	--	0.962	--	--
Lan.cam	--	--	--	0.917	--
Sta.fra	--	--	--	--	0.647
Beta Diversity (w)	1.333	1.560	1.490	1.615	1.495

Discussion

Our study revealed that the plant trait of food resource duration was not associated with network role in our Neotropical plant-pollinator network. Specifically, plants with a longer duration of resource production were not all hubs in our network, as we had predicted based on current plant-animal network science positing that longer resource production should result in the accumulation of more mutualistic partners. With the exception of *H. patens* and *C. pulcherrima*, all sampled plant species, including three of the shrub species with continuous flowering phenology, were peripherals. Evaluation of other plant traits shared by *H. patens*, *C. pulcherrima* and other connector species may reveal underlying influential drivers dictating network roles. Recent studies have observed species strength among other factors in relation to species relative abundances could be driving force of network topology (Kaiser-Bunbury et al, 2014, Schleuning et al. 2014, Watts et al. 2016).

Finding a high degree of network modularity implies either that our study area is relatively well protected or that the loss of specialized interactions has not yet occurred

(Sebastian-Gonzalez 2015) or that owing to the availability of many native flowering plants in open areas of the country-side matrix, either as ornamentals or agricultural use, plant pollinator networks are less vulnerable to defaunation compared to fruit-frugivore networks (Isaacs et al., 2009, Williams and Lonsdorf 2018). Interestingly, partitioned modules of our network did not show clear patterns of floral traits (e.g. morphology or color) however, some pollinator species did. For instance, *S. frantzii* was the only plant within its module, but nearly all pollinators stemmed from two sub-groups, *Euglossa spp. (*Apidae), and two genera of butterflies, *Heliconius spp*. (Nymphalidae) and *Hesperiidae spp* (Hesperidae).

Empirical support for the role of both floral abundance and flowering duration in assigning plant species role in mutualistic network has been found for more seasonal systems and in fruit-frugivore networks (Carstensen et al. 2014, Olesen et al. 2008). In contrast, our network constructed from a more aseasonal shrub-pollinator network demonstrates floral abundance independent of plant identity affects basic diversity parameters but has no influence on network topology (Kaiser-Bunbury et al. 2014). None of the shrubs with continuous flowering phenology (i.e. the longest duration of reproductive activity possible) were hubs in our network, however they differed in relative comparison between other shrubs in regard to pollinator richness and abundance. Shrub species recorded having more abundant interactions were synonymous with focal plants that were deemed connector species in the overall network. However, *S. frantzii* and *C. pulcherrima* were observed having more species rich assemblages. This makes sense because the way modularity scores are calculated, more abundant interactions of the same pollinator and plant are more weighted than few

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interactions with many species and a particular plant. Therefore, in this instance, interaction abundance is more valued for module scores than richness.

Ecological networks have been described as temporally dynamic, changing the inner details from year to year, but always maintaining broad level structural attributes such as nestedness, asymmetry and interaction heterogeneity (Chacoff et al. 2017; Olesen et al. 2008). For our constructed network groupings, temporal variation in network roles was only experienced interannually, and between seasons showed no change in network topology. This may be credited to the differences in network size potentially owing to temporal dynamism of plant-pollinator networks (Alarcon et al. 2008, Burkle and Alarcon 2011, Chacoff et al. 2017, Olesen et al. 2008). Nearly two-fold more interactions were recorded in 2018, which can affect the operability of modularity algorithms as well as other network level attributes (Beckett 2016, Dormann and Strauss 2014). However, it should be noted from the compositional similarity analyses, all focal shrubs aside from *S.frantzii*, exhibited a reliable pollinator assemblage from year to year. Network connector species appeared to have the most compositional similarity between years, but *S. frantzii* appears to provide more opportunistic resources given the low similarity value (see Table 3).

More insight into plant-pollinator network microstructure is needed, perhaps other conceptual theories may be better suited to explain species rich ecological networks in the tropics. Recent studies have found that, contrary to previous expectations, specialization does not increase in tropical latitudes (Ollerton and Crammer 2002, Schleuning et al. 2012). Instead, it is posited that high specialization is an adaptive response to low plant diversity and that tropical systems being species-rich

implies a greater robustness to species extinctions in comparison to temperate systems (Schleuning et al. 2012). Mechanisms of neutral theory and biological constraints have been investigated for their capacity to better explain network properties acting simultaneously (Bascompte and Jordano 2007, Dupont et al. 2003, Krishna et al. 2008). Neutral theory describes network patterns resulting from the relative abundance of species, in the sense that more abundant species (i.e. plants or pollinators) will have a greater interaction strength by receiving more interactions. Vazquez et al. (2009) provided support finding neutral theory and temporal overlap thoroughly predicted several network properties for plant-pollinator mutualisms. Kaiser-Bunbury et al. (2014) further observed species identity and their relative abundances were major factors in explaining the finer structural components of plant-pollinator networks and for fruit-frugivore networks (Gonzalez-Castro et al. 2015). We are only beginning to unravel the topological dynamics of weighted ecological networks. Future avenues of research should explore plant identity and species relative abundances for their degree of impact on network topology in a system where spatiotemporal resource predictability for pollinator communities is unconstrained.

III.   II. The Role of Continuous Flowering Phenology in Neotropical Plant-Pollinator

Interactions Across an Elevation Gradient

Introduction

In the Neotropics there exists an unrivaled diversity of plant-animal mutualisms,

greater than all other terrestrial systems (Fleming and Kress 2013). One hypothesis for

the higher number of species involved in these mutualisms is that the Neotropics hosts a

higher spatial and temporal predictability of fruit and nectar resources compared to the

other regions (Fleming and Kress 2013). The Neotropical understory shrub community

is comprised of a subset of plant species that display a relatively rare phenology

whereby the species produces flowers and fruits during all months of the year. This

reproductive phenology has been labeled the continuous, or "steady state" reproductive

phenology (Gentry 1974, Newstrom et al. 1994) and has been shown to benefit insect

diversity in agroforestry systems (Peters 2014). However, the role of this reproductive

phenology in supporting plant-animal mutualisms temporally has yet to be evaluated.

If shrub species with the continuous flowering phenology support a functionally

diverse pollinator community across different seasons, then they have the potential to

buffer the community against threats associated with global change. Globally, land use

change, pesticide use, invasive species and climate change threaten ecological processes

such as seed dispersal and pollination (Burkle and Alarcon 2011, Hoiss et al. 2015,

Howe 2016). Climate change, in particular, is responsible for the straining of many

mutualistic interactions (Miller-Struttman et al. 2015, Peñuelas and Filella 2001, Post

and Forchhammer 2001). Studies conducted across elevational gradients can reveal

patterns of species response to warming temperatures (Hodkinson 2005, Sundqvist et al. 2013). Elevation gradients contribute to simplifying climate change studies by reducing noise caused by abiotic variation and confounding effects associated with long-term studies or studies conducted over large areas (Sundqvist et al. 2013). In particular, the tropical Americas have been identified as the region with the highest number of thermal zone specialists (Laurence et al. 2011). Despite recent advances, significant knowledge gaps still remain, as only a handful of invertebrate groups have been studied across elevation gradients (e.g. beetles, ants, lepidoptera, mollusks, orthopterans). An even more worrisome prediction for pollinators and pollination services, specifically for mountainous areas of Central America concerns the combined effects of warming temperatures with changing precipitation patterns (McCain and Colwell 2011). In fact, the effects of changing precipitation regimes in the mountainous regions of Central America is expected to pose a substantially higher risk to species compared to warming temperatures alone (McCain and Colwell 2011). Additionally, changing patterns of precipitation associated with global climate change are expected to have the greatest effect on species and communities comprising the Pacific slopes of Mesoamerica (McCain and Colwell 2011)

Changes in phenological events, geographical distributions and thermal specialization have been shown in many taxa, including plants, birds, amphibians and insects (Addo-Bediako et al. 2000, Bartomeus et al. 2011, Chen et al. 2009, Kelly and Goulden 2008, Wilson et al. 2005). Phenological mismatches occur when one species involved in an interaction responds to cues altered by a changing climate, while the other species may not respond at the same rate, the same way, or even to the same cues

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(Harrington et al. 1999, Visser and Both 2005). Such inherent variation in response rates and directionality remain a challenge for predicting the extent of interaction persistence. Mountain-dwelling species are expected to climb an average of 6km per decade as an adaptive response to warming temperatures (Parmesan and Yohe 2003), and tropical insects are theorized to be especially sensitive to warming temperatures owing to stricter physiological constraints (Addo-Bediako et al. 2000, Hegland et al. 2009, Janzen 1967, Wilson et al. 2005). Moreover, the majority of plants in Neotropical systems initiate flowering onset during the late dry season and early rainy season, possibly in response to rainfall (Frankie et al. 1974; Opler et al. 1980), but erratic rainfall patterns associated with climate change could potentially alter the timing of flowering onset and duration. Insects make up the vast majority of pollinators, but it remains uncertain how these groups will respond to warming temperatures and changing patterns of precipitation.

Only two native shrub species with the continuous reproductive phenology, *Hamelia patens* Jacq. (Rubiaceae) and *Stachytarpheta frantzii* Pol. (Verbenaceae), occur naturally across a broad elevation range and throughout the Neotropics. Owing to their widespread distribution, these shrub species have the potential to play important roles in plant-pollinator interactions if plant species with longer flowering seasons accumulate more partners over time in all ecosystems. Support for the idea that species with longer fruiting and flowering seasons play pivotal roles in mutualistic communities has been found in non-tropical systems (Olesen et al 2008) and for the extended fruiting phenology in temperate systems (Yang et al. 2013). However, the continuous reproductive phenology has yet to be evaluated for its role in mutualistic interactions.

Species with the continuous reproductive phenology tend to produce predictable resources in space and time, but at low density (e.g. < 1000 open flowers per plant). As such these plant species may play an important role for a smaller subset of the animal community, specifically traplining animal species, and therefore have a higher consumer specificity compared to plant species with an extended reproductive phenology (i.e. an intermediate phenophase between short term and continuous).

Bee-flower interactions occurring on shrub species with a continuous flowering phenology can be observed to understand this phenology's role in supporting pollinators temporally and across different elevations, as well as to provide insight into how plant-pollinator interactions in the seasonally dry Pacific slopes of Mesoamerica may respond to more erratic precipitation patterns. Throughout the region, only two native plant species produce flowers during all months of the year, *H. patens* and *S. frantzii*. These two plant species produce distinct floral blooms, including purple, shallow flowers and red, tubular flowers. Focusing on these two plant species, our study aimed to understand:

(a) What proportion of the local pollinator assemblage is supported by shrub species with a continuous reproductive phenology?

(b) Does elevation or plant species identity explain more of the variation in bee community composition of the flower visiting assemblage?

(c) Does the bee community that uses floral resources of shrub species with a continuous reproductive phenology change when floral resources are not scarce, i.e. during peak flowering when more generalist plants that exhibit highly abundant, but shorter duration resources are available?

Methods

Study Site

Our study took place in the San Luis de Monteverde region of the Puntarenas province of northwestern Costa Rica (10˚16' N, 84˚48-49' W)

Residing on the Pacific slope of the Tilarán mountain range, the San Luis Valley spreads through two ecosystem types, tropical dry forest, which can be found at the lower elevations and pre-montane forest, which populates higher elevations (Haber 2000). The rainy season occurs May through November resulting in an estimated mean rainfall of 2500 mm annually, subsequently transitioning to the dry season in December (Clark et al. 2000). Mean annual temperature ranges from 17-25˚C. Forest fragments are interspersed between dominant land cover types of shade-grown coffee farms, sugar cane farms, and cattle pastures (Fagan and Picado, 1971), with the addition of rural residences at lower elevations (Harvey and Haber 1999, Sanchez-Azofeifa et al. 2001). A wide gravel road extends throughout the heterogenous matrix and acted as our sampling transect from which we visited all locations where focal shrub individuals were found.

Species Description

Stachytarpheta frantzii Pol. (Verbenaceae) produces small, 5-lobed, purple flowers, seated in the rachis by a slender tube fused to the corolla (Woodson et al. 1973). Flowers of this species, like others of the genus, are arranged in a terminal inflorescence, ranging from 2-12 flowers per cluster (personal observation). This shrub species has been observed flowering and fruiting all months of the year, producing

small, wind-dispersed, dry seeds (Woodson et al. 1973). Geographically, *S. frantzii* has a widespread distribution, occurring naturally throughout subtropical and tropical America, primarily on the Pacific slope, up to 1300 m in elevation. (Zuchowski 2007). Locals often plant them as ornamental species for their association with orchid bees (Apidae, Euglossini), hummingbirds, and skippers (Hesperiidae) (Düster 2018, Khyade 2019). Individuals of this species are often stout, growing only up to two meters tall (Zuchowski 2007).

Hamelia patens Jacq. (Rubiaceae*)* produces orange-red, tubular flowers, reaching roughly an inch in length and grow in cymes (Bawa and Beach 1983). Individual shrubs or treelets vary greatly in size and in floral abundance, having up to five open flowers per cluster a day with nectar production initiating in the early morning (Colwell 1995, Stiles 1978). *H. patens* has a wide geographical distribution, similar to *S. frantzii*, occurring in subtropical and Central America as well as some parts of South America, up to 2000 m in elevation (Croat 1978, Opler et al. 1980). Reproductive behavior of *H. patens* varies between ecosystems and between seasons. In tropical wet forests, *H. patens* individuals fruit and flower during all months of the year, with a slight reduction in the number of open flowers during the dry months; however, in tropical dry forests, individuals flower only during the rainy season (Frankie et al. 1974, Newstrom et al. 1994). Individuals grow in secondary growth, forest edge and disturbed areas, and are oftentimes planted as ornamentals for their association with frugivorous birds and hummingbird pollinators. *H. patens* produces oval, fleshy fruits that begin green and ripen to a dark purple color high in sugar content (Lasso 2003, Peters and Nibbelink 2011, Peters 2014, Thomas et al. 1986, Zurovchak 1997).

Pollinator Sampling

        Individual shrubs of the two species selected for observations were located within the San Luis Valley (elevation range 750m to 1150m) and occurred in a variety of habitats such as agroforests, shade coffee farms, roadsides, and as ornamental plantings. Individual shrubs were located in partial or full sun, as both species are tolerant of disturbed areas and have fewer flowers in shaded areas. Across elevations, an approximately even number of individuals were selected for observation, however within some elevations, we could not find enough individuals of the focal species to observe. A total of 80 individuals of *S. frantzii* were observed across the elevation gradient, with 13 individuals observed between 750 and 850 m elevation, 17 individuals observed between 851 and 950 m elevation, 23 individuals observed between 951 and 1050 m, and 27 individuals observed between 1051 and 1150 m. A total of 89 individuals of *H. patens* we sampled across the gradient, only 2 individuals were observed in the lowest elevation band, 751 to 850 m. At the second elevation band we sampled 21 individuals, 32 observed between 951 and 1050 m, and 34 individuals between 1051 and 11050m. Floral abundance was quantified by estimating the count of open flowers for each shrub individual. The elevation, time of sample, and sun exposure was also recorded during each observation period. Sun exposure was classified into three categories: full sun, partial sun or full shade.

        Pollinator sampling was conducted during the months of June—July and December 2017 and 2018 from 0830 to 1300 h daily. Each individual shrub was sampled by two to three observers that collected all flower visitors to the individual during a 30-minute period. Pollinators were captured using Bioquip mesh nets and jars

charged with ethyl acetate. Lepidopterans were placed in glassine envelopes and bees were placed in 5mL centrifuge tubes filled with 70% Ethanol solution. Butterflies were identified in Costa Rica by J. Montero to the best taxonomic resolution possible, and all bees were exported to KY and identified in the lab. All Ceratinini and Centridini were identified by experts, S. Rehan and J. Pawalek, respectively. All other bees were keyed out to the highest resolution possible using keys in Michener (2000), Mawdsley (2017) for *Xylocopa spp.*, Aguiar and Melo (2011) for *Paratetrapedia spp.*, and Roubik and Hanson (2004) for Euglossini specimens.

The bee species pool that was used to calculate consumer specificity, or the proportion of the pollinator community using a particular shrub species, is the result of 9 years of sampling effort (2009-2018). A variety of methods were used to collect bees throughout the years including hand collection, observations at other flowering plants, malaise traps, bee bowls, vane traps and honey spray solution. Bee bowls were set out once annually from 2012-2016 in either June or January, and 4 times annually from 2016-2019 from 800-1400 hours during the months of June-July and December. Vane traps were set out 4 times annually from 2016-2019 from 800-1400 hours during the months of June-July and December. Malaise traps were set out in coffee agroforests 5 times annually in 2009 and 2010 (Peters 2014). Honey spray solution was used for sampling by spraying vegetation at selected elevations along three replicate transects four times annually during the years 2017-2019 between 800-1300 hours. Hand sampling at flowers along the replicate elevation transects also was conducted four times annually in 2016-2019, between 800 and 1400 hours. The species pool was

classified into four "local" elevation ranges, or bands: 750m-849m, 850m-949m, 950m-1049m and 1050-1150m.

Precipitation and Generalist Plant Sampling

Two angiosperms, a perennial shrub or tree with small white flowers, *Acnistus arborescens* (L.) Schltdl. (Solanaceae), and a shrub or tree with solitary white flowers, *Citrus spp*. (Rutaceae), were observed with open flowers during June 2018, but did not have open flowers during June—July 2017. These two plant species typically flower during the initial start of the rainy season in late April through May (personal observation), but flowered later in the year in 2018. A total of 26 timed observations were conducted at flowering plants of *A. arborescens* (16 observations) and *Citrus spp*. (10 observations) across the elevational gradient in June 2018 during the hours of 800-1400.

Precipitation data were obtained from the University of Georgia Costa Rica weather station located at 1140 m elevation in our study area (www.weather.uga.edu). The data demonstrates a late rainy season in the year of 2018 (Fig. 9).

Data Analysis

To calculate consumer specificity, or the proportion of the local pollinator community that is supported by shrubs with a continuous phenology we compared the visiting assemblage to the local species pool. Consumer specificity was calculated for the two shrub species together, each shrub species separately, and for each subdivided elevation range (e.g. 750m-849m), as well as for the overall gradient sampled (750m-
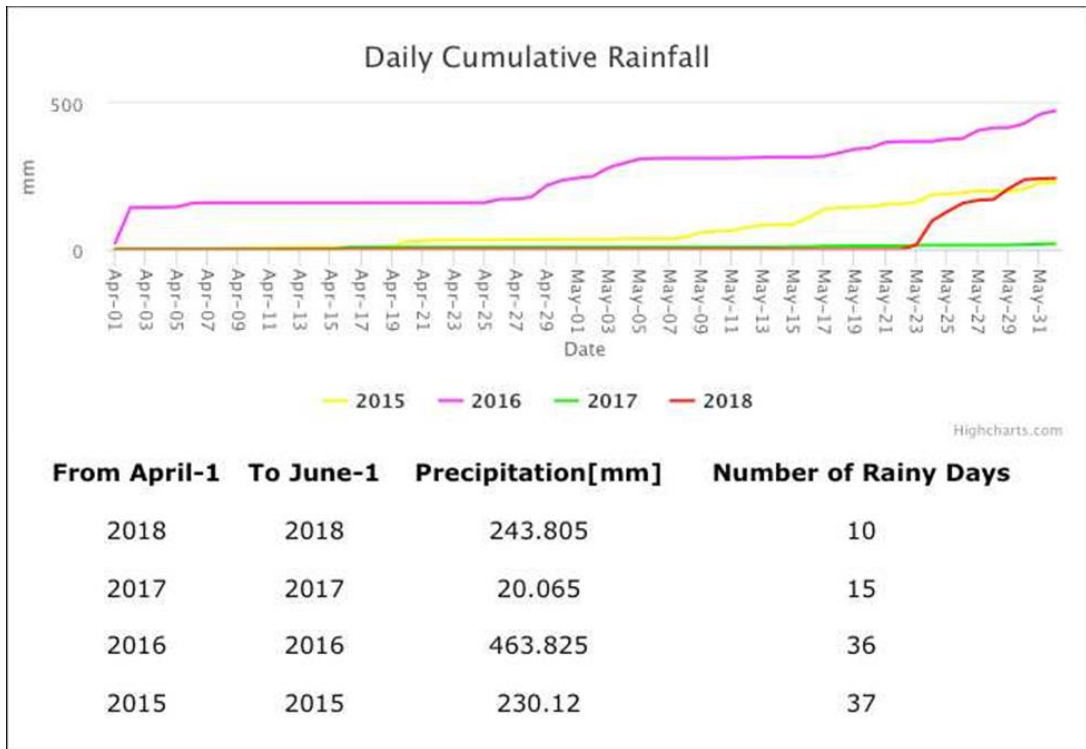
Figure 9. San Luis Valley Precipitation Patterns

1150m elevation). We made this comparison with the inclusion of Lepidopterans and once more excluding Lepidopterans (Apoidea only).

To determine the variation in the pollinator community that is explained by elevation and shrub species identity, we conducted a constrained ordination using the function capscale (CAP) in the vegan package of R version 2.5-4 (Oksanen et al. 2019 revision). Bray-Curtis distance was used and species abundances were transformed to the quarter power to reduce the effect of having species with large abundances drive the results of the analysis. Observations were classified into 50 m elevation bands. Additionally, Lepidoptera species were excluded from this analysis because they didn't appear to show much thermal range specialization within our sampled elevation

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gradient compared to bees. To test for significance of elevation and plant species influence on bee composition we conducted 999 random permutations.

To evaluate the whether the pollinator community remains consistent at our focal shrubs when other more generalist plants are available, we used the Chao similarity index. Similarity scores were calculated comparing samples obtained from focal shrub species during June and July 2018, where June 2018 samples represent the pollinator community of our focal shrubs when alternative, generalist resources are available. We generated an additional Chao similarity index comparing focal shrub pollinator composition to *A. arborescens* and *Citrus spp.* to quantify overlap in pollinator species, using only data collected in June 2018.

Results

The pollinator species pool collected from the San Luis Valley includes 338 pollinator species, of which 188 are bees and 150 are butterflies (Table 4). A total of 130 species (38.5%), 63 bees and 67 butterflies, was observed using flowers of the two focal shrub species. A total of 79 species (23.4%), comprised of 46 bees and 33 butterflies, were recorded visiting *H. patens*, and 92 species (27.2%), comprised of 41 bees and 51 butterflies, visited the flowers of *S frantzii* (Table 5).

Species richness in the pollinator pool was lowest at the 800m elevation range (751-850m: Table 5). A total of 161 pollinator species were found in the species pool at this range (92 bee species, 69 butterfly species), of which 43 species (27%) were observed visiting the focal shrubs. The low visitation rates of pollinators at *H. patens* at

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the 751-850 m elevation band, only five bee species and two butterfly species, is likely

due to the fact that only two individuals of *Hamelia patens* were found naturally

Table 4. Pollinator Species Across Elevation Bands Visiting the Focal Shrubs

Pollinator Species	Plant Species		Elevation			
	H. patens	*S. frantzii*	751-850m	851-950m	951-1050m	1051-1150m
Achlyodes pallida		X				X
Adelpha iphiclus	X				X	
Agapostemon morphospecies 3	X				X	X
Anartia fatima	X	X			X	X
Andinaugochlora morphospecies 1	X					X
Anteos clorinde	X					X
Anthanassa ardys		X				X
Anthanassa otanes		X		X		
Anthanassa tulcis	X				X	
Aphrissa boisduvalii	X			X	X	X
Aphrissa statira	X	X		X	X	X
Apis mellifera	X	X	X	X	X	X
Ascia monuste	X	X	X		X	
Astraptes alardus		X				X
Astraptes morphospecies 1		X		X	X	X
Augochlora morphospecies 1	X		X	X	X	X
Augochlora morphospecies 15	X	X	X		X	X
Augochlora morphospecies 16		X	X		X	
Augochlora morphospecies 18	X			X	X	X
Augochlora morphospecies 6	X		X		X	X
Augochlorella morphospecies 1	X	X			X	
Augochlorella morphospecies 9	X			X	X	X
Augochlorini morphospecies 1	X	X	X	X	X	X
Augochlorini morphospecies 10	X			X	X	
Augochlorini morphospecies 11	X					X
Augochlorini morphospecies 8	X				X	
Augochlorini morphospecies 9	X			X	X	X
Augochloropsis morphospecies 2	X				X	
Augochloropsis morphospecies 3	X					X
Augochloropsis morphospecies 4	X				X	
Bombus pullatus		X				X
Castilia eranites	X			X	X	
Ceratina buscki	X	X	X	X	X	
Ceratina chloris	X		X			
Ceratina cobaltina	X		X	X	X	X
Ceratina dimidiata	X			X	X	X
Ceratina eximia	X				X	
Ceratina rectangulifera	X	X	X	X	X	X
Ceratina trimaculata	X	X		X	X	X

Table 4. (continued)

Pollinator Species	Plant Species		Elevation			
	H. patens	*S. frantzii*	751-850m	851-950m	951-1050m	1051-1150m
Chilicola morphospecies 1	X	X		X	X	
Cissia hermes	X	X		X	X	X
Codatractus iMna	X	X		X		X
Codatractus morphospecies 1		X	X		X	X
Consul fabius	X					X
Corynura morphospecies 1	X	X			X	X
Dione juno	X	X		X		X
Dismorphia amphiona	X					X
Doxocopa cyane	X					X
Eufriesea macroglossa		X	X			
Euglossa azureoviridis		X	X			
Euglossa bursigera		X	X			
Euglossa cyanura	X				X	X
Euglossa despecta	X				X	
Euglossa mixta		X			X	
Euglossa sapphrina		X		X		
Euglossa townsendi		X	X	X	X	
Euglossa tridentata		X	X			
Euglossa variabilis		X			X	X
Euglossa viridissima	X	X	X	X	X	X
Eulema meriana		X				X
Eulema polychroma		X	X			
Eurema nise	X	X			X	
Eurema salome		X		X	X	
Eurema xanthochlora	X	X			X	
Ganyra limona		X		X		
Greta oto	X				X	
Halictini morphospecies 4	X	X			X	X
Halictini morphospecies 5		X			X	
Halictini morphospecies 9	X	X		X		X
Halictus morphospecies 1	X	X	X		X	
Halictus morphospecies 2	X					X
Heliconius charithonia	X	X	X		X	X
Heliconius clysonymus	X				X	
Heliconius erato	X	X	X	X	X	
Heliconius hecale	X	X		X	X	X
Heliconius hewitsoni		X		X		
Heliconius ismenius	X			X		
Heraclides cresphontes		X			X	
Hesperidea morphospecies 1		X				X
Hesperidea morphospecies 10		X		X	X	
Hesperidea morphospecies 11		X			X	
Hesperidea morphospecies 12		X			X	
Hesperidea morphospecies 13		X			X	X
Hesperidea morphospecies 14		X	X			
Hesperidea morphospecies 15		X	X			X
Hesperidea morphospecies 16		X	X			

Table 4. (continued)

Pollinator Species	Plant Species		Elevation			
	H. patens	*S. frantzii*	751-850m	851-950m	951-1050m	1051-1150m
Hesperidea morphospecies 17		X	X			
Hesperidea morphospecies 18		X	X			
Hesperidea morphospecies 2		X	X	X	X	X
Hesperidea morphospecies 4		X	X		X	X
Hesperidea morphospecies 5		X			X	X
Hesperidea morphospecies 6	X	X		X		X
Hesperidea morphospecies 7		X				X
Hesperidea morphospecies 8		X			X	X
Hesperidea morphospecies 9		X			X	X
Hyalyris excelsa		X		X		
Lasioglossum morphospecies 1	X	X	X	X	X	X
Lasioglossum morphospecies 2	X	X			X	X
Leptophobia aripa	X	X	X			X
Mechanitis menapis		X		X		
Melipona fasciata		X		X		
Nanotrigona mellaria	X			X		
Nymphidium ascolia	X			X		
Paratetrapedia calcarata	X	X	X		X	X
Partamona orizabaensis	X	X	X	X	X	X
Peponapis morphospecies 2		X				X
Peponapis morphospecies 3		X				X
Pereute charops		X				X
Phoebis agarithe	X			X		
Phoebis argante	X			X	X	X
Phoebis philea	X				X	
Phoebis rurina	X		X		X	X
Phoebis sennae	X	X	X	X	X	
Plebia frontalis	X	X		X	X	
Plebia pulchra	X	X		X	X	X
Proteides morphospecies 1		X				X
Ptilothrix morphospecies 1		X		X	X	
Pyrrohgyra edocla	X					X
Scaptotrigona mexicana	X					X
Siproeta stelenes		X			X	
Tetragona dorsalis	X	X	X	X	X	X
Tetragonisca angustula	X	X	X	X	X	X
Trigona corvina	X	X		X		X
Trigona fulviventris	X	X	X	X	X	X
Trigonisca buyssoni	X	X	X	X		
Urbanus morphospecies 1	X	X		X	X	X
Urbanus morphospecies 2		X	X	X	X	
Urbanus morphospecies 3		X	X			X
Urbanus morphospecies 4		X	X	X		X
Urbanus teleus	X	X	X	X	X	X

occurring in this elevation band. As elevation increased, pollinator richness of both bees

and butterflies collected within each elevation band also increased (Table 4). In

addition, consumer specificity decreased with elevation, or the proportion of pollinators

utilizing the focal shrubs increased in the higher elevation bands. Consumer specificity

was lowest for the pollinator community visiting the two focal shrub species at the

highest elevation band (1051-1150 m elevation) with 44% of the local (107 of 241

species) using floral resources of the focal shrubs as a result of the high volume of

Table 5. Focal Shrub Visiting Assemblage in Comparison to Pollinator Species Pool

| | Shrubs | | | | | Hamelia patens | | | | Stachytarpheta frantzii | | | |
| | | | Pollinator Species | | | | | Pollinator Species | | | | Pollinator Species | |
Elevation	Species	%	Total	Bees	Butterflies	%	Total	Bees	Butterflies	%	Total	Bees	Butterflies
751-850 m	161	26.7	43	25	18	4.3	7	5	2	23.0	37	21	16
851-950 m	190	29.5	56	29	27	18.4	35	23	12	17.4	33	15	18
951-1050 m	182	42.3	77	41	36	26.9	49	33	16	24.2	44	21	23
1051-1150 m	241	44.4	107	37	70	27.4	66	28	38	27.0	65	16	49
Total	338	38.4	130	63	67	23.4	79	46	33	27.2	92	41	51

butterflies (Table 5). When the analysis was carried out removing the Lepidopteran pollinators, slightly different results were observed (Table 5). Consumer specificity decreased with elevation as well but peaked, increasing once more at the 1051-1150 m elevation band; consumer specificity was lowest for the bee community at the 950-1049 m elevation band, with a total of 118 bee species recorded out of the 182 number of species in the species pool (65%; Table 5).

Elevation and plant species identity together explained 15% of the variation in bee community composition visiting the two plant species across the elevation gradient (Elevation: $F_{1,169} = 1.97$, p = 0.001; Plant species: $F_{1,169} = 4.18$, p = 0.001; Figure 10). Most of the bee species in the community were clustered in the center of the ordination plot. This indicates that these species were less specific to plant species or elevation. Several other bee species, however showed strong relationships with either one of the plant species or with elevation. For example, *Tetragonisca angustula* (Apidae, Meliponini), a very small stingless bee, shows a strong relationship with lower elevational bands. The medium-sized orchid bee, *Euglossa virridissima*, (Apidae, Euglossini) was most strongly associated with the shrub *S. frantzii*. A common stingless bee, *Trigona fulviventris* (Apidae, Meliponini), exhibits a strong association with *H. patens.* Several small-bodied bee species, *Lassioglossum spp*. (Halictidae), *Plebia spp*. (Apidae, Meliponini) and *Ceratina cobaltina* (Apidae, Ceratinini,), as well as several medium-sized species in the genera *Euglossa* (Apidae, Euglossini) and *Tetragona dorsalis* (Apidae, Meliponini) were more abundant at lower elevations. These bee species were only rarely collected from higher elevational bands.
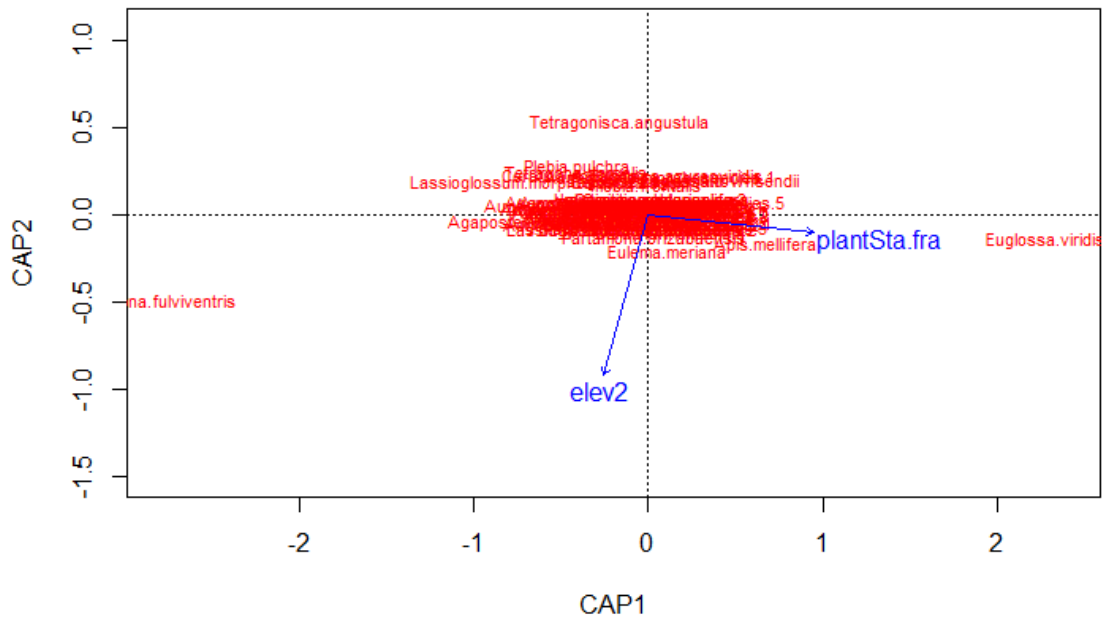
Figure 10. Ordination Demonstrating Drivers of Visiting Bee Community of the Focal Shrubs

Chao similarity indices revealed that *H. patens* and *Acnistus arborescens* had a very high overlap in pollinator community composition (Chao $_{H. patens\ v.\ A.\ arborescens}$ = 0.82, Chao $_{H. patens\ v.\ Citrus\ spp.}$ = 0.41). *H. patens* also showed a high overlap in pollinator community composition between years (Chao $_{June\ v.\ July}$ = 0.78) suggesting that the presence of other generalist, more abundant resources does not deter visitors from *H. patens*. This demonstrates pollinator fidelity to resources provided by individuals of this plant species. In contrast, *S. frantzii* had very low compositional similarity with either of the generalist plants (Table 6). *S. frantzii* also had relatively low similarity in pollinator community composition between years (Chao $_{June\ v.\ July}$ = 0.36).

Table 6. Pollinator Community Similarity in the Presence and Absence of Generalist Plant Species

June, 2018	June, 2018		July, 2018	
	Acnistus Arborescens	*Citrus spp.*	*H. patens*	*S. frantzii*
Hamelia patens	0.82	0.41	0.78	--
Stachytarpheta frantzii	0.08	0.11	--	0.36

Discussion

The two focal shrub species *H. patens* and *S. frantzii,* together, showed low consumer specificity, meaning that they support a very high proportion of the pollinator community (approx. 40%) across the elevation gradient as a whole. Consumer specificity changes across the different elevation bands, where the pollinator assemblage using shrubs with the continuous flowering phenology represents a greater proportion of the pollinator species pool as elevation increases, contrary to what we had anticipated. Tropical premontane systems harbor a high proportion of endemic species and are hypothesized to be one of the more sensitive ecosystems in response to climate, impacted by warming temperatures and irregular precipitation (Enquist 2002). Empirical studies have already documented dramatic plant population declines in recent years (Cascante-Marin et al. 2011). Plant population reductions combined with a highly fragmented landscape could cause reduced resource abundance and diversity thus affecting foraging opportunities for local pollinator communities. In this instance, pollinators would benefit from reduced consumer specificity or more generalized diets. Moreover, specialization has been shown to decrease inversely with altitude, plants and mutualistic animals display more generalist interaction behaviors at higher altitudes

(Maglianesi et al. 2015, Pellissier et al. 2010, 2012). Our results demonstrating lower consumer specificity at the continuous flowering shrubs at higher elevational bands supports findings of fewer specialized interactions at higher elevations. The ability of these focal plants to support a large proportion of the pollinator community across the gradient can be extrapolated to infer their potential across all elevations at which they occur. We also note that we did not sample the shrubs at elevations greater than 1150 m, and a decrease in the pollinator species pool may be observed at higher elevations.

A diverse assemblage of pollinators was observed foraging on the focal shrubs, including small-bodied bee species, *Tetragonisca angustula* and *Trigonisca buyssoni*, that were more constrained by elevation. Effects of climate change may be more extreme on species with restricted thermal ranges such as *T. angustula, Plebia spp*. and *Trigonisca spp*. owing to their small flight foraging distances and even stricter thermal tolerances (Greenleaf et al. 2007; Deutsch et al. 2008, Eickwort and Ginsberg 1980, Oyen et al. 2016). Both focal shrubs were recorded receiving visits from these pollinators and similar species. The interactions of small-bodied species and quasi-specialists with these focal shrubs demonstrate the potential to mitigate impacts of climate change on more sensitive species. Similarity indices revealed that the pollinator community of *S. frantzii* primarily acts as a more opportunistic resource but maintains specialized interactions with a small subset of pollinator species; For instance, many pollinators interacted infrequently with *S. frantzii*, but highly frequent interactions of *Euglossa viridissima* and multiple *Hesperiidae spp*. formed strong, more specialized associations by comprising of over a third of all recorded interactions and little to no interactions with *H. patens* (Table 4). This is further illustrated by the high turnover in

pollinator community composition between months and the low similarity to the pollinator assemblage at highly abundant, generalist floral resources. On the other hand, *H. patens* exemplified a more predictable pollinator assemblage with high similarity even when other generalist plants were flowering.

In tropical systems, more than 90% of flowering plants depend on plant-pollinator interactions for population persistence (Bawa 2003). Mutualists are considered to be among the most threatened species on the planet, as their coexistence depends on the continued existence of other species (Dunn et al. 2009). Furthermore, the changing climatic variables of temperature and precipitation threaten to de-synchronize plant-pollinator mutualisms by inducing spatial and temporal mismatch. The Neotropics, where mutualism diversity is highest (Fleming and Kress 2013), is expected to be the most vulnerable region globally to the changing patterns of precipitation and warming temperatures associated with climate change (Deutsch et al. 200; McCain and Colwell 2011). Our results reveal that throughout the Neotropics, shrub species with a continuous reproductive phenology already support a diverse group of pollinators across a broad elevational range, and these plant species may play an increasingly more important role in supporting pollinators as rainfall patterns change (Chen et al. 2011, Kelly and Goulden 2008, Thomas et al., 2006), however this may be species specific as *H. patens* supported a more consistent generalist pollinator assemblage, while *S. frantzii* supported a more specialized and opportunistic assemblage. Continuously flowering shrubs of our two observed native species occurred in a variety of habitats within the countryside matrix, in addition to being planted commonly as ornamentals. Given their widespread geographical distribution, their high

abundance within their range, and the predictability of food resources both spatially and temporally provided by plant species with a continuous reproductive phenology, these plant species could be used broadly throughout the Neotropics to restore plant-pollinator interactions and protect existing mutualisms from the impacts of land use intensification and climate change.

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