High resource overlap and a consistently generalised pattern of interactions in a bat-flower network in a seasonally dry landscape

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# Abstract

1. Pollination is an ecosystem process that is crucial to maintain biodiversity and ecosystem function. Bats are important pollinators in the tropics and are an integral part of complex plant-pollinator interaction networks. However, network analysis-based approaches are still scarce at the plant species and bat community levels.
2. We used metabarcoding to identify plant taxa present in pollen from fur and faecal samples collected across one year from three nectar-feeding bat roosts in central Mexico. We calculated the frequency of occurrence of plant taxa and assembled a zoocentric network of bat-plant interactions. We constructed a year-long network, encompassing the entire period of sampling, two seasonal networks comprising the wet and dry seasons, and six individual networks from sampling at two-month intervals across the year.
3. Four species of nectar-feeding bats interacted with 36 plant species from 16 families. We found highly generalised interaction patterns across networks corresponding with opportunistic feeding behaviour by bats, with little seasonal variation in network structure. There was high resource overlap between bat species, and bats visited a diverse range of plant species even during periods with a high abundance of particular resources in the landscape.
4. The diverse diet of nectar-feeding bats emphasises the importance of floristically rich natural habitats in the landscape to provide reliable foraging resources year-round in a seasonally variable system. While a generalised network structure is thought to increase robustness, further research is necessary to understand how fluctuations in pollinator abundance and diversity in the face of land use and climate change may impact bat-flower networks and the consequences on plant communities.

**KEYWORDS**

*Anoura geoffroyi,* Bat-flower interactions, *Choeronycteris mexicana, Leptonycteris nivalis,* *Leptonycteris yerbabuenae,* metabarcoding, Mexico, pollination networks

# 1 INTRODUCTION

Pollination is a crucial process for maintaining ecosystem function and biodiversity and is one of the most vulnerable stages to disturbance in the life cycle of plants (Neuschulz et al*.,* 2016). Bats are important pollinators in the tropics and form an integral part of complex plant-pollinator dynamics (Fleming et al., 2009; Kunz et al., 2011). In Mexico, nectar-feeding bats are keystone pollinators of much of the dominant vegetation in tropical forests and arid and semi-arid zones, including columnar cacti (Cactaceae), paniculate agaves (Agavaceae) and canopy trees in the Malvaceae family (Fleming & Valiente-Banuet, 2002; Fleming et al., 2009). The study of plant-pollinator interactions allows for an increased understanding of community structure, with implications for ecosystem function and stability in the face of environmental change (Montoya et al., 2006; Kaiser-Bunbury & Blüthgen, 2015; Zamora-Gutierrez et al., 2021).

Across different ecosystems, plants generally have numerous potential pollinators, which can each visit various plants (Waser et al., 1996; Lucas et al., 2018). Generalised networks are thought to be more robust to fluctuations in pollinator diversity and abundance, allowing plant species to exchange one pollinator for another (Johnson & Steiner, 2000). However, higher specialisation may be beneficial from the perspective of both the plant and pollinator (though particularly when considering functional groups of pollinators rather than species), potentially increasing delivery of conspecific pollen to the plant (Armbruster, 2014) and reducing inter-specific competition for the pollinator (e.g., Maglianesi et al., 2014). Insect pollination networks typically show significantly higher specialisation than other mutualistic networks such as seed dispersal or ant-nectar networks (Blüthgen et al., 2007), while most hummingbird-plant networks across the Americas have moderate specialisation (Dalsgaard et al., 2011). Patterns of bat-flower network structure in the Neotropics vary according to abiotic variables and the bat species assemblage considered (Liévano-Latorre et al., 2023; Gonzalez-Gutierrez et al., 2022). However, studies of nectar-feeding bat diets have revealed broad niches, even for morphologically specialised species (Gonzalez-Terrazas et al., 2012; Diniz & Aguiar, 2023a; Muchhala et al., 2024).

Patterns of generalism in a pollination network are dynamic, as diet breadths of pollinators can shift depending on environmental factors and resource availability (CaraDonna & Waser, 2020). Seasonal differences in plant phenology and pollinator diversity and abundance can impact the properties of network structure (Burkle & [Alarcón](https://bsapubs.onlinelibrary.wiley.com/action/doSearch?ContribAuthorRaw=Alarc%C3%B3n%2C+Ruben), 2011; Souza et al., 2018). Evidence on the effect of resource availability and seasonality on pollination networks is variable. While some studies have found that a higher specialisation of flower visitors is facilitated by high resource availability (e.g., Venjakob et al., 2016), most have found that specialisation increases as resource availability decreases (e.g., Sperr et al., 2011; Tinoco et al., 2017; Souza et al., 2018; Sritongchuay et al., 2019; de Oliveira et al., 2022; Stevens, 2022), and this relationship can be influenced by multiple environmental factors driven by ecosystem seasonality. Across the seasonally dry tropical forests of the Caatinga, Brazil, bat–flower networks have a generalized pattern of interactions across seasons and years, with bat species showing high levels of interaction overlap (Cordero-Schmidt et al., 2021). However, a meta-analysis of 22 Neotropical bat-plant pollination networks found that higher seasonality (particularly in terms of precipitation) resulted in a lower niche overlap between bat species (Liévano-Latorre et al., 2023). Functionally specialised species can switch seasonally to exploit different resources at different times of the year (Bender et al., 2017), and differences in the ability of nectar-feeding bats to track lower-density nectar sources (due to body size, flight characteristics and home range size) can impact interspecific competition and patterns of specialisation in networks (Tschapka, 2004). Interaction patterns can therefore be flexible, influenced by multiple environmental and biological factors, leading to variation in network structure.

Studies on the diet of nectarivorous bats are extensive and there is increasing interest in characterizing bat-plant interactions through network analysis. However, network analysis-based approaches are still scarce at the plant species and bat community levels, with most studies addressing plant identification at coarse taxonomic resolution and focusing on a few interactions (but see Queiroz et al., 2021, Cordero-Schmidt et al., 2021, González-Gutiérrez et al., 2022). Reflecting on the importance of bats as pollinators across the Neotropics, network analysis, particularly at species level, is an important tool to help us identify key pollinator species and assess the vulnerability of plant-pollinator interactions to anthropogenic disturbance (Memmott et al., 2004, 2007; Sritongchuay et al., 2019).

Here, we used metabarcoding to identify plant species in the yearly diet of a nectarivorous bat community to characterise the seasonal dynamics of bat pollinator-plant interactions in a semi-arid tropical landscape in central Mexico. This ecosystem harbours an extremely rich floristic diversity (Banda et al., 2016) and nectarivorous bats (Valiente-Banuet et al., 1996), with a marked seasonality driven by well-defined wet and dry seasons (Macias-Rodriguez et al. 2018). However, compared to other studies conducted in seasonally dry landscapes, our study site is unique as it is located in one of the most important areas for the cultivation of an endemic columnar cactus named “pitayo” (*Stenocereus queretaroensis* F.A.C Weber Buxbaum). Pitayo plants start flowering at the beginning of spring within the dry season, with fruits maturing into the early wet season. In this region, pitayos provide an unusually high availability of nectar, pollen and fruit that is not present in other seasonally dry landscapes.

We recorded the frequency of interactions between bats and plants, and assembled year-long, seasonal, and bi-monthly bat-flower interaction networks. We calculated network indices to describe patterns of specialisation and the foraging behaviour of bat pollinators in all networks. We hypothesized that 1) there would be seasonal variation in network structure, due to likely differences in resource availability; 2) the overall network would be relatively generalist, with low values of H2’ and d’, owing to the broad diet of nectar-feeding bats recorded by previous studies; and 3) pitayo would form the predominant part of the diet of all bat species during the flowering period, owing to the high availability of floral resources provided by pitayo plantations.

# 2 MATERIALS AND METHODS

## 2.1 Study area

Sampling was conducted at three bat roosts (Atoyac 19.99174, -103.50488; San Cayetano 20.13014, -103.5658; del Ermitaño 20.0812, -103.5965) in the Sayula Basin, Jalisco, in central Mexico. The Sayula Basin consists of a seasonal freshwater lagoon, framed by tropical deciduous forest (25% of total area; containing the highest floristic diversity), semi-arid lowland areas with thorn scrub (2%), human settlements (7%) and agriculture (38%, Macías-Rodríguez et al., 2018). The endemic cactus *Stenocereus queretaroensis* is an important regional crop that is mainly bat-pollinated (Tremlett et al., 2020).The average annual rainfall is 660 mm, which mostly falls between June and October (around 65% of total annual rainfall occurs between June and August), with the dry season lasting from November to May (Pimienta-Barrios et al., 2004).

## 2.2 Sample collection and DNA extraction

We visited the three roosts every two months from April 2017 to February 2018, making a total of six sampling trips. We captured bats returning to the roost from feeding (numbers captured depended on activity) and collected samples of pollen from the head, chest and wings of captured bats using a cotton swab dabbed in 96% ethanol. The cotton swabs were subsequently placed in Eppendorf tubes of 1.5 ml with 96% ethanol. Bats were then placed individually in clean cotton bags for a maximum of two hours to collect faecal samples. Faecal samples were placed in tubes with 96% ethanol, which was poured off after 24 to 36 hours and replaced with fine silica gel following Nsubuga et al. (2004). All samples were stored at -20 ºC until further processing in the lab.

We extracted DNA from the faecal samples using a modified CTAB method adapted from Doyle (1991), and from the pollen samples using an ammonium acetate method (Nicholls et al., 2000; Richardson et al., 2001). Further details are available in the Supporting Information.

## 2.3 PCR amplification, sequencing and DNA reference library

We used primer pair UniPlantF and UniPlantR to amplify part of the second internal transcribed spacer of nuclear ribosomal DNA (ITS2), a short region typically of 187-380 base pairs that provides a high taxonomic resolution (Chen et al., 2010; Moorhouse-Gann et al., 2018; Table S1). All samples were processed in duplicate from the first PCR stage (after DNA extraction) resulting in two PCR replicates of each sample. Final pools contained 260 samples (including eight PCR negatives) and were sequenced on an Illumina MiSeq sequencing platform, using 250 bp paired-end reads. Further details are available in the Supporting Information.

To improve taxonomic resolution, reference DNA sequences were generated and submitted to GenBank for some plant species that would potentially be visited by nectarivorous bats in the study region, which were selected after a literature review (Table S2 and Supporting Information).

## 2.5 Bioinformatics

We processed the sequencing data for further analysis using VSEARCH v2.14.2 (Rognes et al., 2016) following the pipeline available at https://github.com/chiras/metabarcoding\_pipeline (Leonhardt et al., 2022). Paired ends of forward and reverse reads were joined, and all reads shorter than 150 bp were removed. We then performed quality filtering (EE<1) as described by Edgar and Flyvbjerg (2015) and *de-novo* chimera filtering following UCHIME3 (Edgar, 2016a). VSEARCH was used to define amplicon sequence variants (ASVs) (Edgar, 2016b). By using VSEARCH against an ITS2 reference database for plant species of the sampled region, reads were directly mapped with global alignments with an identity cut-off threshold of 97%. The reference database was compiled with the BCdatabaser (Keller et al., 2020) based on a list of plant species of Mexico (Villaseñor, 2016) and then curated (Quaresma et al., 2023). To classify remaining reads still without taxonomic allocation at this point, SINTAX (Edgar, 2016c) was used with a reference database comprising global plant species (Sickel et al., 2015; Quaresma et al., 2023).

Reads from the PCR negatives were then checked to provide a baseline for background contamination. The maximum number of reads from each plant species identified in negatives were subtracted from all other samples from the same plate (Drake et al., 2021). Negatives were then excluded from further analyses.

We converted read numbers to relative abundances to account for the variation in read depth both between samples and between sequencing runs, after excluding plant taxa from families not documented to have bat-pollinated members, to mitigate against the likely inclusion of pollen present in samples due to wind drift, pollen present on flowers due to dispersal from other pollinating agents such as birds or insects, or pollen accidentally inhaled or ingested by bats while grooming or drinking from nectar sources (families from Fleming et al., 2009; updated to reflect current taxonomic classification). We specified a minimum sequence percentage threshold of 1% to determine occurrences and retained for analysis plant taxa found in either replicate at above the 1% threshold (Deagle et al., 2018; Drake et al., 2021).

We used published databases of bat-pollinated species (Fleming et al., 2009; Liévano-Latorre et al., 2023) to generate a reference list of species and genera of plants that could be visited by bats for nectar or pollen (i.e., not fruit; Table S3). We excluded species from genera not included in this list. We then manually checked all remaining species to ensure that the taxonomic assignment was appropriate (i.e., the plant species has a geographical distribution within the study area), and the flower morphology has some characteristic associated with chiropterophily (i.e., time of flower opening, flower shape, size and colour, documented records of pollination syndrome), and excluded observations that did not meet these criteria (see Supplementary materials). We therefore took a cautious approach in including plants in the network, with the presence of pollen on the bodies of bats alone insufficient to assume floral visitation), as opposed to contamination due to the potential sources listed above.

We aggregated records of *Ceiba aesculifolia* and *C. acuminata* as we found it is not possible to distinguish between these species with the primers used. Pollen assigned to one taxon each in the *Agave, Bauhinia* and *Calliandra* genera were not classified to species owing to an inappropriate distribution of the species returned by the bioinformatics pipeline. These taxa are therefore shown on the bipartite network as *Agave sp. 1, Bauhinia sp. 1* and *Calliandra sp. 1* but are not included in analyses of network structure*.* All remaining taxa included in the network were classified to species level. Where we found documented flowering phenologies of plant species included in the network, we filtered results to keep occurrences of plant species only within their flowering season, to reduce the possibility of recording instances of frugivory in the bat-flower network (23% of occurrences were lost during this step; Table S3).

## 2.6 Network analyses

We calculated the presence/absence of plant taxa in pollen and faecal samples collected from each bat individual in each month sampled and used this data to create a weighted adjacency matrix showing the summed interactions between bat species and plant taxa. Though occurrence-based diet summaries can over-estimate the importance of food items consumed in small quantities, we opted to use occurrence-based metrics to avoid possible biases in DNA extraction, amplification and sequencing, and a lack of mock community data (Deagle et al., 2018), as well as potential differential digestion rates between pollen of different plant taxa (Herrera and Martinez del Rio, 1998).

We constructed a year-long network, encompassing the entire period of sampling, two seasonal networks comprising the wet and dry season, and individual networks for each bi-monthly sampling period. We calculated three network-level metrics, focussing on quantitative indices which have been found to be less sensitive to sampling intensity and network size:

1) linkage density: represents the diversity of interactions per species, weighted by total interactions (Bersier et al., 2002; Dormann et al., 2009), and computed as the average of the mean number of bat species visiting each plant species and the mean number of plant species visited by each bat species.

2) H2’: a quantitative index of network-level complementary specialisation, which describes how strongly the interactions between bat-plant pairs differ from a random pattern where all bat species have the same preferences (Blüthgen et al., 2006). The expected minimum and maximum specialisation for the fixed diversity and abundance per species (marginal totals of observed network) defines the possible range for this index. Resulting values of H2’ range between 0 and 1, with values close to 1 indicating a highly specialised network (strongest flower species partitioning across bat species), and values close to 0 indicating a highly generalist network (highest overlap).

3) Niche overlap: mean similarity in interaction pattern between bat species, calculated using Horn’s index. Values close to 0 indicate no common use of niches, while values close to 1 indicating complete niche overlap.

We tested values of quantitative metrics (H2’, linkage density and niche overlap) of constructed networks against 1000 iterations of a null model using a Patefield algorithm, which creates null models with marginal totals identical to those of the observed model (Dormann et al., 2009). Comparisons between observed networks and Patefield null models are recommended when considering network metrics sensitive to the abundance and diversity of interaction partners (Blüthgen & Staab, 2024). All interaction network metrics were calculated using the ‘Bipartite’ package (Dormann et al., 2009) in R version 4.2.3 (R Core Team, 2022), including only plant taxa identified to the species level in analyses.

## 2.7 Bat foraging behaviour and resource overlap

To assess the role of the bat pollinators within networks, we also calculated the discrimination/selectivity index d’ for each bat species, which measures how selective a flower visitor is relative to the abundance of available resources. The total number of interactions for each species is used as a measure of partner availability (Blüthgen et al., 2006). Values of d’ closer to 0 indicate an opportunistic flower visitor (i.e., the bat visits similar flowers to all other bat species), while those close to 1 indicate a highly selective flower visitor (i.e., the bat exhibits exclusive preferences for certain flowers). We compared values of d’ calculated for each bat species in each month in the wet season (n = 6) and each bat species in each month in the dry season (n = 8) with a Welch’s t-test.

We visualised resource overlap year-round, and in the wet and dry seasons, using bat species as a predictor in a non-metric multidimensional scaling ordination on a Bray-Curtis matrix, using the Vegan package (Oksanen et al., 2017). We plotted the scaling ordination on two dimensions. We then performed an ANOSIM to investigate whether seasonal resource use differed between bat species.

## 2.8 Sampling completeness

We estimated the sampling completeness of bat-flower interactions in our constructed networks (year-long, dry season and wet season) using the iNEXT package (Hsieh et al., 2016; Chao et al., 2014), considering each combination of bat-plant interaction to be a ‘species’ and their frequency as ‘abundances’. We estimated the completeness of our sampling by dividing the observed interaction richness by the estimated richness. We also plotted individual-based rarefaction and extrapolation curves with Hill numbers for each constructed network, using the same package (Figure S1).

# 3. RESULTS

## 3.1 Nectar-feeding bats

We captured a total of 233 nectar-feeding bats throughout the year across the three roosts: 135 *Leptonycteris yerbabuenae* individuals, 49 *Choeronycteris mexicana,* 35 *Anoura geoffroyi* and 13 *L. nivalis.* *L. yerbabuenae* was present year-round at Roost 1 with fluctuating abundance; low numbers of *C. mexicana* were present year-round at Roost 2 except for during August; and Roost 3 was occupied by *Anoura geoffroyi* in June and October (wet season) and by *L. nivalis* in December and February (dry season). Across all bat species, we collected a total of 149 pollen samples from fur and 113 faecal samples for sequencing (we sequenced both faecal and pollen samples from 63 bats, faecal samples only from 23 bats, and pollen samples only from 76 bats). A higher proportion of captured *Leptonycteris yerbabuenae* bats carried pollen in the dry season, while there was no clear pattern for *Choeronycteris mexicana* (Table S4)*.*  *Leptonycteris nivalis* and *A. geoffroyi* were each caught only in one season.

## 3.2 Bat-plant interactions

We identified 36 plant species in pollen and faecal samples, from 16 plant families. Of these, 32 species were found in pollen samples while 27 species were found in faecal samples. Ten species were found in pollen samples only(*Agave salmiana,* *Cleome spinosa, Combretum farinosum, Cresecentia alata, Croton morifolius, Hibiscus rosa-sinensis, Hintonia latiflora, Ipomoea stans, Pithecellobium dulce* and *Pseudobombax palmeri*) while four species were found only in faecal samples (*Calliandra eriophylla, Cucurbita argyrosperma, Leucaena esculenta* and *Ruellia jaliscana*). Fewer plant species were recorded in samples from the wet season compared to the dry season (a total of 20 and 35, respectively; Figure 1). We identified a mean 5.0 (± 2.5 SD) plant species per bat sampled (pooled across bat species), and a mean of 3.8 (± 1.8 SD) and 3.6 (± 1.5 SD) plant genera and families, respectively.



Figure 1. Percent occurrence of plant species identified from pollen and faecal samples pooled across all bat individuals from all species for each sampling month. Occurences in the wet and dry seasons are shown in blue and orange, respectively.

Estimated sampling completeness of bat-plant interactions was highest when considering the complete year network than for the dry or wet season networks (Table 1; Figure S1).

Table 1. Observed and estimated (95% CL) species richness of plants visited by bat pollinators for the year and seasonal networks.

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Complete** | **Dry** | **Wet** |
| ***Leptonycteris yerbabuenae*** |  |  |  |
| Observed | 34 | 32 | 16 |
| Chao 1 | 36 (34 – 48) | 43 (32 – 64) | 17 (16 – 21) |
| Sampling completeness | 94% | 74% | 94% |
| ***Leptonycteris nivalis*** |  |  |  |
| Observed | 21 | 21 | - |
| Chao 1 | 26 (21 – 43) | 26 (21 – 43) | - |
| Sampling completeness | 81% | 81% | - |
| ***Choeronycteris mexicana*** |  |  |  |
| Observed | 26 | 24 | 16 |
| Chao 1 | 27 (26 – 35) | 32 (24 – 61) | 26 (16 – 54) |
| Sampling completeness | 96% | 75% | 62% |
| ***Anoura geoffroyi*** |  |  |  |
| Observed | 10 | - | 10 |
| Chao 1 | 11 (10 - 20) | - | 11 (10 – 19) |
| Sampling completeness | 91% | - | 91% |

## 3.3 Network analyses

The complete network consisted of 802 occurrences of plant taxa in pollen and/or faeces sampled from bat individuals (Table S5). The three constructed networks showed a highly opportunistic distribution of bat species across plant species visited, with low values of H2’ (network-level specialisation) and linkage density (diversity of interactions) and high values of niche overlap. Patterns of linkage density were significantly non-random (i.e., lower than expected if bats randomly interact with flowers) in the yearly, dry and two of the monthly networks, while H2’ was significantly non-random (i.e., larger than expected if bats randomly interact with flowers) in all networks except April and June (Table 2). There was no difference in H2’ or niche overlap between the wet season months and the dry season months, while linkage density was lower in the wet season (Figure S4).

Table 2. Network metrics across the complete year network, seasonal networks, and bi-monthly networks (sample size too small to compute network metrics in August). Networks include plant species identified in pollen and/or faecal samples.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Network | # samples | # bat species | # plant species | **linkage density** | **H2’** | **niche overlap** |
| Year | 149 | 4 | 36 | 9.74\*\*\*↓ | 0.09\*\*\*↑ | 0.72\*\*\*↓ |
| Dry | 88 | 3 | 35 | 9.05\*\*\*↓ | 0.12\*\*\*↑ | 0.79\*\*\*↓ |
| Wet | 61 | 3 | 20 | 6.40NS↓ | 0.14\*\*↑ | 0.77\*\*↓ |
| Apr | 28 | 2 | 20 | 7.08NS | 0.05NS | 0.96 NS |
| Jun | 12 | 3 | 10 | 4.20NS | 0.14NS | 0.81 NS |
| Aug | 19 | 1 | 11 | - | - | - |
| Oct | 30 | 3 | 13 | 4.55NS | 0.12\*↑ | 0.82 NS |
| Dec | 28 | 3 | 20 | 5.33\*↓ | 0.18\*↑ | 0.77 NS |
| Feb | 32 | 3 | 24 | 6.97\*\*↓ | 0.18\*↑ | 0.66\*↓ |

\*\*\* p ≤ 0.001, \*\* p ≤ 0.01, \* p ≤ 0.05, NS not significant

Observed pattern ↑higher or ↓lower than random associations in null models.

A group of different colored lines

Description automatically generated with medium confidenceFigure 2. Bipartite interaction networks of plant species visted by nectar-feeding bats in the Sayula Basin, Mexico, across the entire year, and during the wet (June to October) and dry season (December to April). Bat species labels correspond to: AnoGeo = *Anoura geoffroyi,* ChoMex = *Choeronycteris mexicana,* LepNiv = *Leptonycteris nivalis*, LepYer = *Leptonycteris yerbabuenae.* Plant species codes are defined in Table S6. Some infrequently encountered plants were removed from the plots for readability, but the interaction network data can be found in Supplementary materials. Bipartite interaction plots for the bi-monthly networks are shown in Figures S2, S3.

## 3.4 Bat foraging behaviour and resource overlap

Low values of the discrimination/selectivity index d’ were observed throughout the year, with no difference between dry season and wet season months (Welch’s t-test: *t =* 0.28, *p* = 0.78; Table 3).

Table 3. Blüthgen’s discrimination/selectivity index d’, calculated for flower-visiting bat species.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Complete | Dry | Wet | Apr | Jun | Oct | Dec | Feb |
| *Leptonycteris yerbabuenae* | 0.06 | 0.08 | 0.12 | 0.05 | 0.16 | 0.08 | 0.18 | 0.16 |
| *Choeronycteris Mexicana* | 0.04 | 0.06 | 0.20 | 0.05 | 0.25 | 0.13 | 0.15 | 0.25 |
| *Anoura geoffroyi* | 0.14 | - | 0.10 | - | 0.03 | 0.18 | - | - |
| *Leptonycteris nivalis* | 0.21 | 0.24 | - | - | - | - | 0.22 | 0.14 |

Bat species was a small but significant predictor of resource use during the wet (*R* = 0.212, *p* < 0.001) and dry (*R* = 0.075, *p* < 0.05) seasons, but not when considering resources used across the entire year (*R* = -0.007, *p* = 0.56; Figure 3). The contribution of bat species to explain resource use was higher in the wet season.

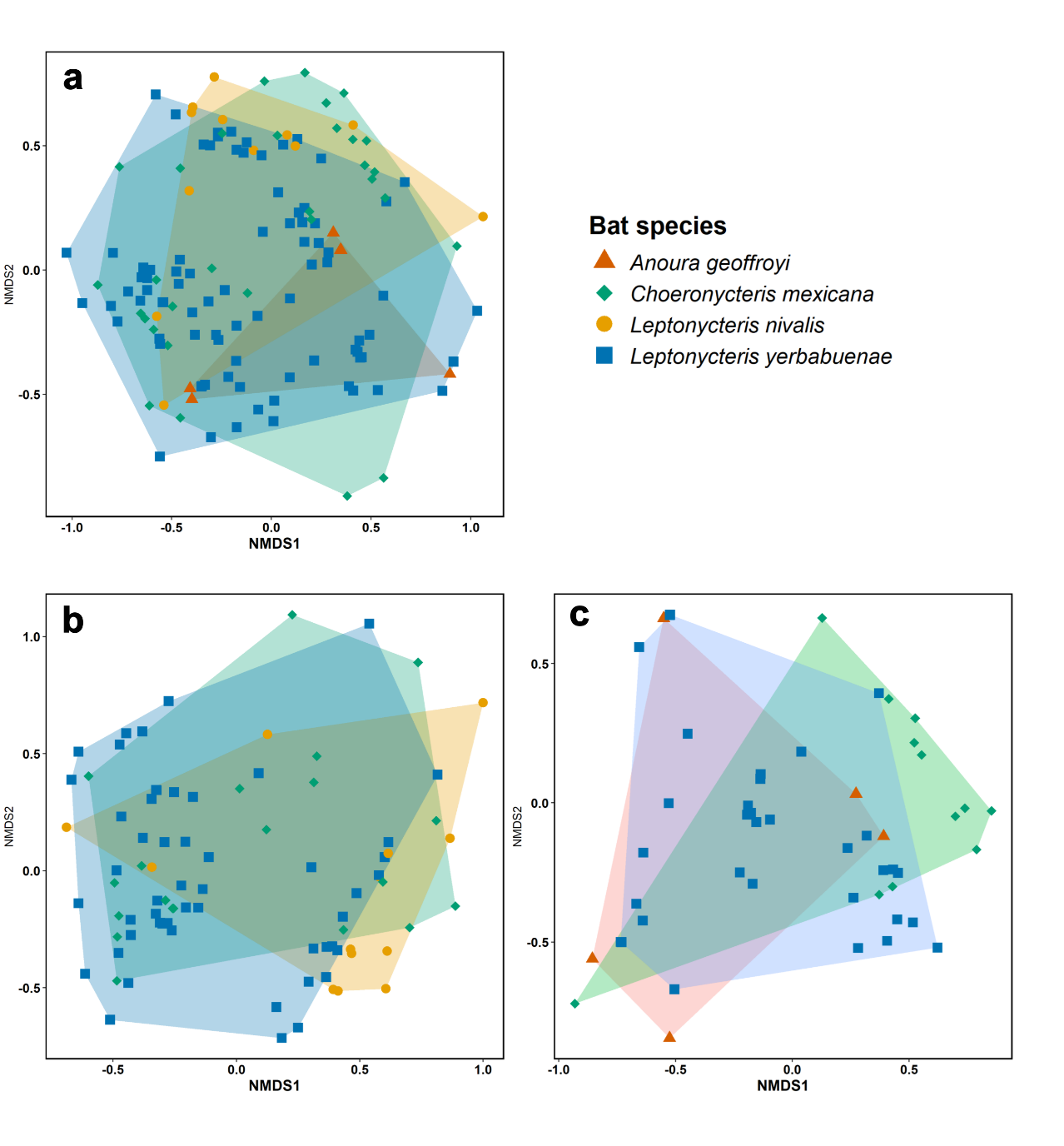


Figure 3. nMDS plots and convex hulls visualising distinctiveness of bat-plant interactions between bat species: a) across the whole year; b) during the dry season months; and c) during the wet season months.

# 4. DISCUSSION

The year-round bat-flower network showed a highly generalised pattern of interactions, despite changes in bat species composition and differing resource overlap in the wet and dry seasons, indicating high overall network stability. Furthermore, low values of discrimination/selectivity (d’) for all bat species, low values of network specialization (H2’) and high niche overlap suggest largely opportunistic feeding behaviour. Our results are consistent with other generalised inter-annual bat-flower networks in the Neotropics and corroborate the relatively high feeding plasticity of nectar-feeding bats (Cordero-Schmidt et al., 2021; Queiroz et al. 2021).

Network structure is influenced by various factors, including study design, the timing of resource availability, phylogenetic relationships between bat species and differences in foraging behaviour. Studies basing network metrics on direct observations of flower visits (e.g., Sritongchuay et al., 2019) tend to report a higher specialisation and lower niche overlap than those identifying plant taxa from pollen samples, as visitation records are less effective at capturing the full range of plant resources used by the bat community (Bosch et al., 2009; Dorado et al., 2011).

Our network focused on specialist nectarivorous bats sampled at the roost on return from foraging trips and included both pollen and faecal samples. We were therefore able to characterise a representative network of flowers visited by these nectarivorous bat species, removing any bias of sampling caused by choice of study sites or habitats. However, this approach also meant that facultative nectar-feeding bats from other guilds (i.e., frugivores) were not included in our network (as these species did not roost communally with the specialist nectar-feeding bats sampled and occur at lower abundances than our focal species), which could impact aspects of network structure in some temporal subsets of the network. For example, bat-flower networks were found to be more specialised in the Brazilian Cerrado, where frugivorous bats dominated the flower-visiting niche in forests, and during times of very low fruit availability (Diniz & Aguiar, 2023b). Thus, niches of flower-visiting bats were driven not only by their ability to exploit certain flower types (i.e., morphology) but also by spatio-temporal overlap of resources and pollinators (Diniz & Aguiar, 2023a). We found the lowest network specialisation in the month where only the two nectar-feeding bat species with resident populations were present (in all other months an additional migratory species was present).

Phylogenetic distance between specialist nectar-feeding bat species may be a more important factor than morphological differences in determining resource partitioning: lower niche overlap was found in communities composed of species from different phylogenetic groups, unrelated to morphological traits (Liévano-Latorre et al., 2023), while morphological specialization appears to allow nectar-feeding bats to access a wider range of floral resources without necessitating switching to other resources such as insects or fruit (Diniz & Aguiar, 2023a; Gonzalez-Terrazas et al., 2012; Muchhala et al., 2024). The low network specialisation and high niche overlap found in our study is likely influenced by the low phylogenetic distance between the four bat species included in our bat-plant network, which are all found within the obligatory nectar-feeding subfamily Glossophaginae (with two species in the same genus; Rojas et al., 2016). Modules of bat-flower genus networks across central-North America were found to be dominated by five bat genera including both specialist and facultative nectar-feeders (*Anoura, Artibeus, Glossophaga, Hylonycteris* and *Leptonycteris*; Gonzalez-Gutierrez et al., 2022). Specialist nectar-feeders acted as hubs, interacting with a high number of plants. Of these modules, only the *Leptonycteris* genus is included within our network, which demonstrated strong associations with the Cactaceae and Asparagaceae plant families.

We found little seasonal difference in network structure, despite seasonal fluctuations in floral resources and bat pollinator diversity and abundance in our system (Lobo et al., 2003; Borchert et al., 2004). A higher seasonality in precipitation is associated with lower niche overlap, likely due to influences on floral resource availability (Liévano-Latorre et al., 2023). The abundance of nectar-feeding bats at the roosts was highest in the dry season, when we also identified a higher number of plant taxa in pollen and faecal samples, and captured a higher percentage of bats carrying pollen, all presumably indicating a higher floral resource availability during this time. The abundance of nectar-feeding bats is highly correlated with food availability, as bat populations time their reproductive activity and local and long-distance migrations to synchronise with peak resource availability (Heideman & Utzurrum, 2003; Stoner et al., 2003; Peñalbaet al., 2006). A higher availability of floral resources during the dry season and the large foraging ranges of bats may allow the convergence on favoured resources and reduce competition (Fontaine et al., 2008; Tinoco et al., 2017; Stevens, 2022). However, though we found (slightly) greater resource overlap in the dry season, consistent with some previous studies of nectar-feeding bats (Sperr et al., 2011; Sritongchuay et al., 2019), both the dry and wet seasons showed a highly generalised pattern of bat-flower interactions. Nevertheless, the inclusion of facultative nectar-feeding frugivore species in the network may impact patterns of specialisation between seasons, depending on the temporal availability of fruit (Diniz & Aguiar, 2023a,b).

Furthermore, nectar-feeding bats in our study visited a high diversity of plants even during times of high abundance of particular resources in the landscape (flowering of monoculture plantations of the columnar cactus *Stenocereus queretaroensis*). Though *S. queretaroensis* appeared to be an important food resource, present in pollen and/or faecal samples from 22 of 32 bats captured in February, we found that 95% of the bat individuals that had visited *S. queretaroensis* had also visited other plant species, and furthermore that these individuals did not visit fewer plant species on average than bats in the same month that had not visited *S. queretaroensis*. In contrast, bats foraging in banana plantations in Costa Rica had a simplified, homogeneous diet compared to those foraging in forests (Alpizar et al., 2020). Fine-scale resource plant selection can be influenced by various factors determining foraging efficiency, including the density and spatial distribution of floral resources in the wider landscape, the quantity and sugar concentration of nectar rewards, flight costs, and inter- and intra-species interactions with coexisting nectar-feeding bats (Tschapka, 2004). *S. queretaroensis* produces a high volume of hexose-rich nectar (Ibarra‐Cerdeña et al. 2005), and the apparent non-limitation of available resources during this time suggests that competitive interactions are unlikely to play a role in determining the foraging behaviour of the bats. We suggest that the concurrent high availability of other food plants (indeed, we observed the highest diversity of plants in the diet in February) during this time allows bats to forage opportunistically on their way to and from key foraging grounds from a diverse number of plant species. However, the use of presence / absence metrics may have underestimated the relative importance of *S. queretaroensis* in the diet, as all plant species are given equal weighting regardless of the proportion of the food intake they represent.

The occurrence of plant taxa in our samples indicated a pattern of sequential flowering of bat-pollinated species throughout the year, and continuous or sub-annual flowering in some species, consistent with that observed in other bat-pollinated flower assemblages (e.g., Heithaus et al., 1975; Bullock & Solis-Magallanes, 1990; Sazima et al., 1999; Stoner et al., 2003; Cortés-Flores et al., 2017). This provides a continuous supply of floral resources for bat pollinators and encourages the availability of bats as pollinating agents that can reside year-round. *Leptonycteris* bats in tropical and sub-tropical areas, including central Mexico, have been found to have a more diverse diet than seasonal populations in northern parts of their range, which feed primarily on Agavaceae and Cactaceae (Fleming & Nassar, 2002). Our study emphasises the importance of maintaining heterogeneous natural habitats in the landscape to provide a diversity of resources for nectar-feeding bats.

Generalised interactions within plant-pollinator networks have traditionally been viewed as competitive because of the cost to plants associated with the delivery of heterospecific, rather than conspecific, pollen (Morales & Traveset, 2008; Flanagan et al., 2009; Ashman & Arceo-Gómez, 2013). However, there is increasing evidence that facilitation via pollinator-sharing can be advantageous to plant communities (Tur et al., 2016; Aparecida Lopes et al., 2021), and generalist pollinators can be vital to meta-network structure and resilience by linking subsets of the network and facilitating gene dispersal (González et al., 2010). A high abundance of generalist pollinators can make an important contribution to pollen transport between conspecific plant individuals (Larsson, 2005). Bat-pollinated species often occur naturally at low densities and tend to be self-incompatible and highly reliant on bats as mobile pollen dispersal agents (Herrerías-Diego et al., 2006; Fleming et al., 2009; Quesada et al., 2013; Ratto et al., 2018). Morphological trait matching also influences pollination success, with diverse flower shapes and designs resulting in differential pollen placement across the body of bats, thus promoting conspecific pollen delivery (Muchhala, 2008; Stewart & Dudash, 2016).

Bats are highly mobile, and the capacity for long-distance travel is a particular characteristic of the bat species assemblage constituting our network, with three of the four species being migratory. *Leptonycteris yerbabuenae* individuals travel up to 100 km per night during foraging trips (Goldshtein et al., 2020), while smaller glossophagines cover 50km or less (Tschapka, 2004; Rothenwöhrer et al. 2011). Bats in our network may therefore travel greater distances than those in other networks in search of resources, increasing the likelihood of encountering, and opportunistically interacting with, a higher number of plant species (Stewart & Dudash 2016). The high mobility of *Leptonycteris* bats also allows them to commute further to forage even when food resources are present nearer to the roost, perhaps to avoid competition and thus reduce niche partitioning (Ober et al. 2005). Additionally, while we found no resource partitioning of plant species, nectar-feeding bat species may display territorial behaviour and partition individual plants or foraging areas (Lemke, 1984; Goldshtein et al., 2020).

Higher generalisation in a plant-pollinator community should increase functional robustness and decrease vulnerability to changes in species diversity and abundance, for both plants and pollinators, as impacts are spread more evenly across the network (Kaiser-Bunbury & Bl**üthgen, 2015).** Changes in climate and land-use are projected to reduce numbers of potential bat-plant interactions in Mexico through changes in plant distributions, with increasing temperatures also predicted to cause a decrease in bat pollinator species richness, particularly in seasonally dry tropical forest (Zamora-Gutierrez et al., 2021). Furthermore, anthropogenic impacts such as deforestation caused mainly by agricultural and cattle ranching activities are exerting a strong pressure on natural vegetation cover in the area (Macías-Rodríguez et al., 2018). *Stenocereus queretaroensis* plantations are largely established in areas originally occupied by flower-rich tropical dry forest, and the area under production is increasing yearly (Macías-Rodríguez et al., 2018; SIAP, 2018). Habitat loss or degradation can result in changes to bat foraging behaviour, which can lead to decreased visitation rates to flowers (Quesada et al., 2004), a more restricted diet (Alpízar et al., 2020), and impact the delivery of conspecific pollen to flowers (Fuchs et al., 2003; Sritongchuay et al., 2019), with possible implications for network structure.

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# CONFLICT OF INTEREST STATEMENT

The authors have no conflict of interest to declare.

# AUTHOR CONTRIBUTIONS

VZG and KSHP conceived the research; VZG, KSHP and CJT designed the study; VZG and CJT collected the data; CJT carried out laboratory work with assistance and advice from MC; KHM advised on bioinformatics; CJT and AK carried out the bioinformatics step; CJT analysed the data with assistance from NB; and CJT wrote the manuscript with input from all co-authors. All authors contributed critically to the drafts and gave final approval for publication.

# STATEMENT ON INCLUSION

Our study brings together authors from a number of different countries, including scientists based in the country where the study was carried out. Whenever relevant, literature published by scientists from the region was cited; efforts were made to consider relevant work published in the local language.

# DATA AVAILABILITY STATEMENT

Data and code available from the Dryad Digital Repository (to be added upon acceptance). Code for bioinformatic processing at https://github.com/chiras/metabarcoding\_pipeline.

# SUPPORTING INFORMATION

# Additional supporting information can be found online in the Supporting Information section at the end of this article.

|  |  |
| --- | --- |
| Filename | Description |
| SupInfo001\_bat-flower network.pdf | Supplemental Methods  DNA extraction  PCR amplification and sequencing  Reference library  Literature cited  Supplemental Results  Supplemental Figures  Figure S1. Rarefaction curves showing sampling completeness of bat- flower interactions  Figure S2. Bipartite interaction networks of plant species visted by nectar-feeding bats in the Sayula Basin, Mexico, across during the dry season months (December, February and April).  Figure S3. Bipartite interaction networks of plant species visted by nectar-feeding bats in the Sayula Basin, Mexico, across during the wet season months (June, August and October).  Figure S4. Network metrics in the dry and wet season months.  Supplemental Tables  Table S1. Sequences of the universal primer pairs used.  Table S2. Spreadsheet with details of herbarium samples and GenBank accession numbers for reference sequences, uploaded separately.  Table S3. Spreadsheet with details of initial reference list of potential plant species in network, and further check of the distribution and likely chiropterophily of species in final network, uploaded separately.  Table S4. Number of bats captured and samples collected, per species and sampling month; and mean number ± SD of plant species identified in individual samples per bat species and sampling month.  Table S5. Bat-plant interaction network, uploaded separately.  Table S6. Codes for plant species used in Figure 2 and Figures S2-3 |
| SupInfo003\_Table S2.xlsx | Spreadsheet with details of herbarium samples and GenBank accession numbers for reference sequences |
| SupInfo003\_Table S3.xlsx | Spreadsheet with details of initial reference list of potential plant species in network, and further check of the distribution and likely chiropterophily of species in final network |
| SupInfo004\_Table S4.csv | Bat-plant interaction network. |