

Title: Testing for niche partitioning and specialization in plant–frugivore interactions using generalized joint attribute modeling

Authors: Laurel R. Yohe^{1,2,3,4}, Leith B. Miller⁵, Zofia A. Kaliszewska⁵, Susan R. Whitehead⁶, Sharlene E. Santana^{5,7}, Liliana M. Dávalos^{3,8}

Affiliations:

¹Department of Bioinformatics and Genomics, University of North Carolina Charlotte, Charlotte, NC, 28223, USA

²North Carolina Research Campus, Kannapolis, NC, 28081, USA

³Department of Ecology & Evolution, Stony Brook University, 650 Life Sciences Building Stony Brook, NY 11794, USA

⁴Department of Earth and Planetary Sciences, Yale University, 210 Whitney Ave. New Haven, CT 06511, USA

⁵Department of Biology, University of Washington, Seattle, WA, 98195, USA

⁶Department of Biological Sciences, Virginia Tech, Blacksburg, VA 24061, USA

⁷Burke Museum of Natural History and Culture, University of Washington, Seattle, WA, 98195, USA

⁸Consortium for Inter-Disciplinary Environmental Research, School of Marine and Atmospheric Sciences, Stony Brook University, 129 Dana Hall, Stony Brook, NY 11794, USA

E-mail Addresses:

LRY: lyohe1@uncc.edu; laurel.yohe@stonybrook.edu; laurel.yohe@yale.edu

LBM: leith1@uw.edu

ZAK: zakalisz@gmail.com

SRW: swhitehead@vt.edu

SES: ssantana@uw.edu

LMD: liliana.davalos@stonybrook.edu

Short-running title (45): Bat–plant trait interactions

Keywords (10): trophic interactions, functional traits, *Piper*, *Carollia*, Bayesian hierarchical models, generalized joint attribute modeling, bats, mutualism, Phyllostomidae, niche partitioning

Type of Article: Letter

Word count: Abstract, Main Text, Text Box

Number of References: 46

Number of Figures: 3

Number of Tables: 1

Number of Text Boxes: 0

Statement of Authorship: LBM, ZAK, SRW and SES collected primary data, and LRY and LMD designed and performed the analyses. All authors contributed to the discussion of the results and the writing of the manuscript.

Data Accessibility Statement: All data, scripts, and results will be deposited onto Dryad.

Corresponding Authors:

Laurel Yohe

9331 Robert D. Snyder Rd.

Charlotte, NC 28223

lyohe@uncc.edu

Liliana M. Dávalos
650 Life Sciences
Stony Brook, NY 11794
liliana.davalos@stonybrook.edu

ABSTRACT

We use generalized joint attribute modeling (GJAM) to estimate how the proclivity to consume different *Piper* species varies among three syntopic *Carollia* species may depend on bat traits (e.g., body size and age) or *Piper* fruit traits. How these ecologically and morphologically similar bats coexist is unclear. Compared to the *Piper*-specialist *Carollia castanea*, generalist *C. perspicillata* had a higher proclivity for several *Piper* species that were the lowest ranked for the specialist, indicating some degree of niche partitioning. *Piper* proclivity estimates were associated with bat forearm length and age after controlling for bat species, reproductive condition and sex. However, neither fruit nor seed shape influence dietary differences among bats. Understanding the mechanisms of coexistence for closely related species with a high degree of morphological and behavioral similarity is complex, but simultaneously controlling for multiple sources of variation among different data types allowed us to discover key differences in resource consumption and traits contributing to niche partitioning among frugivores.

INTRODUCTION

Fruiting plants and frugivores are central to many ecosystems, and their interactions have important ecological and evolutionary consequences (Cordeiro & Howe 2003; Sekercioglu 2006; Schleuning *et al.* 2011). Effective seed dispersal by animals can be critical to the plant life cycle because failure to disperse results in less reproduction and higher probability of extinction (Pratt & Stiles 1985; Harms *et al.* 2000; Nathan & Muller-Landau 2000). In turn, frugivores obtain energy from fruits and benefit from the relative predictability of fruiting plants as a food source (Kalko *et al.* 1996; Wendeln *et al.* 2000; Norconk *et al.* 2009). Advantages to both plants and animals are thought to explain the broad spread of animal-mediated seed dispersal: the seeds of up to 90% of tropical angiosperms are dispersed via consumption and subsequent defecation by birds and mammals (Bascompte & Jordano 2007). The importance and prevalence of these associations, as well as the staggering diversity of plants dependent on seed-dispersing frugivores make the study of seed dispersal mutualisms crucial to understanding how ecological interactions persist.

Neotropical *Piper* plants (Piperales: Piperaceae) and *Carollia* bats (Fig. 1; Chiroptera: Phyllostomidae) are a mutualistic system whose ecology has been well documented. *Piper* are both diverse and abundant in tropical ecosystems worldwide (Gentry 1988) and provide a constant supply of ripe fruit throughout the year through continuous or staggered fruiting patterns among sympatric species (Thies & Kalko 2004). Many neotropical *Piper* species depend on *Carollia* for seed dispersal (Dyer & Palmer 2004), and *Piper* fruits dominate these bats' diets throughout the year and across their range (Fleming 1991). Because of the apparent high dietary overlap and morphological similarity among syntopic *Carollia* species, they are ideal for testing predictions from evolutionary and ecological hypotheses, including ecological character displacement and ecological fitting. In Costa Rica, three species of syntopic *Carollia* feed on at least a dozen *Piper* species, with *C. perspicillata* being the most generalist frugivore, *C. sowelli* being intermediate, and *C. castanea* being the most specialized on *Piper* (Fleming 1991).

From an evolutionary perspective, stable species coexistence instead of competitive exclusion is enabled by (1) enough niche space that can be occupied by a diverse set of species within a guild, (2) differentiation of functional traits, and (3) tradeoffs in species performance across niche axes such that niche differentiation can occur (e.g., Weinstein & Graham (2017) but see Sexton *et al.* (2017)). How do closely related, ecologically, behaviorally, and morphologically similar syntopic species meet these requirements? And how do their realized versus fundamental niches compare to one another as a result? These questions are particularly puzzling in specialists like *Carollia* bats, which live in hyper-diverse ecological communities with seemingly conserved trophic niches.

We develop a framework to simultaneously measure the role of traits of both fruits and bats in structuring their ecological interactions. We use Bayesian generalized joint attribute modeling (GJAM) to contrast realized dietary niche breadths and estimate the proclivity of three syntopic species of *Carollia* for *Piper* species, as well as the influence of bat traits on these estimates. In turn, we relate *Piper* fruit traits to these estimates, testing their influence on bat consumption. Analyzing the trophic interactions among bats and plants, and among competing congeners, requires the integration of several types of ecological data (e.g., continuous traits, presence/absence of food resources), and has been historically challenging to model (Clark 2016; Clark *et al.* 2017). Joint attribute

modeling is able to account for multiple sources of variation and multiple predictors of different data types to obtain robust estimates of responses (Clark *et al.* 2017). We predicted divergence of bat proclivities to consume different *Piper* species and evolved functional traits that minimize competition among congeners. Additionally, we tested if differential proclivity to consume *Piper* species is related to specific *Piper* traits. We discovered two *Carollia* species show realized dietary niches that are divergent, complementary, and associated with divergent morphological traits of the bats. *Piper* traits examined showed no relationships to bat proclivities, suggesting these may not be involved in fruit selection by bats. Despite only subtle trait differences among the bat species studied, our analyses uncovered key differences in resource consumption contributing to niche partitioning among frugivores.

MATERIALS AND METHODS

To investigate mechanisms of co-existence among *Carollia* bats, we collected data from co-occurring individuals of bats and plants at La Selva Biological Station, Sarapiquí, Costa Rica. We used these data to build three types of Bayesian models. The first models link bats and their traits to *Piper* species represented in bat feces. The second model estimates the relationship between bat morphometric (*e.g.*, body size) and performance (*i.e.*, bite force) traits related to feeding, and the third quantifies the effects of *Piper* traits on modeled bat proclivities to consume *Piper* species.

Piper Consumption by Bats

To determine how *Carollia* species and traits relate to particular proclivity to consume *Piper*, we quantified the diets of the three syntopic *Carollia* species at La Selva. All procedures for bat capture and handling were approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Washington, Seattle, USA (protocol #4307-02). We used mist nets to capture bats between 1800–2200 h along trails throughout the forest during the wet season, when there is a greater incidence of fruiting peaks for *Piper* (July and September – December 2015). We collected fecal samples from 318 individuals from the three *Carollia* species (Fig. 1): *C. perspicillata* ($N = 84$), *C. sowelli* ($N = 111$), and *C. castanea* ($N = 123$) by placing individual bats in cloth bags for up to two hours. If the bat defecated, we collected fecal pellets, which we dried in an air-conditioned room for 1–2 days. Samples were then transported to UW for seed identification. We identified seed species in rehydrated fecal pellets using morphological characters and by comparison to a seed reference library that included *Piper* and non-*Piper* species native to La Selva. The reference library was built from seeds removed from ripe fruits collected directly from the parent plant, and plants were identified by LBM, ZAK, Orlando Vargas (OTS), and confirmed via genetic markers (see (Santana *et al.* 2021)). If we could not identify the species of a particular seed, we classified them as a morphotype (*e.g.*, *Piper* Type 1). We coded each plant species as present or absent in the individual fecal sample (Data S1).

Bat Traits

We recorded age class (adult, sub-adult, juvenile), sex (male, female), reproductive condition (reproductive, non-reproductive), mass, and forearm length (Data S1) for each bat that produced a fecal sample. Using these bat-specific variables as covariates, we built a model to estimate the relationship between bat traits and species and *Piper* species

represented in the feces (i.e., to examine how bat traits and species designation influence their dietary records). We call these modeled coefficients of bat species and traits “*Piper* bat proclivities”. Our data set was composed of multiple data types, including a zero-inflated matrix of *Piper* species in the bat fecal samples (e.g., 0 if *Piper* species is not present; 1 if *Piper* species is present) and correlates of those data: discrete categories of *Carollia* species, continuous bat size traits, as well as the categorical traits of sex and reproductive condition. Simultaneously estimating relationships among bat species, their traits, and the *Piper* species consumed by bats, is a challenge to general linear models. We implemented the flexible framework of generalized joint attribute modeling (GJAM) (Clark *et al.* 2017), which uses a Bayesian multivariate approach to infer the parameters of the linear model based on a series of joint distributions of both the bat traits and the *Piper* fecal abundances, while simultaneously accommodating multifarious trait data, in this case from bats.

Generalized Joint Attribute Modeling

For each observation i of n bat individuals, there is a set $\{x_i, y_i\}^n$, in which each x_i observation has Q predictors to result in a vector of predictors $x_{iq}: 1 \dots Q$. In our case $Q = 6$, with predictors species, age class, sex, reproductive condition, mass, and forearm length. The set of responses is a vector of $y_{ip}: 1 \dots P$, where P is the total number of *Piper* species ($P = 18$) observed across all fecal samples. For y_{ip} , each vector of bat individual i is the presence or absence of *Piper* species p . Seven *Piper* species were removed from the analysis, as they accounted for less than 1% of the observations. Most of the observations in y_{ip} are 0, meaning most *Piper* species are not observed in a sample. To accommodate this zero-inflation, GJAM implements a Tobit regression. The representations of x_i and y_i are composed of partitions of discrete and continuous space, and GJAM applies a connection between the two, which we represent as I in our model. Thus, it is possible to estimate a continuous response w_i from multifarious data such that for each observation,

$$w_i | x_i, y_i \sim \mathcal{N}(B', E) * I$$

where B' is the matrix of coefficients and E is a $P \times P$ correlation matrix to represent the covariances among the response variables. For detailed explanations of the calculations of I , E , and w , see further discussion in Clark *et al.* (2017). We estimated the coefficients using the R package *gjam* v. 2.1.6 for 20,000 generations, discarding 4,000 as burn-in. We applied a series of dimension reduction options ($N = 2, 5$, by $r = 2, 5$) to facilitate convergence amidst the multiple dimensions of covariance space and adopted the one that yielded the lowest model deviance. Note that we compared both fractional composition models (continuous on (0,1)) and presence-absence models (discrete). Medians of the posterior distributions of the continuous response w_i were used for further modeling.

Bat Functional Traits

Bite force is a metric of feeding performance linked to the mechanical demands of the food a species can process (e.g., Aguirre *et al.* 2002; Santana *et al.* 2010; Santana & Miller 2016). Following methods by Santana *et al.* (2010), we measured deep bilateral, voluntary bite forces for at least ten wild individuals per *Carollia* species using a piezoelectric force transducer (Kistler 9203; range ± 500 N, accuracy 0.01–0.1 N) attached to a handheld

charge amplifier (Kistler 5995A). The force transducer was mounted between two metal plates covered with medical tape to provide a non-skid biting surface and to protect the bats' teeth. We adjusted the distance between the bite plates for each individual to accommodate a moderate gape angle of approximately 30°, following Santana *et al.* (2010). To avoid variation from age (Santana & Miller 2016) and stress to reproductive females, we only measured adult males and adult non-pregnant, non-lactating females. We recorded five to eight measurements for each bat and chose the highest value to represent maximum bite force. Following bite force measurements, we recorded head length, width, and height measured to the nearest 0.1 mm (Fig. S1B), as well as mass and forearm length for most individuals (Data S1).

Piper Fruit and Seed Traits

Physical traits of fruits and seeds can constrain whether and how bats of different sizes can process them. We collected dimensions of whole *Piper* infructescences (the unit consumed by *Carollia*, herein called “fruits” for simplicity) and individual seeds to estimate how these traits relate to bat proclivities to consume *Piper* species. We measured length and width from five ripe fruits from each *Piper* species to the nearest 0.001 mm, and used ImageJ (Rasband, W.S., ImageJ, U. S. National Institutes of Health, Maryland, USA) to measure seed length and seed width from digital photographs of three seeds from each fruit. Seed photos were taken with a Leica MZ 95 microscope camera coupled with Clemex Captiva software. We used these fruit and seed measurements to calculate a ratio (length/width) as an estimate of fruit and seed shape, respectively.

Bayesian Hierarchical Modeling

After determining that both sex and head length were linear predictors of bite force in regressions with either a sample-wide intercept (male sex coefficient $t_{(27)} = 2.29$, P -value = 0.03, head length coefficient $t_{(27)} = 7.60$, P -value = $3.54e-08$), or species-specific intercepts (male sex coefficient $t_{(27)} = 4.23$, P -value $1.20e-04$, head length coefficient $t_{(27)} = 2.44$, P -value = 0.01), we modeled bite force as a function of bat body size traits while controlling for both sex and head length, which may explain bite force. We used Jags v.3.3.0 (Plummer 2003) to code these models, and ran them in the R package R2jags v.0.04-01 (Su & Yajima 2012). These models included species-specific intercepts whose prior was drawn from a normal distribution. Priors for both between- and within-population variances were modeled as half-Cauchy distributions with variance of at least 100,000. These priors do not make any assumptions about the relative contribution of variation from different levels in the hierarchy (Gelman & Hill 2006). For each model, four independent chains ran for 500,000 iterations with 250,000 iterations as burn-in, and samples were taken every 250 generations. Convergence was assessed by both the effective sampling size of model parameters (>1000 in every case), and the potential scale reduction factor (PSRF), which approaches 1 at convergence (Gelman & Rubin 1992). The models coded measures of error to estimate the variance explained, as outlined by Gelman & Pardoe (2006).

We used the *Piper* traits as regressors in Bayesian models of *Piper* bat proclivities estimated by GJAM analysis. Thus, these models connect the differential use of *Piper* resources by bats (e.g., across species, age class, or body sizes) to the *Piper* traits that might underlie those differences. We used the R package MCMCglmm (Hadfield 2010) to

code the models, and accounted for the correlation structure of the data due to evolutionary relatedness by including a molecular phylogeny of *Piper* (Santana *et al.* 2021) as a species-specific (random) effect. We applied a parameter-expanded prior with the parameters $V = 1$ $\nu = 1$ for the residual variance (Rojas *et al.* 2018), and a proper Cauchy prior defined by $V = 0.5$ $\nu = 1$ and $\alpha.\mu = 0$ and $\alpha.V = 10^3$ for the random term (Hadfield 2019). Each model ran for 200,000 iterations, sampling every 100, with 10,000 generations as burn-in. Convergence of the resulting posteriors was assessed by the effective sampling size of model parameters (>1000 in every case). In total, we ran four models corresponding to the modeled proclivities associated with bat forearm, body mass, and *C. castanea* and *C. perspicillata*.

RESULTS

As expected, the percentage of *Piper* presence in the diet was highest in the specialist *C. castanea* (67.5%) and lowest in the generalist *C. perspicillata* (45.2%). *C. sowelli* was intermediate (60.5%) (Fig. S2). For the period sampled, *Piper* Type 4 was the most common species in the diets of *C. castanea* and *C. sowelli*, while *P. hispidum* was the most common for *C. perspicillata*. Dietary proportions are displayed in Fig. S2 and raw diet data in Data S1.

Bat species proclivities to Piper

Model fit was assessed through DIC (Deviance Information Criterion) and posterior predictive output and the fractional composition model (as opposed to presence-absence) demonstrated a much better fit (Fig. 2A, 2B). In GJAM, the sensitivity of the covariate inputs can be interpreted as the amount of information each input contributes to estimating the model coefficients (Clark *et al.* 2017). Bat species (particularly *C. castanea* and *C. perspicillata*), body size (forearm length), age, and reproductive status all showed sensitivity values greater than one, suggesting they were much more informative than sex or body mass in explaining the presence of *Piper* species in the diet of *Carollia* (i.e., *Piper* bat proclivities; Table S1; Fig. 2C). Figure 3 illustrates the posterior probabilities for each *Piper* species estimated by the proclivity of each bat species to consume that particular *Piper* species (details in Table S2). While the 95% highest posterior density (HPD) credible interval crossing zero corresponds to a weak relationship between the covariate and the *Piper* species, an HPD not overlapping zero can be interpreted as a strong response. Consequently, *C. perspicillata* showed a strong positive response to six species of *Piper* (in order of highest proclivity: *P. hispidum* (median: 0.45; 95% HPD: [0.22, 0.66]), *P. colonense* (0.45 [0.20, 0.66]), *P. silvivagum* (0.45 [0.20, 0.68]), Type 4 (0.36 [0.13, 0.56]), *P. aduncum* (0.35 [0.10, 0.59]), and Type 10 (0.31 [0.001, 0.59])). The *Piper* specialist *C. castanea* also has the lowest proclivities for five of these six species (Fig. 3); *P. colonense* (−0.52 [−0.75, −0.24]), *P. hispidum* (−0.34 [−0.56, −0.09]), *P. silvivagum* (−0.34 [−0.58, −0.07]), *P. aduncum* (−0.33 [−0.58, −0.07]), and Type 4 (−0.32 [−0.54, −0.08]). *C. castanea* also showed a negative proclivity for *P. sancti-felices* (−0.33 [−0.63, −0.01]), towards which *C. sowelli* (the bat species that exhibits intermediate specialization on *Piper*) also demonstrated a positive proclivity (0.22 [0.02, 0.41]). *C. perspicillata* only showed a negative proclivity towards *Piper* Type 1 (−0.29 [−0.58, −0.01]). Table S2 shows coefficient estimates for all *Piper* species.

Influence of bat traits on Piper proclivities

The sensitivity of bat traits and their influence on proclivity to *Piper* species varied (Table S1; Fig. 2C). The magnitude of these coefficients reflects the influence of the trait on the proclivity or response to each *Piper* species. One covariate representing body size (forearm length) had strong sensitivity on responses for *Piper* species (Fig. 2C; Fig. 4). The presence of *P. Type 1* showed the strongest positive response to forearm length (0.70 [0.34, 1.01]). There was a strong positive influence of forearm length in four other *Piper* species (Fig. 4; *P. peracuminatum*: 0.41 [0.13, 0.73]; *P. paulowniifolium*: 0.34 [0.04, 0.70], *P. sancti-felicitis*: 0.27 [0.01, 0.59], and *P. multiplivervium*: 0.26 [0.03, 0.52]). A strong negative response to forearm was estimated for *Piper Type 4* (-0.21 [-0.37, -0.06]), which also had anticorrelated proclivities favoring *perspicillata* (0.36 [0.13, 0.56]) and negative for *castanea* (-0.31 [-0.54, -0.08]). Although age showed the second highest sensitivity among all covariates (Fig. 2C), no *Piper* species had a posterior that entirely excluded zero (Fig. S4), likely because there were few observations of juveniles and subadults. It is worth noting that despite this variation, *P. paulowniifolium* showed the strongest response with adult bats and Type 1 and *P. hispidum* showed the strongest response in juveniles and subadults (Fig. S4). There was no meaningful bat sex or reproductive condition influence on *Piper* species consumed. Table S2 summarizes estimates for each categorical or continuous covariate of this model.

Bat functional traits

We modeled the scaling of bite force with head and body dimensions using hierarchical models and in the natural log scale in every case. Although head length did not differ among species ($F_{2,27} = 1.443$, $p = 0.256$), it was a positive covariate of maximum bite force with high variance explained (multiple regression $R^2 = 0.90$, after controlling for sex), and a consistently positive posterior coefficient distribution (Table S3). Similar results were obtained in combination with body mass (multiple regression $R^2 = 0.90$), and forearm length (multiple regression $R^2 = 0.90$ and lowest deviance), with the forearm length coefficient indicating negative trends with bite force after controlling for head length (Table S4). Head and forearm length were positively correlated ($R = 0.76$, $t_{28} = 6.2306$, $p = 9.862e-07$). In short, once the effect of head size is accounted for, and acknowledging that larger bats have larger heads, the marginal relationship between forearm length and bite force tends to be negative. Male bats always had greater bite force compared to females, even after controlling for head length, or body size (Table S3).

Piper fruit traits and Bat-Piper responses

Phylogenetic hierarchical Bayesian models sought to relate bat responses to *Piper* species to *Piper* traits (seed shape index, fruit shape index). These models examined whether *Piper* traits could predict the relative strength of GJM coefficients reflecting the likelihood that a given bat will consume a given *Piper* species (*i.e.*, *Piper* bat proclivities). Neither of the fruit traits was a statistically significant predictor of *C. castanea* proclivities (Table S4), or of proclivities estimated based on forearm length or body mass for *C. perspicillata*.

DISCUSSION

Niche theory predicts that coexisting, closely related species will select different resources to minimize competition, evolving specializations over time to avoid competitive interactions with phenotypically similar species (Schluter 2000). We built a framework to estimate bat species proclivities to consume plant species within an assemblage of *Piper* and *Carollia* species. Using these estimates, we tested both how bat traits contributed to responses or proclivities to an array of *Piper* species, and whether plant traits showed a reciprocal influence on bat consumption. Our study has three key findings: [1] the *Piper* species most consumed by the *Piper* generalist *C. perspicillata* were the same ones least probable in the diet of the specialist *C. castanea*; [2] body size sometimes influences these interactions and is a weakly negative correlate of feeding performance ability (bite force) at the intrageneric level; and [3] the fruit traits analyzed do not influence *Piper*–*Carollia* interactions. In short, we found evidence for niche partitioning among bat frugivores that is sometimes associated with bat traits, but no corresponding matching by the plant traits examined.

Despite great morphological similarity, we expected syntopic *Carollia* species to use different co-occurring plants and to exhibit traits that match the consumption of these divergent resources. Indeed, of the six *Piper* species with the highest probability of being consumed by the generalist *C. perspicillata* (Fig. 3), five also had the lowest probability of being consumed by the specialist *C. castanea* (Fig. 3). This finding stands in contrast to past work that found no evidence for differential use of *Piper* across *Carollia* species (Maynard *et al.* 2019), suggesting that the models in GJAM that allow for simultaneous inference of multiple covariates with different variance structures help to elucidate the complex patterns of species interaction within this guild. This inverse proclivity to a suite of *Piper* species is strong evidence that *C. perspicillata* is using *Piper* species that *C. castanea* uses very little, contributing to niche partitioning. We interpret these patterns to mean that, rather than support for traditional concept of niche partitioning in which different coexisting species are adapted to eat different resources, rather *C. perspicillata* is eating several *Piper* species that *C. castanea* does not (or rarely does). Nearly all other *Piper* species had overlapping probabilities of being found in the diet of the three bat species (Fig. 3), indicating a relatively high degree of food niche overlap among the *Carollia* species. Niche partitioning between *perspicillata* and *castanea* may also be facilitated by a greater proportion of non-*Piper* species consumed by *perspicillata* (Fig. S2). These two bat species may also use their habitat differently or at different times, or be in active competition on an ecological time scale. We propose that in the presence of a competing species such as *C. perspicillata*, the realized niche is smaller for the specialist *C. castanea*, such that specializing on different *Piper* resources.

Dietary specialization should be reflected in bat traits, and we found bat body size traits to influence consumption probability for some *Piper* species, including two that are differentially consumed by *C. castanea* and *C. perspicillata* (Type 1, Type 4) and one that is differentially consumed by *C. castanea* and *C. sowelli* (*Piper sancti-felicitis*). With head size a reliable positive predictor of bite force, specialization may involve greater bite force by the larger *perspicillata* (which would enable access to harder fruit). This interpretation is indirect, however, as head size could not be directly included in *Piper* consumption models. The traits analyzed here do not exhaust the universe of traits potentially involved in specialization, as there is evidence for plant–bat specialization mediated by bat perception

through the sense of smell (Yohe *et al.* 2021) and fruit scent specifically in *Piper* (Santana *et al.* 2021). Alternatively, these bat species may use habitat differentially, but such partitioning was found to be linked to age (Maynard *et al.* 2019) rather than species, and age also had the highest sensitivity of all bat traits in our study. Together, these findings suggest habitat use across the sampled localities does not correspond to bat species and therefore cannot explain the differential use of certain *Piper* we found. Finally, *perspicillata* may be in direct competition with *castanea* for access to some *Piper* species; even though *castanea* does consume all the *Piper* underrepresented in its diet, these could possibly be more highly represented were it not for the presence of its larger congener. As there were no bat traits consistently associated with species specific proclivities (other than for Type 4), this is also a viable explanation for the niche partitioning found. Experiments excluding *perspicillata* can help distinguish between ongoing strong competition (Maynard *et al.* 2019) and the longstanding hypothesis that *castanea* is a more specialized frugivore than its congeners (Dyer & Palmer 2004; Salazar *et al.* 2013).

While body size may predict consumption of some *Piper* species, we found little evidence that body size independent of head size underlies differences in a functional trait, bite force. Body size —measured by body mass or forearm length— does not explain bite force differences among *Carollia*, but head length and sex do, after controlling for species (Table S3). Bite performance might be important to reduce competition among bats like *Carollia*; higher bite force allows access to tougher foods, thereby expanding the species' fundamental niche (Meyers & Irschick 2015; Villalobos–Chaves *et al.* 2016). The link between bite force and *Piper* proclivities is indirect (as the *Piper* consumption model lacked head length data, and bite force was not measured for individuals therein). But head and forearm length are positively correlated, implying that the influence of forearm length on *Piper* proclivities (Fig. 3) correspond to bite force as well. Structuring of *Piper* proclivities by size —which connects to bite force— aligns our results with comparative analyses for all phyllostomids (Santana *et al.* 2010b). Although nowhere near the relatively high bite forces crucial for phyllostomids that eat hard fruit (Dumont *et al.* 2009), our models indicate that partitioning by body size (forearm length) and positively associated with higher bite force could be important for explaining differential consumption for some *Piper* species.

Yet, *Carollia* feed on ripe *Piper* fruits, most of which become very soft when ripe, and all of which are cylindrical. Hence, gape and consumption speed may be more important than force in food processing (Freeman & Lemen 2007). Indeed, field observations show *Carollia* eat *Piper* fruit like corn on the cob (Fig. 1D), and very rapidly when compared to other phyllostomid frugivores (Fleming 1988). Despite differences in body size (Fig. S4), *Carollia* species have similar head dimensions, and this is reflected in similar posterior distributions for bite force across species (Table S3). In addition to different bite forces, the differences in body size that structure proclivities to *Piper* might indicate differences in dietary niche breadth, because niche breadth can increase with body size in bats (Barclay & Brigham 1991). In the latter case, instead of specialization, *Piper* proclivities might be related to *Piper* distribution and bat dispersal ability. In effect, and although we did not focus on non-*Piper* species, the larger generalist *C. perspicillata* eats fruits from several other types of plants too. Relating niche breadth to body size would thus support ongoing competition among bat congeners.

In the context of coevolution driven by mutualisms, a reciprocal correlation between frugivore phenotype and traits of the food resources is expected, as in the case of beak size and shape, and seed size and hardness in Galapagos finches (Schluter & Grant 1984; Schluter *et al.* 1985). While we found no relationship between *Piper* traits and any proclivity estimates, many other traits might be involved in this plant–bat interaction. Traits such as fruiting time (Thies & Kalko 2004), plant habit, or secondary metabolite profiles (Whitehead *et al.* 2016) have been proposed as being more important to differential consumption than the physical traits of fruit we measured. There is also strong support for chemical communication between plants and bats, with behavioral evidence for *Carollia* using the sense of smell to locate ripe fruit (Thies *et al.* 1998; Leiser–Miller *et al.* 2020), and bat olfactory receptor diversity scaling to dietary diversity (Yohe *et al.* 2021). Chemical bouquet composition both differs sharply and evolved adaptively among *Piper* species (Santana *et al.* 2021), so those traits may affect and better reflect reciprocal adaptation to bat consumption. In short, while we found no evidence of an effect of fruit and seed dimensions on bat consumption, behavioral and chemical evidence suggest scent traits are likely to be more important in structuring niche partitioning across bat species.

Though the inverse relationship in proclivities for *Piper* may indicate ongoing specialization in food resources in two *Carollia* species, there is some indication that behavioral aspects, such as learning, may contribute to differential resource use. While no *Piper* species showed a significant association to bat age, age had high sensitivity and some interesting patterns of contrasting consumption tendencies in adult versus juvenile bats warrant further exploration (Fig. S3). A previous study has found that adults used a lower percentage of mid- to late-successional species than juveniles, partitioning *Piper* by habitat (Maynard *et al.* 2019). We hypothesize that older, more experienced bats can locate and exploit resources better than younger, naïve bats –whether through spatial learning, or familiarity with less conspicuous fruit cues.

Because our model both accounts for several sources of variation and can incorporate many different types of ecological data, we were able to discover partitioning and estimate the influence of various traits on plant–frugivore interactions. Identifying such patterns both uncovered ecological factors contributing to coexistence and provided quantitative evidence of the relationships between differential resource use and frugivore traits. We discovered that, while the use of different fruit resources is related to putatively adaptive differences in body size traits, age may also play an important role in defining the dietary niche of syntopic species. As body size both may confer niche breadth and underlies functional traits such as bite force, our findings are consistent with both specialization through adaptation and ongoing competition. While there was not an effect of the plant traits examined on bat consumption, mounting evidence for plant chemical adaptation and specialization suggests plant–bat interactions may not be mediated by gross fruit morphology. Thus, this approach enabled us to both uncover the most informative predictors of differential plant use, and hint at new mechanisms underlying the evolutionary ecology of fruit–frugivore interactions.

Acknowledgements

This project was made possible by the scientists and administrators at La Selva Biological Research Station in Sarapiquí, Costa Rica. We specifically acknowledge Bernal Rodriguez

470 Hernandez, Bernal Matarrita, Orlando Vargas, David Villalobos Chaves, and Minor Porras for
471 their technical assistance and help with permits. This project was funded via the National
472 Science Foundation DEB-1442142, DEB-1456375, DEB-1856776, and the Postdoctoral
473 Research Fellowship in Biology (NSF DBI-1812035).

References

- Aguirre, L.F., Herrel, A., van Damme, R. & Matthysen, E. (2002). Ecomorphological analysis of trophic niche partitioning in a tropical savannah bat community. *Proc. R. Soc. B Biol. Sci.*, 269, 1271–1278.
- Barclay, R.M.R. & Brigham, R.M. (1991). Prey detection, dietary niche breadth, and body size in bats: why are aerial insectivorous bats so small? *Am. Nat.*, 137, 693–703.
- Bascompte, J. & Jordano, P. (2007). Plant–animal mutualistic networks: The architecture of biodiversity. *Annu. Rev. Ecol. Evol. Syst.*, 38, 567–593.
- Clark, J.S. (2016). Why species tell more about traits than traits about species: Predictive analysis. *Ecology*, 97, 1979–1993.
- Clark, J.S., Nemergut, D., Seyednasrollah, B., Turner, P.J. & Zhang, S. (2017). Generalized joint attribute modeling for biodiversity analysis: Median–zero, multivariate, multifarious data. *Ecol. Monogr.*, 87, 34–56.
- Cordeiro, N.J. & Howe, H.F. (2003). Forest fragmentation severs mutualism between seed dispersers and an endemic African tree. *Proc. Natl. Acad. Sci.*, 100, 14052–14056.
- Dumont, E.R., Herrel, A., Medellín, R.A., Vargas–Contreras, J.A. & Santana, S.E. (2009). Built to bite: Cranial design and function in the wrinkle–faced bat. *J. Zool.*, 279, 329–337.
- Dyer, L.A. & Palmer, A.D.N. (2004). *Piper: a model genus for studies of phytochemistry, ecology, and evolution*. Springer, New York.
- Fleming, T.H. (1988). *The short–tailed fruit bat: a study in plant–animal interactions*. University of Chicago Press.
- Fleming, T.H. (1991). The relationship between body size, diet, and habitat use in frugivorous bats, Genus *Carollia* (Phyllostomidae). *J. Mammal.*, 72, 493–501.
- Freeman, P.W. & Lemen, C.A. (2007). Using scissors to quantify hardness of insects: do bats select for size or hardness? *J. Zool.*, 271, 469–476.
- Gelman, A. & Hill, J. (2006). *Data analysis using regression and multilevel/hierarchical models*. Cambridge university press.
- Gelman, A. & Pardoe, I. (2006). Bayesian measures of explained variance and pooling in multilevel (hierarchical) models. *Technometrics*, 48, 241–251.
- Gelman, A. & Rubin, D.B. (1992). Inference from iterative simulation using multiple sequences. *Stat. Sci.*, 7, 457–472.
- Gentry, A.H. (1988). Tree species richness of upper Amazonian forests. *Proc. Natl. Acad. Sci.*, 85, 156–159.
- Hadfield, J. (2019). MCMCglmm course notes. *Unpubl. Manuscript, Univ. Edinburgh*.
- Hadfield, J.D. (2010). MCMC methods for multi–response generalized linear mixed models: the MCMCglmm R package. *J. Stat. Softw.*, 33, 1–22.
- Harms, K.E., Wright, S.J., Caldero, O. & Herre, E.A. (2000). Pervasive density–dependent recruitment enhances seedling diversity in a tropical forest. *Nature*, 30, 493–495.
- Kalko, E.K. V., Herre, E.A. & Handley Jr., C. (1996). Relation of fig fruit characteristics to fruit–eating bats in the New and Old World tropics. *J. Biogeogr.*, 23, 565–576.
- Leiser–Miller, L., Kaliszewska, Z., Lauterbur, M., Mann, B., Riffell, J. & Santana, S.E. (2020). A fruitful endeavor: scent cues and echolocation behavior used by *Carollia castanea* to find fruit. *Integr. Org. Biol.*, 2.
- Maynard, L.D., Ananda, A., Sides, M.F., Burk, H. & Whitehead, S.R. (2019). Dietary

- resource overlap among three species of frugivorous bat in Costa Rica. *J. Trop. Ecol.*, 35, 165–172.
- Meyers, J.J. & Irschick, D.J. (2015). Does whole-organism performance constrain resource use? A community test with desert lizards. *Biol. J. Linn. Soc.*, 115, 859–868.
- Nathan, R. & Muller-Landau, H.C. (2000). Spatial patterns of seed dispersal , their determinants and consequences for recruitment. *TRENDS Ecol. Evol.*, 15, 278–285.
- Norconk, M.A., Wright, B.W., Conklin-Brittain, N.L. & Vinyard, C.J. (2009). Mechanical and nutritional properties of food as factors in Platyrrhine dietary adaptations. In: *South American Primates* (eds. Garber, P., Estrada, A., Bicca-Marques, J.C., Heymann, E.W. & Strier, K.B.). Springer, New York, NY, pp. 279–319.
- Plummer, M. (2003). JAGS: A program for analysis of Bayesian graphical models using Gibbs sampling. In: *Proceedings of the 3rd international workshop on distributed statistical computing*. Vienna, Austria.
- Pratt, T.K. & Stiles, E.W. (1985). The influence of fruit size and structure on composition of frugivore assemblages in New Guinea. *Biotropica*, 17, 314–321.
- Rojas, D., Pereira, M.J., Fonseca, C. & Davalos, L.M. (2018). Eating down the food chain: generalism is not an evolutionary dead end for herbivores. *Ecol. Lett.*, 21, 402–410.
- Salazar, D., Kelm, D.H. & Marquis, R.J. (2013). Directed seed dispersal of *Piper* by *Carollia perspicillata* and its effect. *Ecology*, 94, 2444–2453.
- Santana, S.E., Dumont, E.R. & Davis, J.L. (2010a). Mechanics of bite force production and its rel to diet in bats. *Funct. Ecol.*, 24, 776–784.
- Santana, S.E., Dumont, E.R. & Davis, J.L. (2010b). Mechanics of bite force production and its relationship to diet in bats. *Funct. Ecol.*, 24, 776–784.
- Santana, S.E., Kaliszewska, Z.A., Leiser-Miller, L.B., Lauterbur, M.E., Arbour, J.H., Dávalos, L.M., *et al.* (2021). Fruit odorants mediate co-specialization in a multispecies plant-animal mutualism. *Proc. R. Soc. B Biol. Sci.*, 288, 20210312.
- Santana, S.E. & Miller, K. (2016). Extreme postnatal scaling in bat feeding performance: a view of ecomorphology from ontogenetic and macroevolutionary perspectives. *Integr. Comp. Biol.*, 56, 1–10.
- Schleuning, M., Blüthgen, N., Flörchinger, M., Braun, J., Schaefer, H.M. & Böhning-Gaese, K. (2011). Specialization and interaction strength in a tropical plant – frugivore network differ among forest strata. *Ecology*, 92, 26–36.
- Schluter, D. (2000). *The Ecology of Adaptive Radiation*. Oxford University Press, New York.
- Schluter, D. & Grant, P.R. (1984). Determinants of morphological patterns in communities of Darwin’s finches. *Am. Nat.*, 123, 175–196.
- Schluter, D., Price, T.D. & Grant, P.R. (1985). Ecological character displacement in Darwin’s finches. *Science (80-.)*, 227, 1056–9.
- Sekercioglu, C.H. (2006). Increasing awareness of avian ecological function. *TRENDS Ecol. Evol.*, 21, 464–471.
- Sexton, J.P., Montiel, J., Shay, J.E., Stephens, M.R. & Slatyer, R.A. (2017). Evolution of ecological niche breadth. *Annu. Rev. Ecol. Evol. Syst.*, 48, annurev-ecolsys-110316-023003.

- Su, Y.-S. & Yajima, M. (2012). R2jags: A Package for running jags from R. *R Packag. version 0.03-08*, URL <http://CRAN.R-project.org/package=R2jags>.
- Thies, W. & Kalko, E.K. (2004). Phenology of neotropical pepper plants (Piperaceae) and their association with their main dispersers, two short-tailed fruit bats,. *Oikos*, 2, 362–376.
- Thies, W., Kalko, E.K. V. & Schnitzler, H.-U. (1998). The roles of echolocation and olfaction in two Neotropical fruit-eating bats, *Carollia perspicillata* and *C. castanea*, feeding on *Piper*. *Behav. Ecol. Sociobiol.*, 42, 397–409.
- Villalobos-Chaves, D., Padilla-Alvárez, S. & Rodríguez-Herrera, B. (2016). Seed predation by the wrinkle-faced bat *Centurio senex*: A new case of this unusual feeding strategy in Chiroptera. *J. Mammal.*, 97, 726–733.
- Weinstein, B.G. & Graham, C.H. (2017). Persistent bill and corolla matching despite shifting temporal resources in tropical hummingbird-plant interactions. *Ecol. Lett.*, 20, 326–335.
- Wendeln, M.C., Runkle, J.R. & Kalko, E.K. V. (2000). Nutritional values of 14 fig species and bat feeding preferences in Panama. *Biotropica*, 32, 489–501.
- Whitehead, S.R., Obando Quesada, M.F. & Bowers, M.D. (2016). Chemical tradeoffs in seed dispersal: defensive metabolites in fruits deter consumption by mutualist bats. *Oikos*, 125, 927–937.
- Yohe, L.R., Leiser-Miller, L.B., Kaliszewska, Z.A., Donat, P., Santana, S.E. & Dávalos, L.M. (2021). Diversity in olfactory receptor repertoires is associated with dietary specialization in a genus of frugivorous bat. *G3 Genes/Genomes/Genetics*, 11, jkab260.

Figure Legends

Figure 1. Headshots of the three sympatric short-tailed fruit bats (*Carollia*) found in our study locality in Costa Rica: (A) *Carollia perspicillata*, (B) *C. sowelli*, and (C) *C. castanea*. (D) *C. perspicillata* feeding on *Piper sancti-felicis*. Photo credit: David Villalobos Chaves (A–C) and Susan Whitehead (D).

Figure 2. Parameters (A) richness and (B) diversity calculated as posterior predictive checks of model fit for the relative abundance fractional composition model. Model fit of the generalized joint attribute modeling in predicting *Piper* bat proclivities. The brown histogram in (A) and (B) is the distribution of the observed data and the dashed lines are the 1:1 diagonals of the observed values and predictions. (A) Richness represents a predictive posterior check, such that richness is responses predicted that are greater than 0. (B) More diverse “sites” had a better fit, as less diverse “sites” are more rare. (C) Sensitivity of the covariate inputs can be interpreted as the amount of information each input contributes to estimating the model coefficients. The higher the sensitivity, the more informative the covariate to the model.

Figure 3. Posterior distributions of bat species responses for each *Piper* species (*Piper* bat proclivities), ordered by median. Bat species response to *Piper* can be interpreted as the probability of a particular *Carollia* species to show a proclivity towards a particular *Piper* species. Asterisks and black species names refer to *Piper* in which 95% of the highest posterior density intervals did not cross zero, indicating a strong positive or negative response.

Figure 4. Posterior distributions of bat body size trait responses of forearm length for *Piper* species that indicate strong positive or negative responses (from Fig. 3), determined by the entire 95% highest posterior density being entirely above or below zero.