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Interactions between nutrients and fruit secondary metabolites shape bat foraging behavior and protein absorption

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Abstract

Frugivore foraging behavior is largely influenced by two key groups of chemical traits: nutrients and secondary metabolites. Many secondary metabolites function in plant defense, but their consumption can negatively impact both mutualistic and antagonistic frugivores, often due to toxic properties of the metabolites or through nutrient absorption interference. Frugivores are assumed to maximize nutrient acquisition while avoiding or minimizing toxic metabolite intake, but the relative roles of co-occurring nutrients and secondary metabolites in foraging behavior are not well understood. Here, we used a neotropical fruit bat to investigate the interactive effects of nutrients and a broadly bioactive fruit secondary metabolite, piperine, on two essential processes in nutrient acquisition, namely foraging behavior and nutrient absorption. Through the manipulation of nutrient and piperine concentrations in artificial diets, we showed that captive fruit bats prioritize nutrient concentrations regardless of the levels of piperine, even though piperine is a strong deterrent on its own. Furthermore, our findings reveal that while piperine has no detectable influence on total sugar absorption, it reduces protein absorption, which is a crucial and limited nutrient in the frugivore diet. Overall, our results demonstrate the importance of considering the interaction between co-occurring chemical traits in fruit pulp to better understand frugivore foraging and physiology.

KEYWORDS

Carollia, chemical ecology, foraging, frugivores, nutrient absorption, nutritional ecology, piperine

INTRODUCTION

The ecological interactions among fleshy fruits, mutualists, and antagonists are influenced in numerous ways by fruit chemical traits (Nelson & Whitehead, 2021). Fruit pulp exhibits a remarkable chemical complexity (Whitehead et al., 2021), containing primary metabolites or nutrients (sugars, proteins, and lipids) alongside diverse mixtures of secondary or specialized metabolites. Some secondary metabolites can function as attractive traits such as scent (Nevo & Ayasse, 2020; Rodríguez et al., 2013) and coloration (Nevo et al., 2018; Valenta et al., 2017), while many others function as defensive traits and possess deterrent, toxic, or antimicrobial properties (Schoonhoven et al., 2005).

Some secondary metabolites in ripe fruit pulp that function primarily in defense also have broad bioactivity toward mutualists (Baldwin & Whitehead, 2015; Maynard et al., 2020; Whitehead et al., 2016; but see Tewksbury & Nabhan, 2001). Thus, both mutualist and antagonist frugivores face complex foraging decisions, balancing the benefits of nutrient acquisition against the potential costs of secondary metabolite ingestion. These cost–benefit analyses are nontrivial because both frugivore foraging behavior and its physiological consequences are influenced by interactions between co-occurring nutrients and defensive metabolites (Cazetta et al., 2008; Rojas et al., 2021). For instance, the role of nutrients in modulating the effects of secondary metabolites on several performance metrics in both invertebrate and vertebrate herbivores is well documented (Deans et al., 2016, 2017; Perkovich & Ward, 2020; Simpson & Raubenheimer, 2001; Wang et al., 2022). Nevertheless, most studies on frugivore preference have independently evaluated the effect of nutrients or secondary metabolites, leaving the trade-offs that frugivores face between nutrient acquisition and toxin avoidance poorly explored.

Several hypotheses have been proposed to explain the covariation of nutrients and secondary metabolites that function primarily in defense in fruit pulp, along with related predictions about frugivore foraging behavior when encountering co-occurring nutrients and toxins (Cipollini & Levey, 1997b). Among these, the "nutrient-toxin titration hypothesis" predicts that frugivores will select high-nutritional fruits, regardless of the concentration of defensive traits, as nutrients can offset potential adverse effects from toxin consumption. The interactive effect between nutrients and secondary metabolites has been investigated by evaluating frugivore removal rates in natural and artificial conditions (Cazetta et al., 2008; Cipollini & Levey, 1997b; Schaefer et al., 2003) and by assessing the correlation between nutrients and various groups of defensive metabolites within fruit pulp (Gelambi & Whitehead, 2023; Izhaki et al., 2002). Some of these studies align with the predictions of the nutrient-toxin titration hypothesis, but its applicability seems to depend on the system and the type of secondary metabolites studied.

After consumption, a critical mechanism through which nutrients and secondary metabolites continue to interact is the modification of nutrient absorption by secondary metabolites (Kessler & Kalske, 2018; Mithöfer & Boland, 2012). Various ratios of co-occurring nutrients and defensive metabolites could potentially modify nutrient absorption through different mechanisms. One basic mechanism by which secondary metabolites reduce nutrient absorption is simply by diluting the nutrients per unit of plant tissues, a phenomenon known as the "dilution effect" (Guglielmo et al., 1996). Furthermore, secondary metabolites and nutrients can directly interact (Mueller-Harvey, 2006), often leading to a reduction in the availability of nutrients for absorption. Upon ingestion, certain secondary metabolites can modify the activity of several digestive enzymes (Platel & Srinivasan, 2001), while also inducing changes in the integrity, nutrient transport, and permeability of the gastrointestinal epithelium, thereby impacting nutrient absorption (Patra et al., 2019). Our understanding of the effects of defensive metabolites on nutrient absorption has primarily come from the agricultural sciences, with tannins being the most extensively studied class of defensive metabolites (Foley & Moore, 2005). However, the narrow focus on tannins has limited our understanding of how other classes of defensive metabolites may affect nutrient absorption in nonruminant animals.

Here, we investigate the effects of nutrients and a representative fruit secondary metabolite on the Seba's short-tailed bat, *Carollia perspicillata* (Linnaeus, 1758), a key seed disperser of *Piper* spp. and other pioneer plants throughout the tropics in Central and South America (Fleming, 1988). First, we determined the relative role of nutrients and secondary metabolites in bat preference (objective 1). Specifically, we asked (1.1) Do bats have a preference for high- over low-nutrient diet? (1.2) Do bats avoid fruit secondary metabolites? (1.3) Does a high-nutrient diet mitigate the deterrent effects of high concentrations of secondary metabolites, as postulated by the "nutrient-toxin titration hypothesis"? (1.4) Do bats adjust their overall nutrient or secondary metabolite intake based on the food options available? Then, we investigate the effect of a secondary metabolite on nutrient absorption (objective 2). Specifically, we asked: Do fruit secondary metabolites modify the absorption of (2.1) total and individual sugars and (2.2) total protein?

METHODS

Study site and study organisms

Preference and absorption experiments were conducted at La Selva Biological Station, province of Heredia, Costa Rica, during June and July 2021. The station comprises 1536 ha of primary and secondary lowland tropical rainforest managed by the Organization for Tropical Studies (OTS). As a study organism, we used *C. perspicillata*, one of the most abundant bat species in the Neotropics (Fleming, 2004). *Carollia* diet is mainly ripe infructescences (hereafter fruits) of *Piper* spp. (York & Billings, 2009). When feeding on ripe *Piper* fruits, bats encounter numerous structurally diverse secondary metabolites, including amides, lignans, neolignans, phenylpropanoids, and chalcones (Dyer & Palmer, 2004; Uckele et al., 2021; Whitehead et al., 2021).

Secondary metabolite selection

As a representative fruit secondary metabolite, we used the piper amide piperine. Piper amides are a group of nitrogenous metabolites found in several plant species from the genus *Piper* (Salehi et al., 2019; Uckele et al., 2021; Whitehead et al., 2013). In several neotropical *Piper* species, amides play a defensive role in the meditation of ecological interactions with antagonist pests (Dyer et al., 2003; Whitehead & Bowers, 2014). Captive experiments have demonstrated that bats are deterred by amides, resulting in both reduced fruit removal and a decreased amount of fruit consumed (Whitehead et al., 2016). Therefore, amides are ideal candidates for studying the trade-off between nutrient acquisition and secondary metabolite avoidance. Specifically, piperine, an amide present in black pepper (*Piper nigrum*) and other *Piper* species, is commercially available and has been extensively investigated for its pharmacological effects on mammalian physiology (Haq et al., 2021). Multiple studies suggest that piperine can modify nutrient metabolism in mammals through different mechanisms, including the inhibition of key digestive enzymes (Haq et al., 2021; Suresh & Srinivasan, 2010). Although piperine has not been detected in the more abundant native *Piper* at La Selva, it has been reported in other neotropical species (de Oliveira Chaves et al., 2003; Leitão da-Cunha & de Oliveira Chaves, 2001), and *Carollia* bats may also frequently encounter piperine when consuming *P. nigrum* from cultivated plants (Hernández-Leal & Sánchez, 2021). It is also structurally similar to other piper amides that are an abundant part of the *Carollia* diet (Salehi et al., 2019; Whitehead et al., 2013).

Bat capture and maintenance

Two males and 18 nonreproductive female *C. perspicillata* bats were captured using mist nets placed in clearings and secondary forest sites in June 2021. We kept two rounds of 8 and 10 bats in captivity, housed in individual flight cages (2 × 1 × 1 m) located in the forest. Bats acclimated for at least two nights when we fed them with a synthetic maintenance diet based on the formulation described in Denslow et al. (1987) consisting of water, agar powder (Eco-Taste), mashed ripe banana, soy protein isolate powder (Bulk Supplements), NaCl, $CaHPO_4$ (Eisen-Golden Laboratories), vegetable oil, and wheat germ (Bob's Red Mill). Consecutive trials were then conducted with each bat over a period of 9 days (see below), and after every night's trial, we offered water and 35 g of an unsupplemented maintenance diet to each bat. After finishing all the trials, we released the bats at the site of capture. All experiments were approved by the Comisión Nacional para la Gestión de la Biodiversidad (resolution no. R-007-2021-OT-CONAGEBIO) and Virginia Tech Institutional Animal Care and Use Committee protocols (approval no. IACUC 20-212).

Objective 1: Determine the relative role of nutrients and secondary metabolites in bat preference

We conducted three sets of trials, each one consisting of two-choice preference experiments comparing: (1.1) low nutrients versus high nutrients, both without piperine; (1.2) low piperine versus high piperine, both with high nutrients and (1.3) high nutrients, high piperine versus low nutrients, low piperine. The first group (8 bats) participated in trials 1.1 and 1.3, whereas the second group (10 bats) participated in trials 1.1, 1.2, and 1.3. Each bat participated in two or three paired choice tests in random order on consecutive nights. We started the trials at 6:00 PM by placing two Petri dishes approximately 30 cm inside the flight cage door. This represented the first food of the day. To avoid the influence of previous experience, we randomized the position (left or right) of the dishes for each trial. After 30 min, we quantified the amount of food eaten in each dish. We used the difference in total consumption between the two dishes as the main measure of preference.

Each petri dish in the trials contained 5 g of the artificial diet described above with different amounts of nutrients and toxins. The high-nutrient option was prepared with the same proportions of ingredients used for the maintenance diet. Based on the average nutritional values reported for bananas in previous studies (Anyasi et al., 2013), and the nutritional information provided in the packages of the protein powder and wheat germ, we estimate that 5 g (wet mass) of diet contained approximately 96 mg of sugars, 76 mg of proteins, and 5 mg of lipids. The low-nutrient option was prepared based on the same ingredients, maintaining the same ratios for NaCl, $CaHPO_4$, oil, and wheat germ, but leaving out the protein powder and reducing the bananas by half. Thus, the 5 g of diet contained approximately 54 mg of sugars, 7 mg of proteins, and 4 mg of lipids. We added 0.1% dry mass of piperine (≥95%, Sigma-Aldrich) in the low-piperine option and 2% dry mass in the high-piperine option, which corresponds to the natural variation range of amides in ripe neotropical *Piper* spp. fruits (Baldwin & Whitehead, 2015; Whitehead et al., 2013). After completing all trials, bats were fed the unsupplemented maintenance diet for one night and then participated in the experiments for objective 2.

Objective 2: Investigate the effect of a secondary metabolite on sugar and protein absorption

We added four different concentrations of piperine (≥95%, Sigma-Aldrich), 0.1%, 0.5%, 1.5%, and 2% dry mass, directly to 10 g of the artificial diet described above. Each bat was randomly assigned one of the four concentrations and a control (unsupplemented diet) for five consecutive nights. After the bats consumed the 10 g of diet, within approximately 2–3 h, we collected fecal samples from the bottom of the cage using a clean spatula. Nutrients remaining in the feces indicate the amount of the nutrient that has been excreted and therefore has not been absorbed. We stored fecal samples at −80°C for later analysis in the laboratory, except for transportation from Costa Rica to Virginia, USA, on dry ice. We split each fecal sample collected in two to quantify (2.1) individual and total sugars and (2.2) total proteins. Because bat fecal samples are potentially contaminated with the respiratory pathogen *Histoplasma capsulatum*, we decontaminated samples prior to analysis by adding 1 mL of isopropanol, vortexing the mix, and evaporating the isopropanol in a SpeedVac (vacuum: 13332 Pa, temperature: 65°C). Finally, samples were freeze-dried and ground to a fine powder.

Sugar extraction and quantification

We modified the extraction and quantification of sugars using high-performance liquid chromatography (HPLC) described in Lord et al. (2021). We weighed 10 mg of dried fecal sample and added 1 mL of HPLC water as the extraction solvent. Subsequently, we sonicated the samples for 20 min. Then, we centrifuged the homogenates at 10,000×*g* for 10 min at room temperature. We transferred 0.5 mL of supernatant into another microcentrifuge tube and added 0.7 mL of acetonitrile (ACN) and let the mix sit for 1 h at room temperature. We centrifuged the homogenates at 10,000×g for 10 min. We mixed 100 μL of the supernatant with 900 μL of 65% ACN, and the solution was filtered into a vial using a syringe with an IC Millex-LG 13-mm mounted 0.2-μm hydrophilic millipore polytetrafluoroethylene membrane filter (Millipore). Samples were injected into an HPLC instrument, Agilent 1260 Infinity series (Agilent Technologies) with an apHera NH2 Polymer, 5-μm analytical column, and a 1260 Infinity ELSD detector (Agilent Technologies). We used ACN/water (65:35, v/v) as the elution solvent with a flow rate of 1.0 mL min^{-1}. To calculate the area and retention time of the peaks, we used the software ChemStation using the default integration parameters. We aligned the peaks based on the retention time using the R package GCalignR (Ottensmann et al., 2018). We calculated the total amount of soluble sugars adding the areas of all the individual sugar peaks found in the sample and expressed the concentration as glucose equivalents based on a standard curve for glucose. Each sample was extracted and analyzed once.

Protein extraction and quantification

We modified the extraction in Bonjoch and Tamayo (2001), a specific protocol to extract and quantify protein from plant tissues. We weighed 10 mg of dried fecal sample and added 1 mL of 0.05 M Tris buffer (pH 8.0), containing ascorbic acid 0.1% (m/v), cysteine hydrochloride 0.1% (m/v), polyethylene glycol (1%), citric acid monohydrate 0.1% (m/v), and 2-mercaptoethanol 0.008% (v/v). We sonicated the samples for 20 min and centrifuged the homogenates at 10,000×g for 20 min at 4°C. We quantified the total amount of protein in the supernatant using a commercial colorimetric assay (Bio-Rad, 5000002) based on the Bradford method (Bradford, 1976), adapted to a 96-well plate. We expressed the total protein concentration as bovine serum albumin equivalents. Each sample was extracted and analyzed in duplicate, and the two duplicate measures were averaged.

Statistical analysis

We performed all statistical analyses in R v. 4.1.3 (R Core Team, 2021). For objective 1 (1.1, 1.2, and 1.3), we conducted a comparison of the amount of food consumed in each Petri dish per bat using paired *t*-tests, one per trial. We used the t.test() function, setting the null hypothesis as the mean difference to 0 (no preference between the two options). For objective 1.4, we assessed whether bats adjust their absolute nutrient or piperine intake depending on the available food options by comparing consumption across the three different trials.

We calculated the amount (in milligrams) of nutrients and piperine eaten per bat in each trial and fitted two separate linear models, using the lm() function, to estimate differences in nutrients and piperine consumption between the three trials.

For objective 2, we first fitted two separate generalized linear mixed models (GLMMs) to estimate the differences in (2.1) sugar and (2.2) protein excretion between the control and the four concentrations of piperine tested. Each model consisted of the proportion of total proteins or total sugars per unit fecal mass excreted by bats as the response variable, and each piperine concentration, namely 0%, 0.1%, 0.5%, 1%, and 2%, as the predictor variable. Then, to assess the effect of piperine on each sugar, we fitted separate GLMMs to estimate the differences in individual sugar excretion (Appendix S1: Figure S1, Table S1) between the control and the four concentrations of piperine tested. Each model consisted of the proportion of the individual sugars per unit fecal mass excreted as the response variable, and each piperine concentration, namely 0%, 0.1%, 0.5%, 1%, and 2%, as the predictor variable. As we conducted repeated measurements on the same bats and used a fresh artificial diet every evening with a different banana, we included the bat identity and the trial date as random effects in all the models. To contrast each concentration of piperine with the control, we used the emmeans() function from the emmeans package (Lenth et al., 2021) to obtain estimated marginal means for each level of the treatment variable. GLMMs were performed using the glmmTMB package (Magnusson et al., 2017). To address the non-normality of residuals in the GLMMs, we used the beta distribution and the logit link function, which are suitable for response variables expressed as proportions (Smithson & Verkuilen, 2006).

RESULTS

Objective 1: The relative role of nutrients and secondary metabolites in bat preference

We found that the relative concentrations of fruit secondary metabolites and nutrients modify bat preferences (Tables 1 and 2, Figure 1). In the first trial (1.1), comparing different nutrient concentrations, we found that bats consumed more of the high-nutrient option relative to the low-nutrient option ($p = 0.012$). In the second trial (1.2), comparing different piperine concentrations, bats consumed more of the low-piperine option relative to the high-piperine option ($p = 0.001$). For the final trial (1.3),

TABLE 1 Paired *t* test results investigating bat preference for different concentrations of nutrients and a representative defensive metabolite, piperine.

Trial	*t*	df	Mean difference	95% CI (low, high)	*p*
High nutrients versus low nutrients (1.1)	2.821	17	1.663	0.419, 2.906	**0.012**
2% piperine versus 0.1% piperine (1.2)	−4.400	9	−2.988	−4.453, −1.447	**0.001**
High nutrients, 2% piperine versus low nutrients, 0.1% piperine (1.3)	3.866	17	1.995	0.906, 3.084	**0.001**

Note: The *p* values <0.05 appear in boldface.

TABLE 2 Linear models estimating the differences in nutrients and piperine consumption between the three trials.

Predictor variable	Coefficient	SE	95% CI (low, high)	*t*	*p*
Nutrients consumed					
Trial 1.1, intercept	134.38	12.49	109.18, 159.58	10.76	**<0.001**
Trial 1.2	23.17	20.91	−18.99, 65.33	1.11	0.274
Trial 1.3	−11.07	17.67	−46.71, 24.56	−0.63	0.534
R^2	0.059				
Piperine consumed					
Trial 1.2, intercept	2.62	1.12	0.31, 4.93	2.33	**0.028**
Trial 1.3	6.39	1.40	3.52, 9.28	4.57	**<0.001**
R^2	0.45				

Note: The *p* values <0.05 appear in boldface.

we simulated a trade-off between the acquisition of nutrients and avoidance of defensive metabolites, and we found that bats consumed more of the high-nutrient, high-piperine option, relative to the low-nutrient, low-piperine option ($p = 0.001$). When we compared the quantities of nutrients and piperine consumed between trials (Table 2, Figure 2), we observed no statistically significant differences in nutrient consumption among the three trials. In contrast, we found variations in piperine consumption, with bats consuming a significantly higher amount of piperine in trial 1.3 ($p < 0.001$, Table 2).

Objective 2: The effect of piperine on sugar and protein absorption

Our results suggest that 2% piperine interferes with protein absorption in fruit bats (Table 3, Figure 3). We found that bats excreted, on average, 34% more protein when consuming 2% of piperine, relative to the control ($p = 0.009$). The lower piperine concentrations tested did not differ significantly from the control in the amount of protein excreted (Table 3, Figure 3).

None of the piperine concentrations tested significantly affected total sugar excretion (Table 4, Figure 3). In the samples analyzed, we quantified 12 unique sugars (Appendix S1: Table S1, Figure S1). Among these, the most abundant and frequent sugars, present in at least 20% of the fecal samples, were labeled as "A," "B," "F," "G," and "K" (Appendix S1: Table S1, Figure S1). These five sugars were significantly correlated in our samples (Appendix S1: Figure S2). None of the piperine concentrations tested had a statistically significant effect on the sugar composition of the fecal samples ($p = 0.969$, Appendix S1: Figure S3; PERMANOVA). When analyzing the effect of piperine on each sugar, we found that bats excreted, on average, 9% and 30% less A and K sugars, respectively, when consuming 2% of piperine, relative to the control (A, $p = 0.012$; K, $p = 0.046$; Figure 4, model parameters presented in Appendix S1: Tables S3 and S7). In contrast, we also found

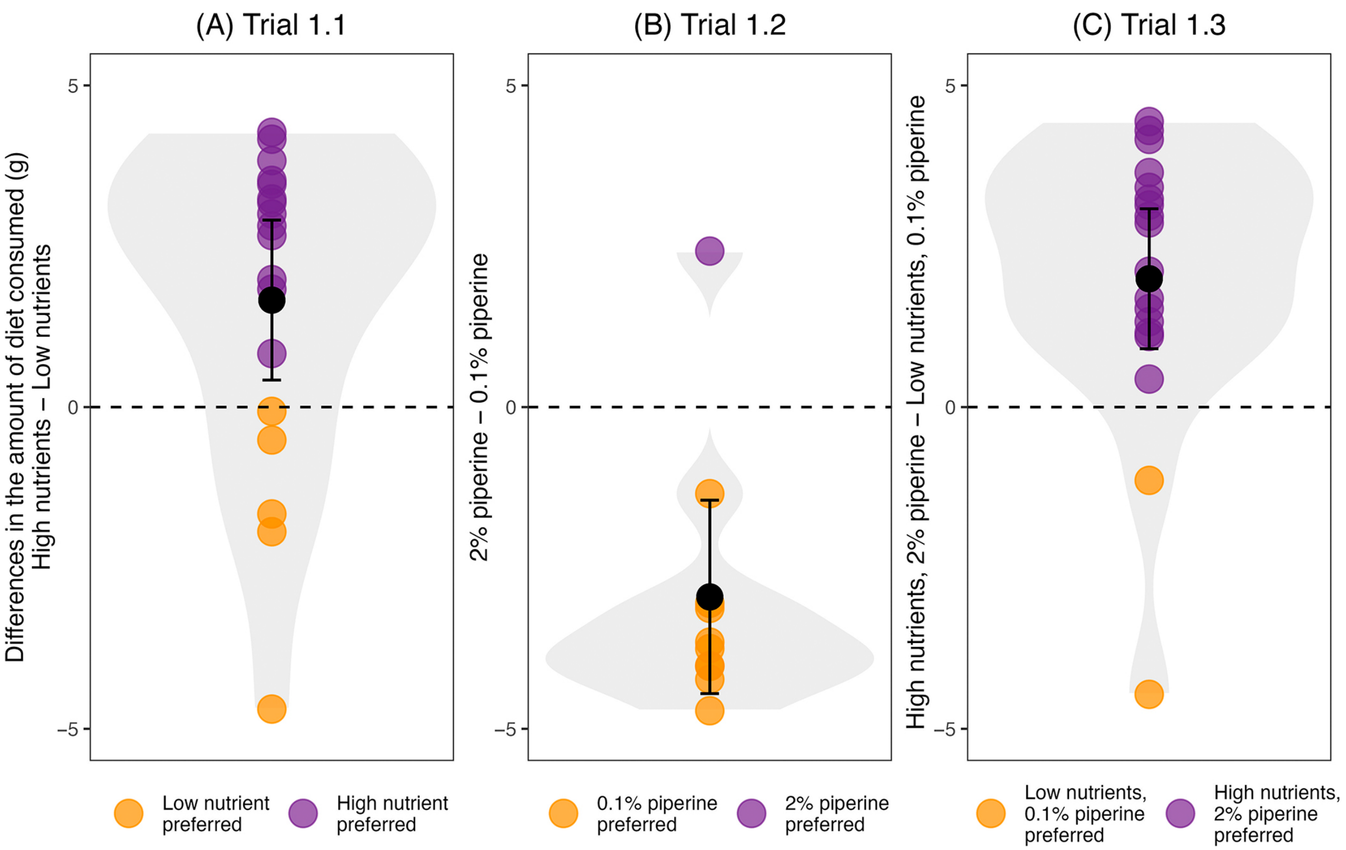

FIGURE 1 Two-choice feeding experiments testing the effect of varying concentrations of nutrients and a representative defensive metabolite, piperine, on bat preference. Each data point represents a single bat and shows the difference in consumption over 30 min between two simultaneous options: (A) low nutrients versus high nutrients ($N = 18$ bats), (B) 0.1% piperine versus 2% piperine ($N = 10$ bats), and (C) low nutrients, 0.1% piperine versus high nutrients, 2% piperine ($N = 18$ bats). Positive values, depicted in purple, indicate a preference for the high-nutrient and/or 2% piperine option, while negative values, depicted in orange, indicate a preference for the low-nutrient and/or 0.1% piperine option. A value of zero would indicate no preference for either diet. Black points and error bars represent mean difference and 95% CIs computed using the paired *t* test.

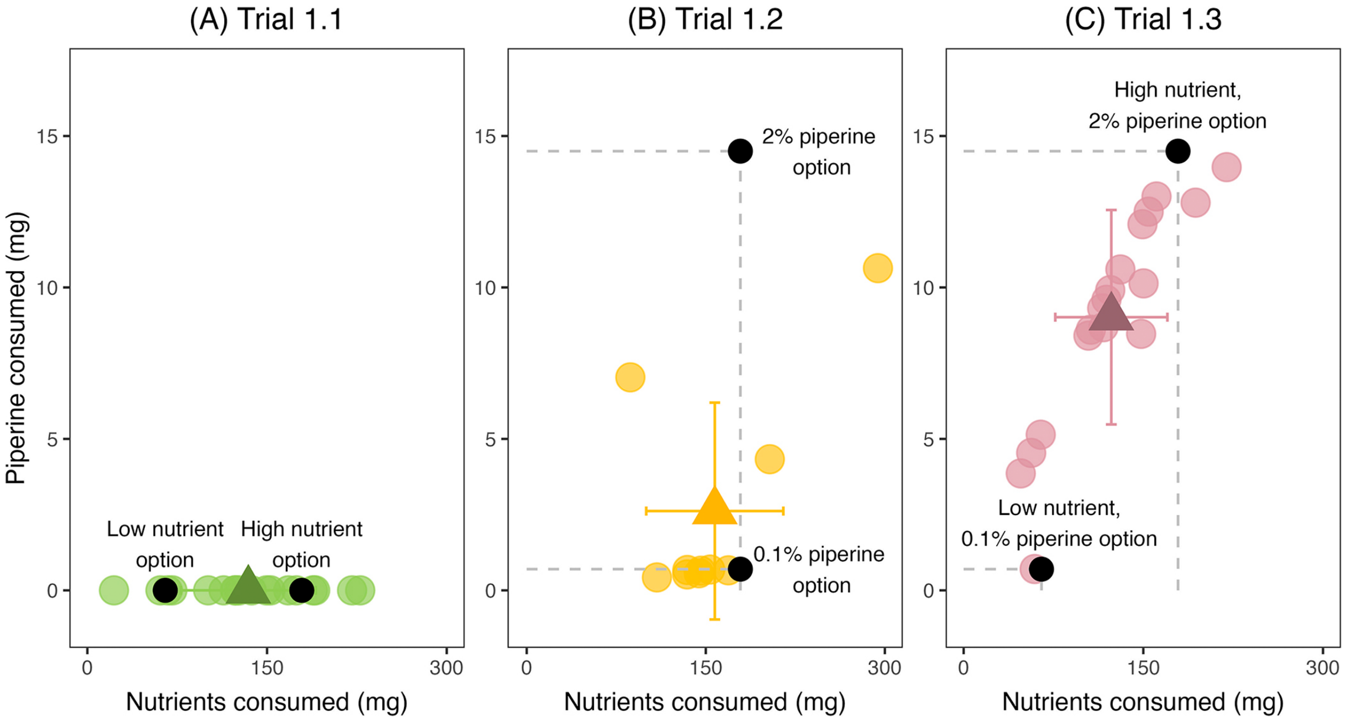

FIGURE 2 Comparison of nutrient and piperine consumption across three trials. Each point represents the amount of nutrient and piperine consumed per individual bat in each trial, from both Petri dishes. Triangles, along with SDs, indicate the average consumption of nutrients and piperine. Each black point represents the two options available per 5 g of trial. (A) Low nutrients versus high nutrients ($N = 18$ bats), (B) 0.1% piperine versus 2% piperine ($N = 10$ bats), and (C) low nutrients, 0.1% piperine versus high nutrients, 2% piperine ($N = 18$ bats).

TABLE 3 Generalized linear mixed model output estimating the effect of different piperine concentrations on total protein excretion.

Predictor variable: Treatment	Coefficient	SE	95% CI (low, high)	z	p
Control, intercept	−4.12	0.16	−4.43, −3.80	−25.93	**<0.001**
0.1% piperine	−0.03	0.12	−0.27, 0.20	−0.30	0.766
0.5% piperine	0.06	0.11	−0.16, 0.28	0.52	0.605
1.5% piperine	−0.10	0.12	−0.33, 0.13	−0.82	0.410
2% piperine	0.29	0.11	0.07, 0.51	2.59	**0.009**
Conditional R^2	0.697				
Marginal R^2	0.053				

Note: The bat identity and trial date were included as random effects in the models. The p values <0.05 appear in boldface.

that bats excreted, on average, 24% more G sugars, when consuming 2% of piperine, relative to the control ($p = 0.028$, Figure 4, model parameters presented in Appendix S1: Table S6). Finally, piperine did not significantly influence the excretion of the other individual sugars (Figure 4, Appendix S1: Tables S2, S4, and S5).

DISCUSSION

Fleshy fruits contain complex mixtures of nutrients and secondary metabolites, but little is known about the interactive effects of these two key classes of chemical traits on frugivore foraging and physiology. Our findings suggest that nutrients and secondary metabolites can interact to modify both animal foraging strategies and nutrient absorption. We showed that our focal species, *C. perspicillata*, makes foraging decisions based on both nutrients and secondary metabolites. Our trials showed that nutrients appear to be the primary foraging driver, and relatively high-nutrient concentrations can mask or reduce the deterrent effect of piperine. In our absorption experiments, we found that relatively high concentrations of piperine (2%) significantly increased protein

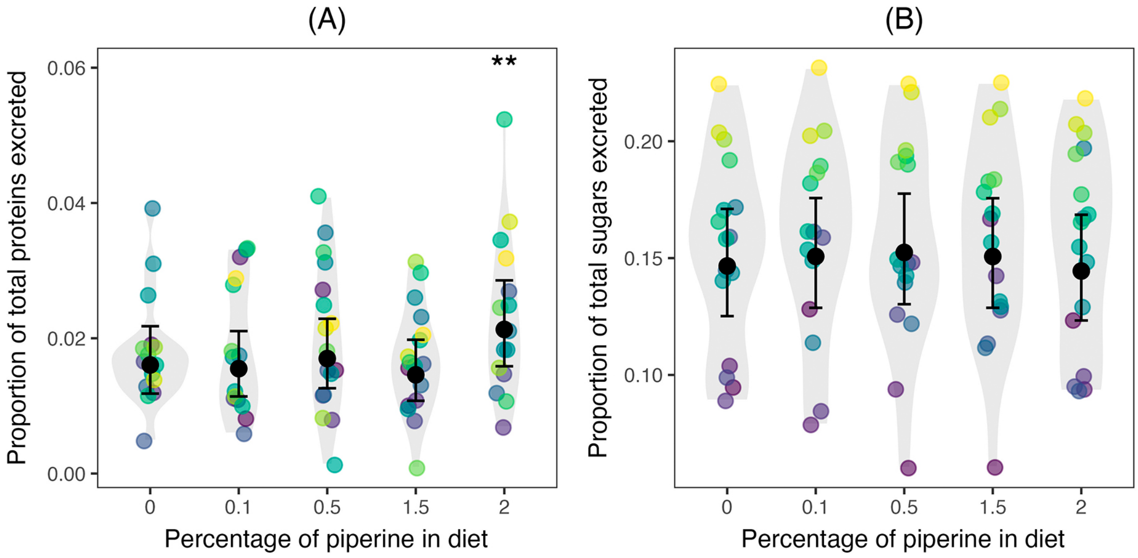

FIGURE 3 Proportions of (A) total proteins and (B) total sugars excreted in fecal samples from bats fed on different experimental diets with varying concentrations of piperine. Each point is colored by bat ID. Black points and error bars represent the estimated marginal means and 95% CIs predicted by the generalized linear mixed models, back-transformed from the logit scale. Significance levels of each treatment in comparison to the control are denoted by asterisks (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$).

TABLE 4 Generalized linear mixed model output estimating the effect of different piperine concentrations on total sugar excretion.

Predictor variable: Treatment	Coefficient	SE	95% CI (low, high)	z	p
Control, intercept	−1.76	0.09	−1.98, −1.58	−18.85	**<0.001**
0.1% piperine	0.03	0.03	−0.03, 0.09	1.04	0.297
0.5% piperine	0.05	0.03	−0.01, 0.11	1.49	0.135
1.5% piperine	0.03	0.03	−0.03, 0.09	1.05	0.292
2% piperine	−0.02	0.03	−0.08, 0.04	−0.59	0.557
Conditional R^2	0.959				
Marginal R^2	0.005				

Note: The bat identity and trial date were included as random effects in the models. The p values <0.05 appear in boldface.

excretion by 34%, while none of the tested piperine concentrations affected total sugar absorption. The relatively higher concentrations of piperine, 1.5% and 2%, exhibited both negative and positive effects on the excretion of some individual sugars.

Our first trial (1.1) revealed that bats preferred a high-nutrient diet. The ability to distinguish between different nutrient concentrations and optimize nutrient intake has been reported for different frugivores, including fruit bats (Nelson et al., 2005) and birds (Bosque & Calchi, 2003; Schaefer et al., 2003). The primary distinction between our high-nutrient and low-nutrient diet treatments was the lowered levels of protein and soluble carbohydrates achieved by reducing the amounts of protein powder and banana in the diet. In addition, the reduction in banana content in the diet could have led to alterations in other components, including micronutrients and volatile organic compounds. While our findings indicate that the primary factor influencing bat foraging is diet nutrient composition, further investigation is required to determine the specific role of different macronutrients, micronutrients, and other attractants in shaping bat preferences.

In our second trial (1.2), we showed the strong deterrence effect of piperine in a nutrient-constant condition, while our third trial (1.3) showed that the negative effect of piperine is eliminated under high-nutrient conditions. Despite the deterrent effect of piperine, bats consistently consumed similar amounts of nutrients across all three trials, even when this resulted in higher piperine intake, suggesting that nutrients are the primary foraging driver. Previous studies have demonstrated a similar deterrent effect of piper amides (Whitehead et al., 2016) and other classes of secondary metabolites present in ripe *Piper* pulp (Hernández-Leal & Sánchez, 2021; Maynard et al., 2020). Our results highlight how this deterrence

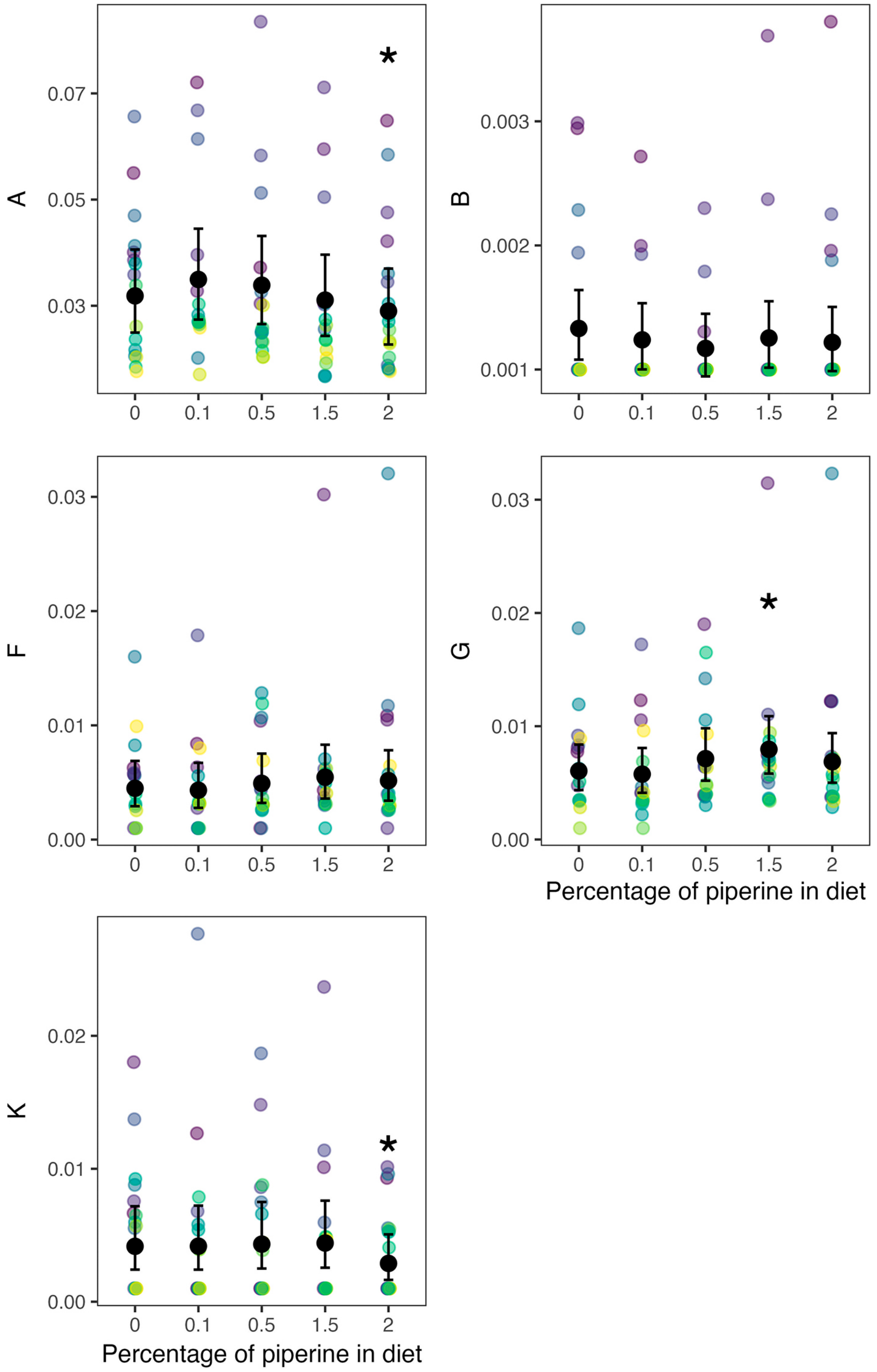

FIGURE 4 Proportions of the most abundant individual sugars (A, B, F, G, and K; details in Appendix S1) excreted in fecal samples from bats fed on different experimental diets with varying concentrations of piperine. Each point is colored by bat ID. Black points and error bars represent the estimated marginal means and 95% CIs predicted by the generalized linear mixed models, back-transformed from the logit scale. Significance levels of each treatment in comparison to the control are denoted by asterisks (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$).

effect is context-dependent and is influenced by the nutritional composition of the food. There are two possible mechanisms for this shift in bat preference. First, nutrients can alter the consumer taste perception of potentially deterrent secondary metabolites, reducing or masking unpleasant tastes such as bitterness and astringency (Behmer et al., 2002; Lerch-Henning & Nicolson, 2015; Simpson & Raubenheimer, 2001). However, it is notable that bats were able to detect and discriminate against piperine in our second trial (1.2), which contained the same nutritional composition as the high-nutrient option in the third trial (1.3), suggesting this is not the only mechanism explaining our results. Second, frugivores can exhibit flexible foraging decisions, even when detecting potentially toxic secondary metabolites, likely based on the relative costs and benefits of consuming different ratios of secondary metabolites and nutrients (Cipollini & Levey, 1997b).

Taken together, our preference experiments provide support for the nutrient-toxin titration hypothesis (Cipollini & Levey, 1997b), which argues that nutritional rewards can offset any negative effects of toxins on mutualists, leading to a positive correlation between nutrients and defenses in fruit pulp. A higher nutrient intake during the digestion of well-defended plant material may provide the necessary energy for detoxification processes, which are often energetically demanding (Iason, 2005). As reported for some mammal species consuming different toxin classes (Iason, 2005), piperine may also impose an additional energetic cost allocated to detoxification pathways. Therefore, when feeding on a well-defended fruit, bats might optimize nutrient intake to fuel detoxification reactions. The consumption of secondary metabolite can lead to amino acid losses, which often stimulate the consumer to forage for additional protein (Au et al., 2013; Wang et al., 2022). A similar mechanism might be operating in our study system, as our absorption experiment indicated that bats consuming higher (2%) piperine concentrations exhibited increased protein excretion. Additionally, past evidence from herbivores and frugivores has shown that high-nutrient concentrations did not fully compensate for the deterrent effects of the secondary metabolites (glycoalkaloids in Cipollini & Levey, 1997a, 1997b and 1,8-cineole in Bedoya-Pérez et al., 2014). Even under high-nutrient conditions, these metabolites continued to deter frugivores to some extent. Our study highlights the complexity of how animals balance the consumption of nutrients and secondary metabolites, suggesting that fruit bat foraging is particularly influenced by nutrient content, with limited generalizability to other plant consumers.

Our results reveal that while the tested piperine concentration did not significantly affect total sugar absorption, 2% piperine increased total protein excretion by 34%, indicating a potential interference with protein absorption. Likewise, piperine significantly modified the excretion of some individual sugars, resulting in both increased and decreased excretion. Piperine could alter total protein and sugar absorption in fruit bats by several mechanisms. First, piperine may affect enzymes involved in nutrient absorption. Evidence from pharmaceutical trials in mice and other mammals suggests that piperine can stimulate and inhibit several different digestive enzymes (Mhaske et al., 2018; Srinivasan, 2007). Second, it has been shown that amides cause a significant reduction in gut retention time in *Carollia* (Baldwin & Whitehead, 2015), and other mammals (Srinivasan, 2007), and shorter retention times can reduce nutrient absorption (Karasov, 2011). Regardless of the mechanism, interfering with protein absorption likely has ecological implications for frugivores, as numerous studies indicate that fleshy fruits are often nutritionally imbalanced, being high in carbohydrates but low in proteins (Lei et al., 2021).

In addition to the consequences for frugivores, interactions between nutrients and secondary metabolites can have complex consequences for plant fitness that may not always be obvious. For example, while deterring effective seed dispersers may initially appear detrimental to the plant's reproductive success, a frugivore's early departure can be beneficial in some cases. For instance, the protein assimilation hypothesis proposes that certain secondary metabolites that interfere with protein assimilation may force frugivores to leave the plant earlier and search for alternative high-protein food sources (Cipollini & Levey, 1997a). During the new foraging events, seeds would be dispersed over longer distances, away from the parent plant, reducing competition for resources. While our results are consistent with the protein assimilation hypothesis, further research is necessary to confirm whether an increased protein excretion is detrimental to the bats and whether it triggers additional foraging bouts. Furthermore, we did not find a significant effect on total sugars excreted. From the plant's perspective, selectively modifying nutrient absorption could benefit the seed dispersal process. By limiting the absorption of proteins while still providing high concentrations of easily assimilable sugars, plants could energetically fuel the locomotion of seed dispersers, promoting future foraging bouts.

Future studies should focus on disentangling the individual effects of nutrients and other attractants to elucidate the primary drivers of bat foraging behavior. A comprehensive exploration of diverse secondary metabolites is crucial, considering the broad range of compounds found in species such as *Piper* spp. Our study focuses on piperine, as a representative secondary metabolite, demonstrating the broad potential for interactions between nutrients and defenses in shaping bat foraging behavior. Examining a wide range of ecologically relevant secondary metabolites will deepen our understanding of these interactions' diverse and complex consequences for frugivory and seed dispersal. Also, future studies should consider incorporating different ratios between nutrients and secondary metabolites that were not tested here (i.e., high-nutrient low-secondary metabolites and low-nutrient low-secondary metabolites) to improve our understanding of animal foraging decisions.

In conclusion, our study shows interactive effects between nutrients and a representative secondary metabolite, piperine, on fruit bat behavior and physiology. Our study expands upon prior research examining the trade-off between nutrient acquisition and secondary metabolite avoidance by testing a new metabolite class (amides) in a key frugivore, highlighting the importance of integrating both nutrients and secondary metabolites in chemical and nutritional ecology.

AUTHOR CONTRIBUTIONS

Mariana Gelambi and Susan R. Whitehead led project design. Mariana Gelambi and Estefania Morales-M. collected the data. Mariana Gelambi conducted chemical and data analyses. Mariana Gelambi led the writing, with input from all other authors.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

All data, metadata, and R scripts used to generate results and figures (Gelambi & Whitehead, 2024) are available from Zenodo: https://doi.org/10.5281/zenodo.10845888.

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REFERENCES

Anyasi, T. A., A. I. O. Jideani, and G. R. A. Mchau. 2013. "Functional Properties and Postharvest Utilization of Commercial and Noncommercial Banana Cultivars." *Comprehensive Reviews in Food Science and Food Safety* 12(5): 509–522. https://doi.org/10.1111/1541-4337.12025.

Au, J., K. J. Marsh, I. R. Wallis, and W. J. Foley. 2013. "Whole-Body Protein Turnover Reveals the Cost of Detoxification of Secondary Metabolites in a Vertebrate Browser." *Journal of Comparative Physiology B* 183(7): 993–1003. https://doi.org/10.1007/s00360-013-0754-3.

Baldwin, J. W., and S. R. Whitehead. 2015. "Fruit Secondary Compounds Mediate the Retention Time of Seeds in the Guts of Neotropical Fruit Bats." *Oecologia* 177(2): 453–466. https://doi.org/10.1007/s00442-014-3096-2.

Bedoya-Pérez, M. A., I. Isler, P. B. Banks, and C. McArthur. 2014. "Roles of the Volatile Terpene, 1,8-Cineole, in Plant–Herbivore Interactions: A Foraging Odor Cue as Well as a Toxin?" *Oecologia* 174(3): 827–837. https://doi.org/10.1007/s00442-013-2801-x.

Behmer, S. T., S. J. Simpson, and D. Raubenheimer. 2002. "Herbivore Foraging in Chemically Heterogeneous Environments: Nutrients and Secondary Metabolites." *Ecology* 83(9): 2489–2501. https://doi.org/10.1890/0012-9658(2002)083[2489:HFICHE]2.0.CO;2.

Bonjoch, N. P., and P. R. Tamayo. 2001. "Protein Content Quantification by Bradford Method." In *Handbook of Plant Ecophysiology Techniques*, edited by M. J. Reigosa Roger, 283–295. Dordrecht: Springer.

Bosque, C., and R. Calchi. 2003. "Food Choice by Blue–Gray Tanagers in Relation to Protein Content." *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* 135(2): 321–27. https://doi.org/10.1016/S1095-6433(03)00080-1.

Bradford, M. M. 1976. "A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding." *Analytical Biochemistry* 72(1–2): 248–254. https://doi.org/10.1016/0003-2697(76)90527-3.

Cazetta, E., H. M. Schaefer, and M. Galetti. 2008. "Does Attraction to Frugivores or Defense against Pathogens Shape Fruit Pulp Composition?" *Oecologia* 155(2): 277–286. https://doi.org/10.1007/s00442-007-0917-6.

Cipollini, M. L., and D. J. Levey. 1997a. "Secondary Metabolites of Fleshy Vertebrate-Dispersed Fruits: Adaptive Hypotheses and Implications for Seed Dispersal." *American Naturalist* 150(3): 346–372. https://doi.org/10.1086/286069.

Cipollini, M. L., and D. J. Levey. 1997b. "Why Are Some Fruits Toxic? Glycoalkaloids in Solanum and Fruit Choice by Vertebrates." *Ecology* 78(3): 782–798. https://doi.org/10.2307/2266058.

de Oliveira Chaves, M. C., A. G. F. de Júnior, and B. V. O. de Santos. 2003. "Amides from *Piper tuberculatum* Fruits." *Fitoterapia* 74(1–2): 181–83. https://doi.org/10.1016/S0367-326X(02)00321-0.

Deans, C., S. T. Behmer, A. E. Tessnow, P. Tamez-Guerra, M. Pusztai-Carey, and G. A. Sword. 2017. "Nutrition Affects Insect Susceptibility to *Bt* Toxins." *Scientific Reports* 7(1): 39705. https://doi.org/10.1038/srep39705.

Deans, C., G. Sword, and S. Behmer. 2016. "Nutrition as a Neglected Factor in Insect Herbivore Susceptibility to *Bt* Toxins." *Current Opinion in Insect Science* 15: 97–103. https://doi.org/10.1016/j.cois.2016.04.005.

Denslow, J., D. Levey, T. Moermond, and B. Wentworth. 1987. "A Synthetic Diet for Fruit-Eating Birds." *The Wilson Bulletin* 99(1): 131–35.

Dyer, L. A., C. D. Dodson, J. O. Stireman, III, M. A. Tobler, A. M. Smilanich, R. M. Fincher, and D. K. Letourneau. 2003.

"Synergistic Effects of Three Piper Amides on Generalist and Specialist Herbivores." *Journal of Chemical Ecology* 29(11): 2499–2514. https://doi.org/10.1023/A:1026310001958.

Dyer, L. A., and A. D. N. Palmer, eds. 2004. *Piper: A Model Genus for Studies of Phytochemistry, Ecology, and Evolution*. New York: Springer US. https://doi.org/10.1007/978-0-387-30599-8.

Fleming, T. H. 1988. *The Short–Tailed Fruit Bat: A Study in Plant–Animal Interactions*. Chicago, IL: University of Chicago Press.

Fleming, T. H. 2004. "Dispersal Ecology of Neotropical *Piper* Shrubs and Treelets." In *Piper: A Model Genus for Studies of Phytochemistry, Ecology, and Evolution*, edited by L. A. Dyer and A. D. N. Palmer, 58–77. Boston, MA: Springer US. https://doi.org/10.1007/978-0-387-30599-8_4.

Foley, W. J., and B. D. Moore. 2005. "Plant Secondary Metabolites and Vertebrate Herbivores – From Physiological Regulation to Ecosystem Function." *Current Opinion in Plant Biology* 8(4): 430–35. https://doi.org/10.1016/j.pbi.2005.05.009.

Gelambi, M., and S. R. Whitehead. 2023. "Multiscale Variability in Nutrients and Secondary Metabolites in a Bat-Dispersed Neotropical Fruit." *Ecology and Evolution* 13(9): e10453. https://doi.org/10.1002/ece3.10453.

Gelambi, M., and S. R. Whitehead. 2024. "Data from: 'Interactions between Nutrients and Fruit Secondary Metabolites Shape Bat Foraging Behavior and Nutrient Absorption' (Version V1)." Zenodo. Dataset. https://doi.org/10.5281/zenodo.10845889.

Guglielmo, C. G., W. H. Karasov, and W. J. Jakubas. 1996. "Nutritional Costs of a Plant Secondary Metabolite Explain Selective Foraging by Ruffed Grouse." *Ecology* 77(4): 1103–15. https://doi.org/10.2307/2265579.

Haq, I.-U., M. Imran, M. Nadeem, T. Tufail, T. A. Gondal, and M. S. Mubarak. 2021. "Piperine: A Review of Its Biological Effects." *Phytotherapy Research* 35(2): 680–700. https://doi.org/10.1002/ptr.6855.

Hernández-Leal, O. F., and F. Sánchez. 2021. "Black Pepper's Essential Oils Affect the Foraging Behavior of Seba's Fruit-Eating Bat." *International Journal of Pest Management* 69(4): 299–305. https://doi.org/10.1080/09670874.2021.1918355.

Iason, G. 2005. "The Role of Plant Secondary Metabolites in Mammalian Herbivory: Ecological Perspectives." *Proceedings of the Nutrition Society* 64(1): 123–131. https://doi.org/10.1079/pns2004415.

Izhaki, I., E. Tsahar, I. Paluy, and J. Friedman. 2002. "Within Population Variation and Interrelationships between Morphology, Nutritional Content, and Secondary Compounds of *Rhamnus alaternus* Fruits." *New Phytologist* 156(2): 217–223. https://doi.org/10.1046/j.1469-8137.2002.00515.x.

Karasov, W. H. 2011. "Digestive Physiology: A View from Molecules to Ecosystem." *American Journal of Physiology—Regulatory, Integrative and Comparative Physiology* 301(2): R276–R284. https://doi.org/10.1152/ajpregu.00600.2010.

Kessler, A., and A. Kalske. 2018. "Plant Secondary Metabolite Diversity and Species Interactions." *Annual Review of Ecology, Evolution, and Systematics* 49: 115–138. https://doi.org/10.1146/annurev-ecolsys-110617-062406.

Lei, B., J. Cui, C. Newman, C. D. Buesching, Z. Xie, D. W. Macdonald, and Y. Zhou. 2021. "Seed Dispersers Shape the Pulp Nutrients of Fleshy-Fruited Plants." *Proceedings of the Royal Society B: Biological Sciences* 288(1953): 20210817. https://doi.org/10.1098/rspb.2021.0817.

Leitão da-Cunha, E. V., and M. C. de Oliveira Chaves. 2001. "Two Amides from *Piper tuberculatum* Fruits." *Fitoterapia* 72(2): 197–99. https://doi.org/10.1016/S0367-326X(00)00271-9.

Lenth, R., H. Singmann, J. Love, P. Buerkner, and M. Herve. 2021. "Emmeans: Estimated Marginal Means, Aka Least-Squares Means." R Package Version 1 (2018).

Lerch-Henning, S., and S. W. Nicolson. 2015. "Effects of Nicotine on the Digestive Performance of Nectar-Feeding Birds Reflect Their Relative Tolerance to This Alkaloid." *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* 190: 47–53. https://doi.org/10.1016/j.cbpa.2015.08.015.

Lord, N., C. Shang, L. Rosso, and B. Zhang. 2021. "Development of Near-Infrared Reflectance Spectroscopy Calibration for Sugar Content in Ground Soybean Seed Using Perten DA7250 Analyzer." *Crop Science* 61(2): 966–975. https://doi.org/10.1002/csc2.20358.

Magnusson, A., H. Skaug, A. Nielsen, C. Berg, K. Kristensen, M. Maechler, K. van Bentham, B. Bolker, M. Brooks, and M. M. Brooks. 2017. "Package 'glmmtmb'." R Package Version 0.2.0.

Maynard, L. D., H. L. Slinn, A. E. Glassmire, B. Matarrita-Carranza, C. D. Dodson, T. T. Nguyen, M. J. Burroughs, L. A. Dyer, C. S. Jeffrey, and S. R. Whitehead. 2020. "Secondary Metabolites in a Neotropical Shrub: Spatiotemporal Allocation and Role in Fruit Defense and Dispersal." *Ecology* 101(12): e03192. https://doi.org/10.1002/ecy.3192.

Mhaske, D. B., S. Sreedharan, and K. R. Mahadik. 2018. "Role of Piperine as an Effective Bioenhancer in Drug Absorption." *Pharmaceutica Analytica Acta* 9(7): 1–4. https://doi.org/10.4172/2153-2435.1000591.

Mithöfer, A., and W. Boland. 2012. "Plant Defense against Herbivores: Chemical Aspects." *Annual Review of Plant Biology* 63(1): 431–450. https://doi.org/10.1146/annurev-arplant-042110-103854.

Mueller-Harvey, I. 2006. "Unravelling the Conundrum of Tannins in Animal Nutrition and Health." *Journal of the Science of Food and Agriculture* 86(13): 2010–37. https://doi.org/10.1002/jsfa.2577.

Nelson, A. S., and S. R. Whitehead. 2021. "Fruit Secondary Metabolites Shape Seed Dispersal Effectiveness." *Trends in Ecology & Evolution* 36(12): 1113–23. https://doi.org/10.1016/j.tree.2021.08.005.

Nelson, S. L., D. V. Masters, S. R. Humphrey, and T. H. Kunz. 2005. "Fruit Choice and Calcium Block Use by Tongan Fruit Bats in American Samoa." *Journal of Mammalogy* 86(6): 1205–9. https://doi.org/10.1644/05-MAMM-A-004R1.1.

Nevo, O., and M. Ayasse. 2020. "Fruit Scent: Biochemistry, Ecological Function, and Evolution." In *Co-Evolution of Secondary Metabolites*, edited by J.-M. Mérillon and K. G. Ramawat, 403–425. Cham: Springer International Publishing. https://doi.org/10.1007/978-3-319-96397-6_33.

Nevo, O., K. Valenta, D. Razafimandimby, A. D. Melin, M. Ayasse, and C. A. Chapman. 2018. "Frugivores and the Evolution of Fruit Colour." *Biology Letters* 14(9): 20180377. https://doi.org/10.1098/rsbl.2018.0377.

Ottensmann, M., M. A. Stoffel, H. J. Nichols, and J. I. Hoffman. 2018. "GCalignR: An R Package for Aligning Gas-Chromatography

Data for Ecological and Evolutionary Studies." *PLoS One* 13(6): e0198311. https://doi.org/10.1371/journal.pone.0198311.

Patra, A. K., S. Amasheh, and J. R. Aschenbach. 2019. "Modulation of Gastrointestinal Barrier and Nutrient Transport Function in Farm Animals by Natural Plant Bioactive Compounds – A Comprehensive Review." *Critical Reviews in Food Science and Nutrition* 59(20): 3237–66. https://doi.org/10.1080/10408398.2018.1486284.

Perkovich, C., and D. Ward. 2020. "Protein:Carbohydrate Ratios in the Diet of Gypsy Moth *Lymantria dispar* Affect Its Ability to Tolerate Tannins." *Journal of Chemical Ecology* 46(3): 299–307. https://doi.org/10.1007/s10886-020-01161-x.

Platel, K., and K. Srinivasan. 2001. "Studies on the Influence of Dietary Spices on Food Transit Time in Experimental Rats." *Nutrition Research* 21(9): 1309–14. https://doi.org/10.1016/S0271-5317(01)00331-1.

R Core Team. 2021. *R: A Language and Environment for Statistical Computing*. Vienna: R Foundation for Statistical Computing. https://www.R-project.org/.

Rodríguez, A., B. Alquézar, and L. Peña. 2013. "Fruit Aromas in Mature Fleshy Fruits as Signals of Readiness for Predation and Seed Dispersal." *New Phytologist* 197(1): 36–48. https://doi.org/10.1111/j.1469-8137.2012.04382.x.

Rojas, T. N., O. A. Bruzzone, I. C. Zampini, M. I. Isla, and P. G. Blendinger. 2021. "A Combination of Rules Govern Fruit Trait Preference by Frugivorous Bat and Bird Species: Nutrients, Defence and Size." *Animal Behaviour* 176: 111–123. https://doi.org/10.1016/j.anbehav.2021.04.001.

Salehi, B., Z. A. Zakaria, R. Gyawali, S. A. Ibrahim, J. Rajkovic, Z. K. Shinwari, T. Khan, et al. 2019. "Piper Species: A Comprehensive Review on Their Phytochemistry, Biological Activities and Applications." *Molecules* 24(7): 1364. https://doi.org/10.3390/molecules24071364.

Schaefer, H. M., V. Schmidt, and H. Winkler. 2003. "Testing the Defence Trade-Off Hypothesis: How Contents of Nutrients and Secondary Compounds Affect Fruit Removal." *Oikos* 102(2): 318–328. https://doi.org/10.1034/j.1600-0706.2003.11796.x.

Schoonhoven, L. M., J. J. A. Van Loon, and M. Dicke. 2005. *Insect–Plant Biology*. Oxford: Oxford University Press.

Simpson, S. J., and D. Raubenheimer. 2001. "The Geometric Analysis of Nutrient–Allelochemical Interactions: A Case Study Using Locusts." *Ecology* 82(2): 422–439. https://doi.org/10.1890/0012-9658(2001)082[0422:TGAONA]2.0.CO;2.

Smithson, M., and J. Verkuilen. 2006. "A Better Lemon Squeezer? Maximum-Likelihood Regression with Beta-Distributed Dependent Variables." *Psychological Methods* 11(1): 54–71. https://doi.org/10.1037/1082-989X.11.1.54.

Srinivasan, K. 2007. "Black Pepper and Its Pungent Principle-Piperine: A Review of Diverse Physiological Effects." *Critical Reviews in Food Science and Nutrition* 47(8): 735–748. https://doi.org/10.1080/10408390601062054.

Suresh, D., and K. Srinivasan. 2010. "Tissue Distribution & Elimination of Capsaicin, Piperine & Curcumin Following Oral Intake in Rats." *Indian Journal of Medical Research* 131(5): 682–691.

Tewksbury, J. J., and G. P. Nabhan. 2001. "Directed Deterrence by Capsaicin in Chillies." *Nature* 412(6845): 403–4. https://doi.org/10.1038/35086653.

Uckele, K. A., J. P. Jahner, E. J. Tepe, L. A. Richards, L. A. Dyer, K. M. Ochsenrider, C. S. Philbin, et al. 2021. "Phytochemistry Reflects Different Evolutionary History in Traditional Classes Versus Specialized Structural Motifs." *Scientific Reports* 11(1): 17247. https://doi.org/10.1038/s41598-021-96431-3.

Valenta, K., O. Nevo, C. Martel, and C. A. Chapman. 2017. "Plant Attractants: Integrating Insights from Pollination and Seed Dispersal Ecology." *Evolutionary Ecology* 31(2): 249–267. https://doi.org/10.1007/s10682-016-9870-3.

Wang, P., D. G. Vassão, B. Raguschke, M. J. Furlong, and M. P. Zalucki. 2022. "Balancing Nutrients in a Toxic Environment: The Challenge of Eating." *Insect Science* 29: 289–303. https://doi.org/10.1111/1744-7917.12923.

Whitehead, S. R., and M. D. Bowers. 2014. "Chemical Ecology of Fruit Defence: Synergistic and Antagonistic Interactions among Amides from Piper." *Functional Ecology* 28(5): 1094–1106. https://doi.org/10.1111/1365-2435.12250.

Whitehead, S. R., C. S. Jeffrey, M. D. Leonard, C. D. Dodson, L. A. Dyer, and M. D. Bowers. 2013. "Patterns of Secondary Metabolite Allocation to Fruits and Seeds in *Piper reticulatum*." *Journal of Chemical Ecology* 39(11–12): 1373–84. https://doi.org/10.1007/s10886-013-0362-4.

Whitehead, S. R., M. F. Obando Quesada, and M. D. Bowers. 2016. "Chemical Tradeoffs in Seed Dispersal: Defensive Metabolites in Fruits Deter Consumption by Mutualist Bats." *Oikos* 125(7): 927–937. https://doi.org/10.1111/oik.02210.

Whitehead, S. R., G. F. Schneider, R. Dybzinski, A. S. Nelson, M. Gelambi, E. Jos, and N. G. Beckman. 2021. "Fruits, Frugivores, and the Evolution of Phytochemical Diversity." *Oikos* 2022: oik.08332. https://doi.org/10.1111/oik.08332.

York, H. A., and S. A. Billings. 2009. "Stable-Isotope Analysis of Diets of Short-Tailed Fruit Bats (Chiroptera: Phyllostomidae: Carollia)." *Journal of Mammalogy* 90(6): 1469–77. https://doi.org/10.1644/08-mamm-a-382r.1.

SUPPORTING INFORMATION

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

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