

RESEARCH ARTICLE

Poison frog chemical defences are influenced by environmental availability and dietary selectivity for ants

Nora A. Martin¹ | Camilo Rodríguez¹  | Aurora Alvarez-Buylla¹ | Katherine Fiocca¹ |
Colin R. Morrison²  | Adolfo Chamba-Carrillo³ | Ana B. García-Ruilova⁴ |
Janet Rentería⁵ | Elicio E. Tapia⁶ | Luis A. Coloma⁶ | David A. Donoso^{7,8}  |
Lauren A. O'Connell¹

¹Department of Biology, Stanford University, Stanford, California, USA; ²Department of Integrative Biology, The University of Texas at Austin, Austin, Texas, USA; ³Programa de Posgrado en Biodiversidad y Cambio Climático, Universidad Indoamérica, Quito, Ecuador; ⁴División de Entomología, Instituto Nacional de Biodiversidad, Quito, Ecuador; ⁵School of Biological Sciences, University of Bristol, Bristol, UK; ⁶Centro Jambatu de Investigación y Conservación de Anfibios, Fundación Jambatu, Quito, Ecuador; ⁷Departamento de Biología, Escuela Politécnica Nacional, Quito, Ecuador and ⁸Grupo de Investigación en Ecología y Evolución en los Trópicos -EETrop-, Universidad de las Américas, Quito, Ecuador

Correspondence

David A. Donoso

Email: david.donosov@gmail.com

Lauren A. O'Connell

Email: loconnel@stanford.edu

Funding information

National Science Foundation, Grant/Award Number: DGE-1656518, IOS-1557684 and 2337580; New York Stem Cell Foundation; Howard Hughes Medical Institute, Grant/Award Number: GT13330; Pew Charitable Trusts

Handling Editor: Alison Davis Rabosky

Abstract

1. The ability to use small molecule alkaloids as defensive chemicals, often acquired via trophic interactions, has evolved in many organisms. Animals with diet-derived defences must balance food choices to maintain their defence reservoirs along with other physiological needs. Poison frogs accumulate skin alkaloids from their arthropod diet, but whether they show selectivity for specific prey remains unexplored.
2. We investigated the role of leaf litter prey availability and dietary selectivity in shaping poison frog chemical defences along a geographic gradient. Specifically, we examined skin alkaloid composition, stomach contents and leaf litter ants in aposematic diablito frogs (*Oophaga sylvatica*) at five sites in north-western Ecuador and in sympatric, cryptic Chimbo rocket frogs (*Hyloxalus infraguttatus*) at one site.
3. Our results show that differential availability of leaf litter ants influenced alkaloid profiles across diablito populations, and low levels of alkaloids were observed in the sympatric, 'undefended' Chimbo rocket frog. Ants were the primary dietary component of the defended species, while the 'undefended' species ate other prey categories including beetles and larvae in addition to ants.
4. A prey selection analysis suggested that defended and 'undefended' frogs both feed on a high proportion of specific small ant genera that naturally contain alkaloids, suggesting that selectivity for toxic prey is not restricted to classically aposematic and highly toxic species.

Nora A. Martina and Camilo Rodríguez joint first authorship.

© 2025 The Author(s). Journal of Animal Ecology © 2025 British Ecological Society.

5. These findings suggest that poison frogs' use of feeding resources relative to availability may be an understudied and important selection factor in the evolution of acquired defences.

KEYWORDS

alkaloids, Dendrobatidae, diet, prey availability

1 | INTRODUCTION

Many organisms use chemical defences to protect themselves from predators or pathogens (Mebs, 2002). These defences often involve small molecule alkaloids synthesized by plants or microbes, and some taxa can acquire them through dietary sequestration (Agrawal et al., 2012; Roberts & Wink, 1998; Santos et al., 2016). Phytophagous insects represent the most well-studied taxa, including some species that specialize in particular plant species and accumulate specific secondary metabolites for chemical communication or defence (Beran & Petschenka, 2022; Roberts & Wink, 1998; Walsh & Tang, 2017). Although our understanding of chemical defence in vertebrates is more sparse than invertebrates, poison frogs are a well-known example of chemical defences acquired through an arthropod-based diet (Savitzky et al., 2012). A key component of poison frog diets includes alkaloid-rich arthropods, and these frogs have evolved the physiological mechanisms to tolerate and integrate the toxin into their tissues to deter predators (Alvarez-Buylla et al., 2023). Yet, it remains unclear whether the ecological principles underlying arthropod diet specialization on toxic plants also apply to vertebrates that sequester their chemical defences from arthropods.

The chemical repertoire of alkaloid-defended species varies within and between populations. For example, the composition and concentration of piperidine alkaloids in the Norwegian spruce (*Picea abies*) differ by location (Virjamo & Julkunen-Tiitto, 2016) and in fire ants (*Solenopsis* spp.) vary within species (Deslippe & Guo, 2000). In species with acquired chemical defences, variation in alkaloid profiles is generally attributed to spatio-temporal shifts in the availability of alkaloid-containing food, often resulting from environmental variation in temperature, rainfall and other climatic factors. For instance, in Argentine *Melanophryniscus* toads (Daly et al., 2007), Malagasy *Mantella laevis* frogs (Moskowitz et al., 2018) and various species of Neotropical poison frogs (Family Dendrobatidae; Saporito et al., 2006; Saporito, Donnelly, Norton, et al., 2007; Prates et al., 2019; Moskowitz et al., 2020), differences in the composition of skin alkaloids across localities and seasons correspond with differences in stomach contents and leaf litter arthropod communities. Dietary selectivity may also influence the alkaloid profile of chemically defended species by favouring the consumption of food items that contain specific defensive alkaloids. Yet, organisms must make dietary decisions based on handling time and nutritional value, in addition to food availability and maintenance of chemical defences. For instance, *Chiasmocleis leucosticta* frogs preferred smaller ants over larger, more aggressive genera (Meurer et al., 2021), while lab-reared

non-toxic *Dendrobates tinctorius* preferred protein-rich larvae over other prey types including ants (Moskowitz et al., 2022). As these studies suggest, variation in the environment, the availability of alkaloid-containing prey, prey phenotype and foraging behaviour are likely important factors in diet-acquired defence evolution. However, there is limited understanding of how these factors interact to influence species' food choices and their ability to sequester chemical defences from specific dietary sources.

Neotropical poison frogs acquire alkaloids from alkaloid-containing arthropods rather than synthesizing them de novo (Daly et al., 1994). Chemical defences in dendrobatids have evolved independently at least four times in parallel with dietary specialization on ants and mites (Darst et al., 2005; Santos et al., 2003). However, recent evidence suggests that diet specialization alone does not explain the defended phenotype, as 'undefended' species often consume alkaloid-containing arthropods and have low but detectable alkaloid levels (Sanches et al., 2023; Tarvin et al., 2024). Yet, it is unclear if selectivity for specific alkaloid-containing prey plays a role in the ability of poison frogs to dietarily acquire their chemical defences, as most studies focus on diet without assessing environmental availability of arthropod prey (McElroy & Donoso, 2019). This is especially important as temporal and geographic variations in abiotic factors such as temperature, altitude and precipitation affect the composition and richness of leaf litter arthropod communities (Basset et al., 2023; Brühl et al., 1999; Gibb et al., 2015; Hoelen et al., 2022; Silva & Brandão, 2014; Tiede et al., 2017). Thus, we currently lack a framework for understanding the evolution of diet-acquired defences in poison frogs, as the role of prey availability in diet specialization has not been studied in depth.

Here, we tested whether dendrobatid poison frogs that acquire chemical defences from their diet exhibit dietary prey selectivity. To test this hypothesis, we sampled stomach contents, skin alkaloids and surrounding leaf litter ant communities from five populations of the aposematic, chemically defended *Oophaga sylvatica* and one sympatric population of the cryptic, chemically 'undefended' *Hyloxalus infraguttatus*. We compared skin alkaloid profiles among poison frog populations along a geographical gradient, predicting intraspecific variation linked to environmental factors, such as altitude, temperature and precipitation, and higher alkaloid composition in the sympatric defended species. We compared stomach contents across poison frog populations and predicted within and between species variation, where chemically defended frogs would eat more ants and mites than 'undefended' frog species. We sorted ants by genus from stomachs and

leaf litter, characterized their morphology and predicted that sympatric defended and 'undefended' frogs will show distinct dietary selectivity for ant genera despite access to the same ant communities. We further predicted that differences in ant communities across localities correspond with differences in chemical defence between poison frogs. Together, our between- and within-species comparisons of poison frog stomach contents and prey availability aim to disentangle the relationship between dendrobatid diet and alkaloid acquisition, which has broader implications for our general understanding of trophic interactions and the evolution of chemical defences across taxa.

2 | METHODS

2.1 | Study system and sample collection

Diablito frogs (*Oophaga sylvatica*) were collected in May 2019, during daylight in secondary forests near the towns of Ceiba ($N=10$; 207 m.a.s.l.), Cristóbal Colón ($N=11$; 221 m.a.s.l.), Puerto Quito ($N=11$; 302 m.a.s.l.), Santo Domingo de los Tsáchilas ($N=10$; 632 m.a.s.l.) and La Maná ($N=20$; 480 m.a.s.l.), in north-western Ecuador (Figure 1a). As *O. sylvatica* suffers from illegal poaching for the pet trade, coordinates for collection can be obtained from the corresponding authors. Chimbo rocket frogs (*Hyloxalus infraguttatus*) were collected during daylight from La Maná ($N=9$). While behavioural observations were not performed, frogs were collected during active foraging hours (06:00–19:00; AmphibiaWeb Database Search, 2025; Funkhouser, 1956). Frogs

were anaesthetized 3–6 h after collection with 20% benzocaine gel applied to the ventral skin and euthanized. For each individual, the dorsal skin was dissected and stored in methanol in glass vials. The stomach contents were stored in 100% ethanol in 1.5 mL plastic tubes. Remaining frog tissues were either preserved in 100% ethanol or RNAlater (Thermo Scientific, Waltham, MA, USA) or deposited at an amphibian collection in Ecuador (see ethical permits for details).

2.2 | Alkaloid extraction and quantification

Skins were removed from methanol with forceps and weighed. From the methanol in which the skin was stored, 1 mL was syringe filtered through a 0.45- μ m PTFE filter (Thermo Scientific, 44504-NP) into a new glass vial (Wheaton, PTFE caps, 60940A-2) supplemented with 25 μ g (–)-nicotine (Sigma Aldrich, N3876-100ML). All tubes were then capped, vortexed and stored for 24 h at -80°C to precipitate lipids and proteins. After precipitating for 24 h, the supernatant was filtered through a 0.45- μ m PTFE syringe filter into a new glass vial. A 100- μ L aliquot was added to a gas chromatography/mass spectrometry (GC/MS) autosampler vial, and the remaining solution was stored at -80°C .

Alkaloid detection was performed using gas chromatography–mass spectrometry (GC–MS) following the protocols described elsewhere (Saporito et al., 2010), and using a Shimadzu GCMS-QP2020 instrument with a Shimadzu 30 m $\times$ 0.25 mm ID SH-Rxi-5Sil MS column. In brief, the separation of alkaloids was achieved with helium as the carrier gas (flow rate: 1 mL/min) using

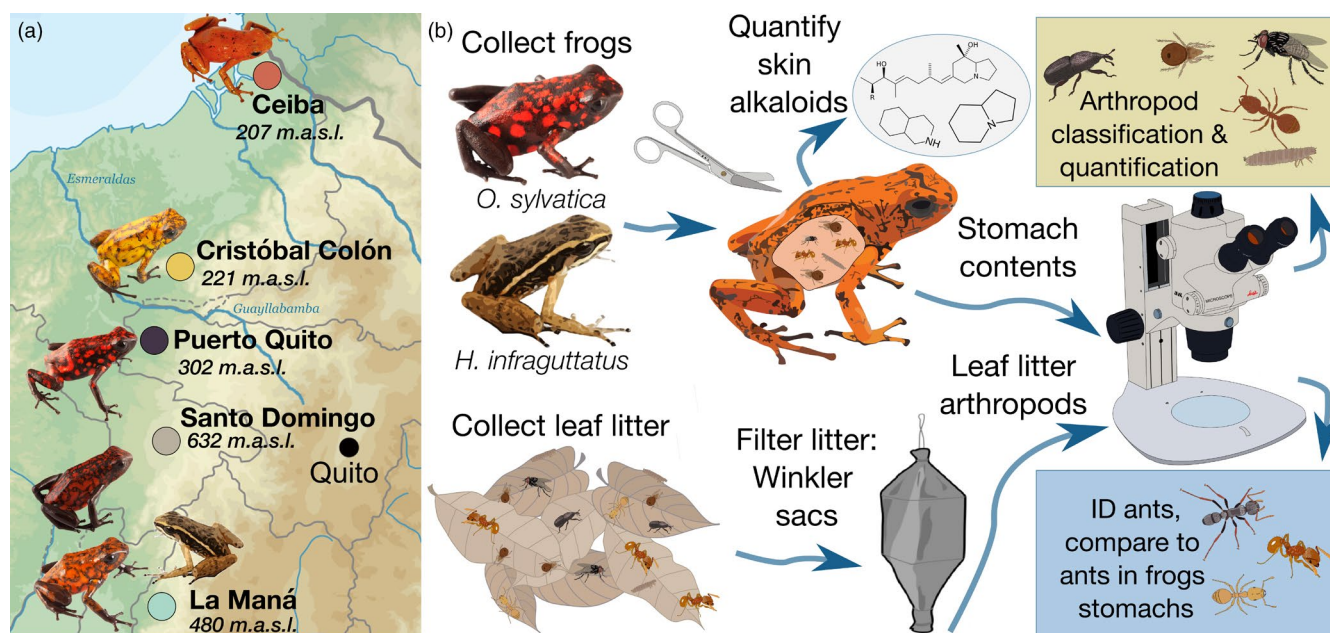


FIGURE 1 Map of frog populations and experimental workflow. (a) Collection sites are shown along a geographical gradient on a topographic map of western Ecuador. Note that both *Hyloxalus infraguttatus* and *Oophaga sylvatica* were collected from La Maná. m.a.s.l. = metres above sea level. (b) A flowchart depicts the main steps of data collection for frog stomach content and leaf litter samples, in order to compare ant genera abundances between these groups.

a temperature programme increasing from 100 to 280°C at a rate of 10°C/min. This was followed by a 2-min hold and an additional ramp to 320°C at a rate of 10°C/min for column protection reasons, and no alkaloids appeared during this part of the method. Compounds were analysed with electron impact-mass spectrometry (EI-MS). The GC-MS data files were exported as .CDF files, and the Global Natural Products Social Molecular Networking (GNPS) software was used to perform the deconvolution and library searching against the AMDIS (NIST) database to identify compounds (Aksenov et al., 2021; Wang et al., 2016). For deconvolution (identification of peaks and abundance estimates), the default parameters were used. Through the deconvolution process, molecular features were reported as rows/observations, while m/z intensities were reported as columns/variables. Automatic library search was obtained from reference libraries of natural products (NIST, Wiley, University of CORISICA, GNPS), and our resulting dataset was filtered to keep only the nicotine standard and alkaloids previously found in poison frogs or compounds with the same base ring structure and R group positions as those classes defined in the Daly poison frog alkaloid database (Daly et al., 2005). Once the feature table from the GNPS deconvolution was filtered to include only poison frog alkaloids and nicotine, the abundance values (ion counts) were normalized by dividing by the nicotine standard and skin weight. The resulting filtered and normalized feature table was used for all further analyses and visualizations.

2.3 | Frog stomach contents identification

Whole stomachs were stored in 100% ethanol at -20°C until processing. Stomach contents were sorted and photographed. Prey items in the photographs were identified to the lowest possible taxonomic rank and then grouped into broad diet categories: ants, mites, larvae, beetles and 'other' (i.e. any non-larval arthropods not described by the other four groups). Note that most larvae belong to Dipteran or Coleopteran taxa, but are considered in our distinct 'larvae' category given their differences in appearance from adults. The vast majority of identifiable prey remained whole, with the exception of ants, whose heads frequently detached from their bodies upon ingestion. To prevent overcounting, only whole ant specimens, partial ant specimens with heads or individual ant heads were counted. Ant specimens were identified to genus using a reference collection of Ecuadorian ants (Donoso, 2017; Donoso & Ramón, 2009; Salazar et al., 2015).

2.4 | Leaf litter communities and ant morphology

Leaf litter samples were extracted and collected using 18–20 Winkler sacs from all localities except for *La Ceiba* due to time and resource limitations. Samples were collected in May–June 2019 within one square metre of where a frog had been previously collected that day. Leaf litter arthropods were extracted from 1m² and hung within

Winkler sacs for 24h, during which the arthropods were collected into 70% ethanol. Only ants were identified, as described above. Collection of ant specimens was done under permits issued by the Ministerio de Ambiente de Ecuador to Museo de Historia Natural Gustavo Orcés at Escuela Politécnica Nacional (MAE-DNB-CM-2017-0068). Ant morphology was characterized by 17 traits related to size, texture, spine count and coloration for leaf litter ant genera that were also found in frog stomach contents. Morphological traits were measured at the species level using data from the Global Ants Database, which provides standardized trait information across ant species (Parr et al., 2017).

2.5 | Data analysis

Statistical analyses and figures were generated using R (version 4.3.1) in R Studio (version 2025.05).

2.5.1 | Alkaloid comparisons

We used a Kruskal–Wallis test to examine overall differences between *O. sylvatica* and *Hyloxalus infraguttatus* populations in summed toxicity across the 79 alkaloids in the 13 structural families. A pairwise Wilcoxon test was used to determine *O. sylvatica* population differences between the 13 structural families in both species, with p-values adjusted for multiple testing using the false discovery rate control (FDR).

2.5.2 | Frogs' diet comparisons

We visualized interactions between arthropod prey taxa and poison frog species in a bipartite network based on the average abundance of arthropod prey for every frog population. We used the species specificity index (ssi), as implemented in the specieslevel() function of the 'bipartite' package, to quantify the variability in dietary interactions between arthropod prey taxa and poison frog species in a bipartite network. This index reflects the degree to which each frog species interacts unevenly across prey taxa, with values ranging from 0 (indicating low variability and generalist behaviour) to 1 (indicating high variability and specialist behaviour). Additionally, we used generalized linear mixed models (GLMM) to test for compositional differences of frog diet categories using the function glmmTMB() within the package 'glmmTMB' (Brooks et al., 2017). We used a negative binomial distribution appropriate for count data with overdispersion. We tested for diet differences by including species/population, prey type and their interaction as main effects. Frog individual tags were included as a random variable to account for repeated sampling of prey categories within individuals. We computed estimated marginal means to test for pairwise comparisons between populations using the 'emmeans' package (Lenth, 2023).

2.5.3 | Ant- versus mite-derived alkaloids

To infer potential dietary origins of frogs' skin alkaloids, we cross-referenced the structural classes of alkaloids with the determined arthropod source reported by Santos et al. (2016), which assigns compounds as derived from ants, mites or both. We tested whether the proportion of ant-based alkaloids is greater than the proportion of mite-based alkaloids across frog populations using an ANOVA followed by a Tukey post hoc test for multiple comparisons. We visualized the proportion of ant- and mite-derived alkaloids using a chord diagram. Additionally, we tested whether total alkaloid abundance, as well as abundance within structural families, correlated with the number of ants and mites consumed across populations.

2.5.4 | Skin alkaloids versus leaf litter ant communities along a geographical gradient

We analysed compositional differences in skin alkaloid profiles of *O. sylvatica* populations and *H. infraguttatus*, and their surrounding leaf litter ant communities in two separate non-metric multidimensional scaling (NMDS), using the function `metaMDS()` within the package 'vegan' (Oksanen et al., 2022). Statistical differences between and within populations were assessed using a permutational multivariate analysis of variance (PERMANOVA) on Bray–Curtis dissimilarities. P-values for pairwise comparisons were adjusted using the function `pairwise.adonis()`. Additionally, we used the `envfit()` function to understand the influence of altitude, ambient temperature and precipitation on both alkaloid composition and leaf litter ant communities. These environmental variables were selected to capture spatial and temporal variation in ecological conditions that could influence ant availability and frogs' foraging behaviour. Ambient temperature and precipitation data were extracted from WorldClim 2.1, at a spatial resolution of 30 arc-seconds (~1 km²) for each study site (Fick & Hijmans, 2017). Similarly, we used `envfit()` to explore the contribution of specific alkaloid classes and ant genera to the ordination spaces. We used Procrustes analysis to test similarities between the NMDS ordinations of skin alkaloids and ant community composition across sites. The Procrustes correlation was calculated using the `procrustes()` function from the 'vegan' package, which aligns the two ordinations by scaling, rotating and translating one configuration to best match the other. Statistical significance of the correlation was evaluated with 999 permutations using the `protest()` function.

2.5.5 | Stomach versus leaf litter ant communities and frogs' selectivity for ant genera

To test for within and between species differences in ant abundance between leaf litter and stomach contents, we employed a negative binomial generalized linear model using the `glm.nb()` function from

the 'MASS' package (Venables & Ripley, 2002). The model used total ant abundance per sample as the response variable, with group (stomach vs. leaf) and population as predictors. Pairwise comparisons were conducted using estimated marginal means. To examine if frogs show selectivity for consuming specific ant genera, we calculated a linear selectivity index by subtracting the relative abundance of ants found on the leaf litter from those found in frog stomach contents (Strauss, 1979). The interpretation of these selectivity values was guided by the methodology in McElroy and Donoso (2019), which involves generating a null distribution of selectivity values via simulation for each ant species. By comparing the observed selectivity values to this null distribution, we classified the ants as 'selected' if the values were above, 'neutral' if they were within, and 'avoided' if they were below the null distribution, thereby delivering a statistically robust assessment of the frogs' selective foraging behaviours.

2.5.6 | Ant morphology versus frog selectivity

To determine whether the morphological and life-history traits of ant species in the leaf litter influence frog selectivity, we performed a principal component analysis (PCA). The PCA aimed at reducing the dimensionality of 17 morphological and life-history traits across 17 ant genera found both in the leaf litter and in *O. sylvatica* stomachs. To build the PCA, we used the function `dudi.pca()` from the 'ade4' package (Dray & Dufour, 2007). Scores of components with eigenvalues higher than 1 were selected and used as response variables in a pairwise Wilcoxon test to determine differences between selectivity categories for ants in all diablito populations. p-values were adjusted for multiple testing using the FDR control.

3 | RESULTS

3.1 | Alkaloids differ between species and across diablito frog populations

Skin extracts consisted of 79 alkaloids. The summed amount of alkaloids varied across species and populations (Kruskal–Wallis; $\chi^2(5)=41.542$, $p<0.001$; Figure 2A), with *O. sylvatica* having more alkaloids than *H. infraguttatus* (*H. infraguttatus* vs. all other *O. sylvatica* populations, $p<0.001$; Table S1). Within *O. sylvatica*, the Ceiba population had fewer toxins than all others ($p<0.001$; Table S1), while frogs from Santo Domingo had on average the highest alkaloid load (Table S1).

We next visualized overall alkaloid compositional differences across *O. sylvatica* populations and *H. infraguttatus* using an NMDS (Figure 2B). The NMDS suggested a two-dimensional solution (stress=0.156) and showed distinct clusters of alkaloid composition. The abundance of all 79 alkaloids varied significantly across groups (PERMANOVA, $F_{(4)}=10.178$, $p<0.001$). A post hoc pairwise comparison indicated significant differences between all possible

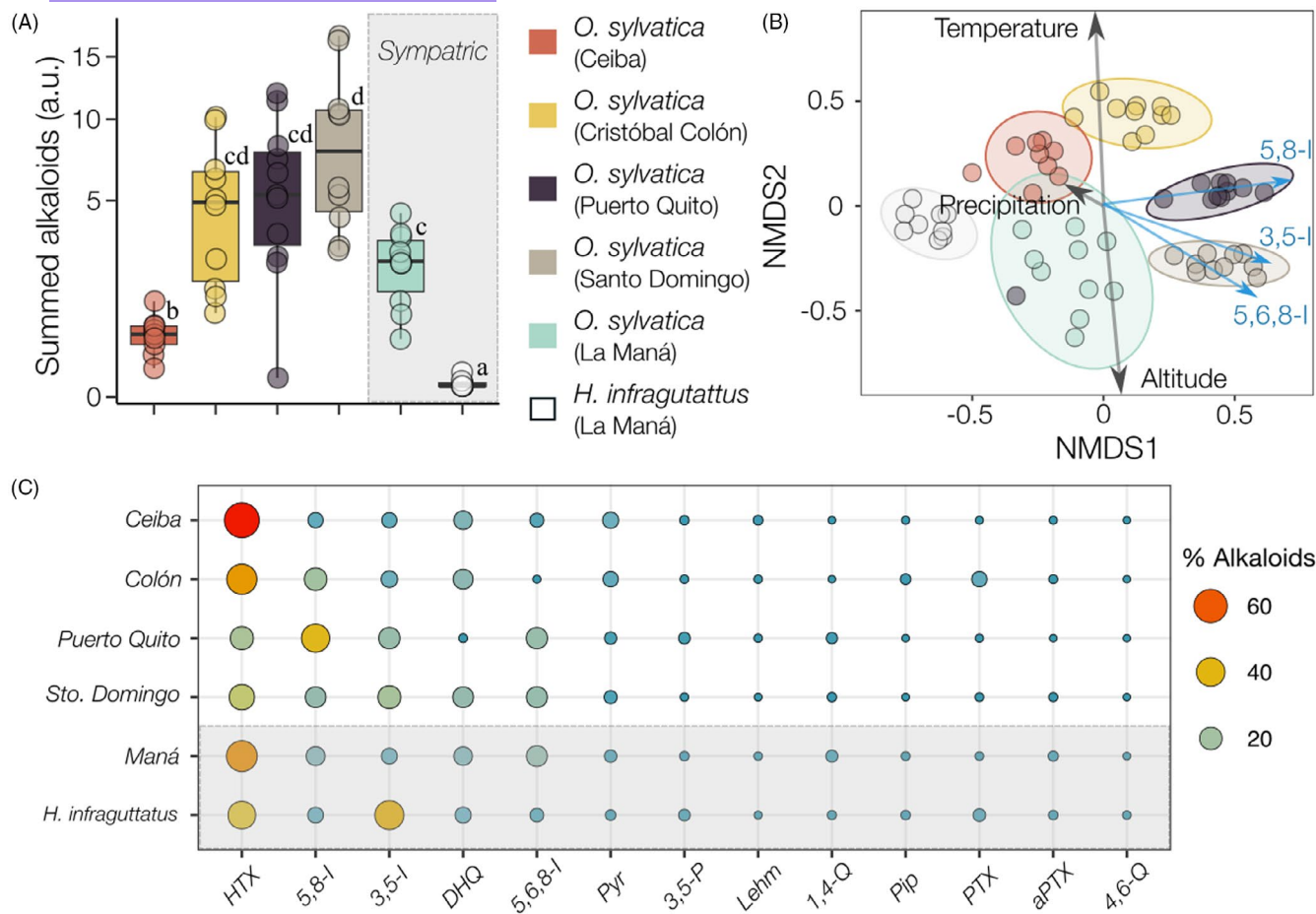


FIGURE 2 Alkaloid profiles differ between species and populations. (A) The summed relative abundances of 79 alkaloids are visualized as box plots, where each dot represents a frog. The y-axis is square-root transformed for visual clarity and summed alkaloids are expressed in arbitrary units (a.u.). Populations are represented with different colours. *Hyloxalus infraguttatus*, collected near the town of La Maná, is shown on the far right. Groups not connected by the same letter are significantly different (Table S1). (B) Non-metric multidimensional scaling (NMDS) biplot based on Bray-Curtis dissimilarities show alkaloid profile differences between populations based on the relative abundances of 79 unique alkaloids (Stress=0.16). Each dot represents a single frog's alkaloid profile. Ellipses represent 95% confidence intervals. Environmental factors (black arrows) and alkaloids (blue arrows) in correlation with the ordination are shown. (C) Bubble heatmap shows percent of all summed alkaloids grouped by alkaloid structural class and population. Histrionicotoxins (HTX); 5,8-disubstituted indolizidines (5,8-I); 3,5-disubstituted indolizidine (3,5-I); Decahydroquinoline (DHQ); 5,6,8-trisubstituted indolizidines (5,6,8-I); Pyrrolidine (Pyr); 3,5-disubstituted pyrrolizidine (3,5-P); Lehmizidine (Lehm); 1,4-disubstituted quinolizidine (1,4-Q); Piperidine (Pip); Pumiliotoxin (PTX); Allopumillotoxin (aPTX); 4,6-disubstituted quinolizidine (4,6-Q).

population pairs (Table S2; Figure 2B), suggesting each group has a unique alkaloid profile. Fitting environmental variables into the NMDS indicated that altitude ($r^2=0.72$, $p=0.001$) and temperature ($r^2=0.68$, $p=0.001$) significantly influenced alkaloid composition across the geographical gradient (Figure 2B). Given their strong inverse correlation and closely aligned vectors in NMDS space, we report both as reflecting a shared environmental gradient.

Skin alkaloids fell into one of 13 structural families: Histrionicotoxins (HTX); 5,8-disubstituted indolizidines (5,8-I); 3,5-disubstituted indolizidine (3,5-I); Decahydroquinoline (DHQ); 5,6,8-trisubstituted indolizidines (5,6,8-I); Pyrrolidine (Pyr); 3,5-disubstituted pyrrolizidine (3,5-P); Lehmizidine (Lehm); 1,4-disubstituted quinolizidine (1,4-Q); Piperidine (Pip); Pumiliotoxin (PTX); Allopumillotoxin (aPTX); 4,6-disubstituted quinolizidine (4,6-Q). When examined more closely, histrionicotoxins,

decahydroquinolines and indolizidines were the most abundant alkaloid classes relative to the total alkaloid content in both *H. infraguttatus* and all *O. sylvatica* populations sampled (Figure 2C). Several indolizidines, including 5,6,8-I ($r^2=0.54$, $p=0.001$), 5,8-I ($r^2=0.54$, $p=0.001$) and 3,5-I ($r^2=0.46$, $p=0.001$) contributed significantly to alkaloid ordination (Figure 2B).

3.2 | Defended frogs consumed more ants relative to other prey types and compared to the diet of the undefended species

We found that the number of prey consumed in different categories differs significantly across populations and between species (GLMM, population $\times$ prey type: $\chi^2(20)=83.59$,

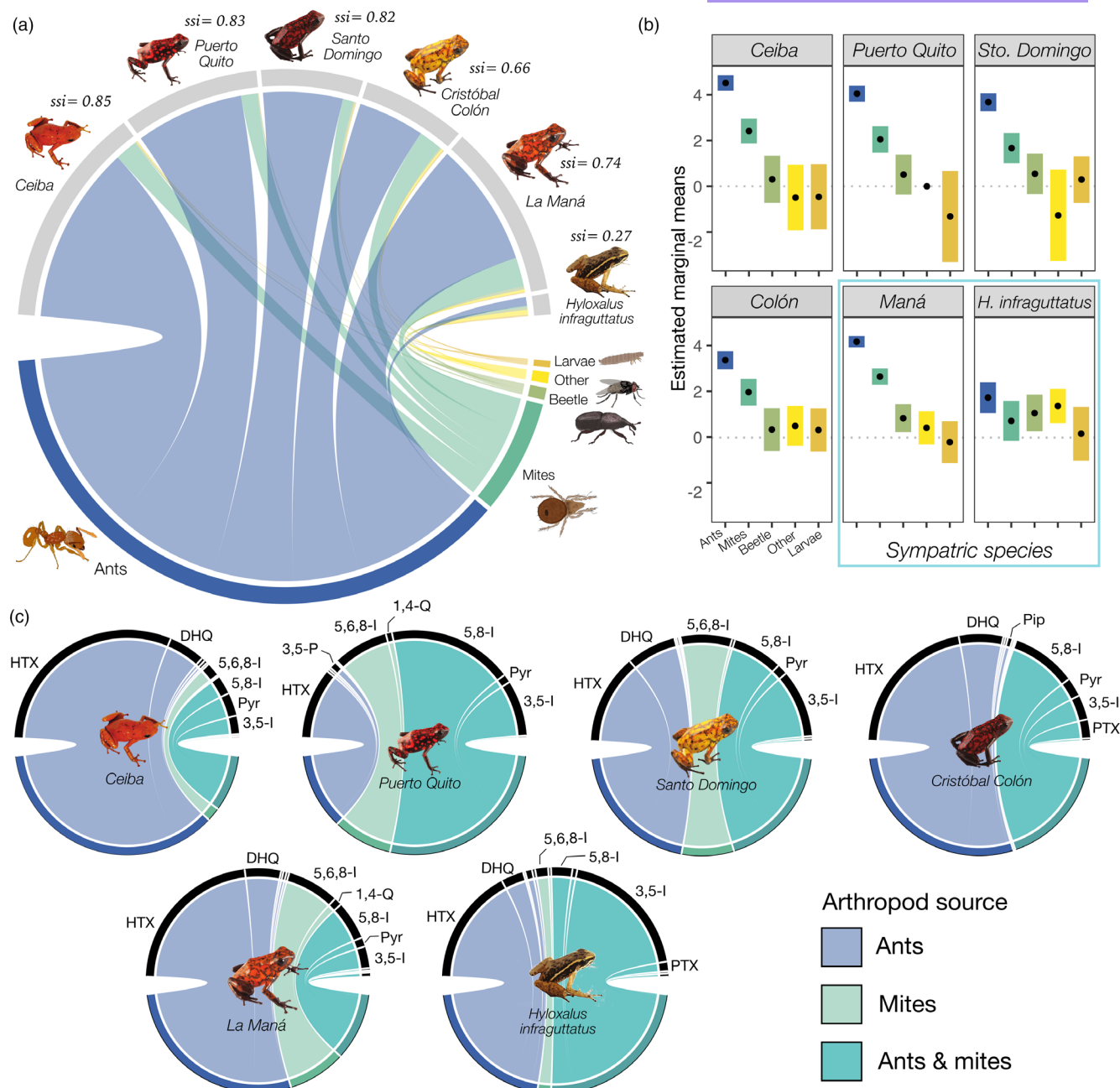


FIGURE 3 Diet differs between frog species and across diablo frog populations. (a) Chord diagram showing a bipartite network of the dietary interactions between two frog species, the non-toxic *Hyloxalus infraguttatus* and the toxic *O. sylvatica*, and their arthropod prey in five localities. The thickness of the connecting bars represents the average number of prey items consumed, indicating its relative importance in the network. Species specificity index (ssi) is shown next to each frog species, ranging from 0 (generalist) to 1 (specialist). (b) Pairwise comparison of prey item consumption between frog species. Dots represent the mean and bars represent 95% confidence intervals for the estimated marginal means. Non-overlapping bars indicate statistically significant differences. (c) Chord diagrams showing the most abundant alkaloid families and their putative arthropod source, across frog populations (sensu Santos et al., 2016). The thickness of the connecting bars represents the summed alkaloid abundance per structural class. Histrionicotoxins (HTX); 5,8-disubstituted indolizidines (5,8-I); 3,5-disubstituted indolizidine (3,5-I); Decahydroquinoline (DHQ); 5,6,8-trisubstituted indolizidines (5,6,8-I); Pyrrolidine (Pyr); 3,5-disubstituted pyrrolizidine (3,5-P); Lehmizidine (Lehm); 1,4-disubstituted quinolizidine (1,4-Q); Piperidine (Pip); Pumiliotoxin (PTX); Allopumiliotoxin (aPTX); 4,6-disubstituted quinolizidine (4,6-Q).

$p < 0.001$; Figure 3a,b). Post hoc pairwise comparisons and the species selectivity index (ssi) showed that all *O. sylvatica* populations consumed significantly more ants than other prey categories ($\bar{x} = 75\%$; $\text{emmeans}_{(\text{ants vs. all prey})}$; $p\text{-value} = < 0.001$;

$\text{ssi}_{\text{range}} = 0.66\text{--}0.85$; Figure 3b, Table S3), whereas *H. infraguttatus* showed a more generalist dietary pattern, consuming a smaller but diverse array of arthropods including ants (45%), beetles (14%) and 'other' arthropods (25%; $\text{emmeans}_{(\text{all prey comparisons})}$;

p -value = >0.05 ; $ssi = 0.27$; Figure 3b, Table S3). It is worth noting that only one *H. infraguttatus* individual had 18 ants in the stomach, which accounts for nearly half of the total consumed for this species in our dataset. When removing this individual, ants made up 36% of the total diet, followed by 'other' arthropods (29.6%) and beetles (16.5%).

3.3 | Frogs' alkaloid composition suggests a predominantly ant-based diet

We found that, across all frog populations, including the 'non-toxic' species *H. infraguttatus*, ant-derived alkaloids appeared to represent the most abundant component of their chemical profile, while a smaller but important contribution was attributed to mite-derived alkaloids (Figure 3c). Tukey's post hoc test revealed that the

proportion of ant-derived alkaloids was significantly higher than mite-derived alkaloids ($p < 0.001$). Neither total alkaloid abundance nor abundance within structural families showed a significant correlation with the number of ants or mites consumed across populations (all p -values > 0.05 ; Table S4).

3.4 | Alkaloid diversity among sites is associated with variation in leaf litter ant communities

As the diet of *O. sylvatica* is mainly composed of ants, we looked at compositional differences in leaf litter ant communities across the geographical gradient. A total of 46 ant genera were recovered from Winkler traps in leaf litter communities. The NMDS suggested a two-dimensional solution (stress = 0.130), representing partially overlapping clusters of ant communities across localities

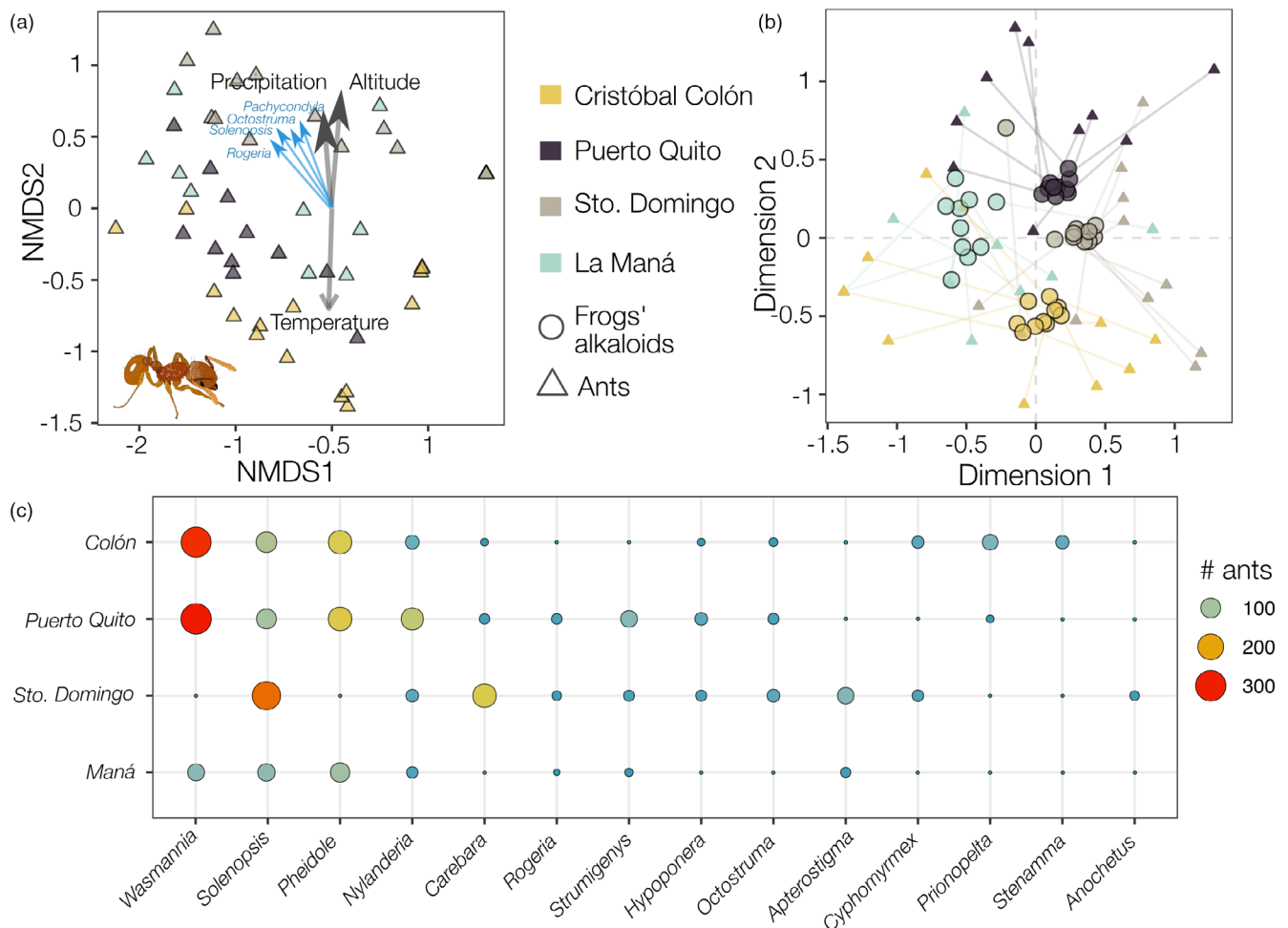


FIGURE 4 Ant community composition varies across localities. (a) Non-metric multidimensional scaling (NMDS) biplot based on Bray–Curtis dissimilarities showing ant communities across locations based on the relative abundances of 113 ant species (Stress = 0.156). Each triangle represents ant communities at one sampling point. Environmental factors (black arrows) and ant genera (blue arrows) in correlation with the ordination are shown. (b) Procrustes ordination plot showing correspondence between frog alkaloids and ant communities ordinations across localities. Procrustes residuals (lines) connect paired points between frog alkaloid profiles (circles) and ant community composition (triangles) at each site. Shorter lines indicate better alignment between the ordinations, suggesting stronger correspondence. (c) Bubble heat map shows the number of ants, grouped by genus (with ≥ 10 individuals), across frog populations. Each ant community was sampled using a trap placed next to the corresponding frog's capture site.

in the ordination space (Figure 4a). PERMANOVA and post hoc comparisons suggested significant differences in the composition of ant communities between all pairs of localities (PERMANOVA, $F(3) = 5.0525$, $p < 0.001$; Table S5, Figure 4a). Precipitation ($r^2 = 0.41$, $p = 0.001$), altitude ($r^2 = 0.48$, $p = 0.001$) and temperature ($r^2 = 0.49$, $p = 0.001$) significantly influenced ant availability across the geographical gradient (Figure 4b). A subset of ant genus, including *Octostruma* ($r^2 = 0.59$, $p = 0.001$), *Pachycondyla* ($r^2 = 0.54$, $p = 0.001$), *Rogeria* ($r^2 = 0.51$, $p = 0.001$) and *Solenopsis* ($r^2 = 0.48$, $p = 0.001$) significantly contributed to the ordination. We next asked whether the availability of leaf litter ant communities between locations is associated with skin alkaloid diversity. The procrustes analysis suggested that variation in alkaloid diversity among sites is significantly correlated with variation in the availability of ant communities ($r = 0.47$, p -value = 0.001, 999 permutations; Figure 4b).

3.5 | Frogs show different dietary selectivity for particular ant genera

We next asked if frogs eat specific ant genera selectively or if their ant diet reflects the genera of the surrounding leaf litter communities. From the 46 ant genera recovered from Winkler traps in leaf litter communities, only 17 of these were consumed by frogs across different species and populations. Our results indicated no significant differences in the total abundance of ants between frog stomach contents and leaf litter across all populations, except for Santo Domingo and *H. infraguttatus* where frogs had a lower abundance of ants in their stomachs compared to the leaf litter (Table S6; Figure 5a). We found that *Solenopsis* was the most selected ant genus across all *O. sylvatica* populations, while *Pheidole* was the only selected ant genus by *H. infraguttatus*. Particularly, frogs from the Cristóbal Colón population showed selectivity for *Paratrachymyrmex*, *Crematogaster* and *Pheidole*, whereas

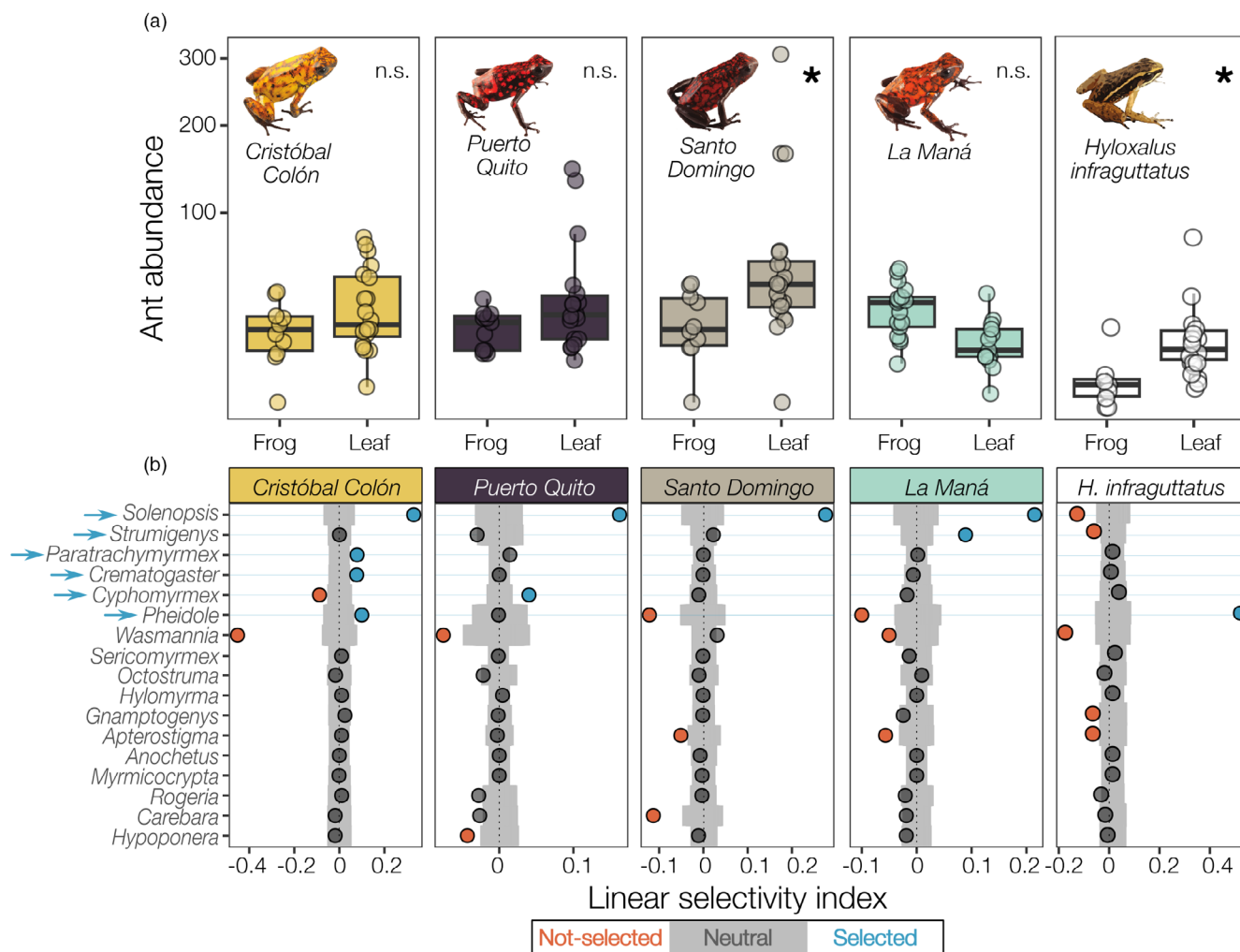


FIGURE 5 Relative abundance and selectivity for ant genera differs across species and within diablito localities. (a) Boxplot showing differences across populations in total abundance of ants within 17 ant genera found in both leaf litter and frog stomach samples. The y-axis is square-root transformed for visual clarity. n.s. = non-significant. * p -value < 0.05 (b) Linear selectivity index for 17 ant genera eaten by the toxic *Oophaga sylvatica* populations and the non-toxic *Hyloxalus infraguttatus*. Grey bars denote simulated null distribution. Points denote categorical selectivity as follows: 'not-selected' if they are below the null distribution (red dots), 'neutral' if they are within (black dots) and 'selected' if the values are above (blue dots). Blue arrows indicate overall selected ant genera.

Strumigenys and *Cyphomyrmex* were selected in La Maná and Puerto Quito populations, respectively (Figure 5b).

On the other hand, the remaining ants were either not selected or occasionally consumed (Figure 5). For example, *Wasmannia* ants were avoided by all populations, except for Santo Domingo frogs where they were consumed in proportion to their availability (i.e. neutral). Similarly, *Apterostigma* ants were avoided by frogs in Santo Domingo and in the sympatric populations of *O. sylvatica* and *H. infraguttatus* in La Maná.

3.6 | Ant morphology influences prey selectivity in frogs

Finally, we asked whether *O. sylvatica* frogs select for specific ant traits. A principal components analysis on 17 morphological and life-history ant traits yielded three principal components, each with eigenvalues exceeding 1. After inverting the scores of these components to aid interpretability, we found that the first component accounted for 55.66% of the total variance, primarily reflecting size and texture traits, with higher scores indicating larger ants. Component number two explained 11.02% of the variance and was strongly associated with colour traits, where higher scores corresponded to ants with dark-brown body coloration. The third principal component, explaining 7.15% of the variance, was largely indicative of the presence of spines, with higher scores representing spiny ants (Figure 6). A pairwise Wilcoxon test on these principal components revealed that ants categorized as selected were significantly smaller than those with neutral selectivity (p -value=0.03; Figure 6A; Table S7), but not than those not selected (p -value=0.162; Figure 6a). Contrary, no differences were found in the traits of colour and the presence of spines across the categories of selectivity (all p values >0.05; Figure 6B,C; Table S7).

4 | DISCUSSION

We found that *O. sylvatica* alkaloid profiles varied between populations, corresponding with changes in the availability of leaf litter

ants along a geographical gradient of temperature, precipitation and altitude. Our results align with previous studies (McGugan et al., 2016; Moskowitz et al., 2020; Myers & Daly, 1976; Prates et al., 2019; Saporito et al., 2006; Stuckert et al., 2014) and provide further evidence of the importance of environmental availability of alkaloid-containing prey in shaping the chemical repertoire in poison frogs. Overall, diablito populations with higher alkaloid loads were found at cooler, high-elevation sites, where leaf litter ant community composition was more diverse, except in La Maná, where both frog alkaloids and leaf litter ants were low despite the high altitude. As altitude and temperature vary along geographical gradients, they can drive changes in frog alkaloid profiles indirectly by shaping the composition and diversity of their arthropod prey (Brühl et al., 1999; Mackay et al., 1986; Moses et al., 2021; Silva & Brandão, 2014; Wise & Lensing, 2019). This is consistent with previous work showing that alkaloid profiles, diet and surrounding leaf litter communities in the diablito population from Santo Domingo were more abundant in frogs from a cooler, humid forest than in a hot, dry pasture (Moskowitz et al., 2020). Other factors like chemical diversity of arthropod prey have been shown to influence alkaloid variability in diablito frogs (McGugan et al., 2016). Future work comparing environmental arthropod chemistry is necessary to better understand the interplay between chemical repertoire and environmental availability of prey in organisms with diet-acquired defences.

We found that *H. infraguttatus*, which is typically considered chemically 'undefended', has lower yet detectable amounts of alkaloids in the skin, compared to the sympatric chemically defended *O. sylvatica*. Our results align with the hypothesis that 'undefended' dendrobatids can accumulate alkaloids even in small amounts, and that the ability to acquire alkaloids for defence may be common across dendrobatids (Tarvin et al., 2024). In this study, histrionicotoxins and indolizidines made up the highest proportion of the alkaloid profiles in both *O. sylvatica* populations and *H. infraguttatus*, which correspond with what has been found in other dendrobatids, including *Oophaga* and *Hyloxalus* frogs (Jeckel et al., 2019; McGugan

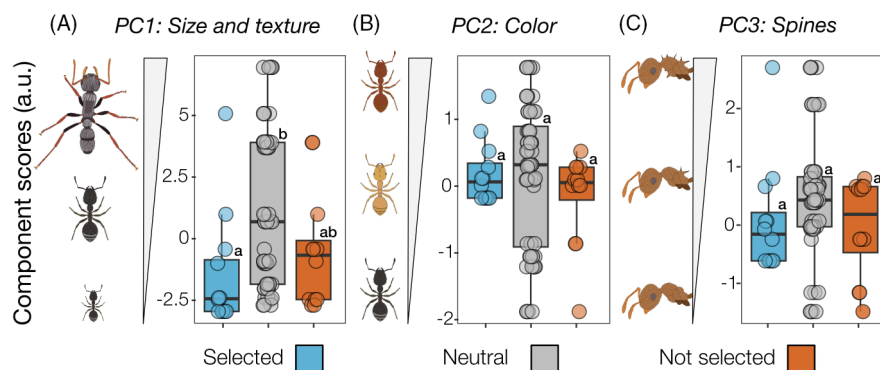


FIGURE 6 Boxplots showing differences between categorical selectivity in principal component scores on 17 ant traits. (A) First principal component (PC1) representing size and texture traits. High score values depict larger ants and low score values depict smaller ants. (B) Second principal component (PC2) representing body coloration. High score values depict dark-brown ants, middle scores depict light-brown ants and low score values depict black ants. (C) Third principal component (PC3) representing number of spines. High score values depict spiny ants, while low score values depict non-spiny ants. Groups not connected by the same letter are significantly different.

et al., 2016; Moskowitz et al., 2020; Saporito et al., 2006; Tarvin et al., 2024). Few studies have shown the variability of individual alkaloids and their influence on frog fitness. The potency or organismal effect of most poison frog alkaloids is unknown, and there are debates about which assays are most appropriate to test potency (Weldon, 2017). Some poison frog alkaloids are toxic, while other alkaloids are more noxious (Daly et al., 2005; Nayik & Kour, 2022; Santos et al., 2016). Birds avoided eating food associated with skin secretions from one *Dendrobates tinctorius* population, and this difference was partly explained by 15 alkaloids, many of them indolizidines (Lawrence et al., 2023). Given the variation in *O. sylvatica* alkaloid profiles, it is likely that there are differences in palatability to predators, although more studies on alkaloid ingestion with natural predators are needed to understand this relationship.

Organisms with diet-derived defences must maintain their defensive reservoirs through balancing food choice and availability. In our study, sympatric groups of defended *O. sylvatica* ate more ants and mites than *H. infraguttatus*, which ate a less specialized diet of ants, beetles and arthropods gathered in the 'other' category, demonstrating dietary differences between species with similar prey access. Additionally, although *O. sylvatica* differed overall in diet and alkaloid profiles across populations, the majority of their stomach contents were constituted by ants, consistent with previous studies (McGugan et al., 2016; Moskowitz et al., 2020). Our results are also consistent with a broader dietary study of dendrobatids in which chemically defended species, such as *Epipedobates anthonyi*, *Dendrobates auratus* and *O. pumilio*, showed a greater degree of dietary specialization on ants than 'undefended' species, such as *Allobates femoralis*, *Allobates zaparo* and *Hyloxalus infraguttatus* (Caldwell, 1996; Darst et al., 2005). However, some 'undefended' dendrobatid species eat mainly ants, and some chemically defended species have a broader diet than expected (Darst et al., 2005; Tarvin et al., 2024; Toft, 1995). Our data are consistent with growing evidence that ants make up a large portion of the diet in many *Hyloxalus* species (Darst et al., 2005; Sánchez Loja et al., 2023; Tarvin et al., 2024). Overall, our results support the broad trend of high alkaloid-bearing dendrobatids being ant and mite specialists, while providing further evidence that poison frogs with low alkaloid levels also consume important amounts of ants.

Consuming specific arthropod prey at rates disproportionate to their availability can influence poison frogs' alkaloid profile, as certain alkaloid classes have known origins in specific arthropod taxa (Blum et al., 1980; McGugan et al., 2016; Santos et al., 2016; Saporito et al., 2004; Saporito, Donnelly, Jain, et al., 2007; Spande et al., 1999). In all *O. sylvatica* populations, frogs consistently selected *Solenopsis* ants, while *Strumigenys*, *Paratrachymyrmex*, *Crematogaster*, *Pheidole* and *Cyphomyrmex* ants were selectively consumed in specific populations, a pattern consistent with our previous findings (McGugan et al., 2016; Moskowitz et al., 2020). These ant genera are known sources of several alkaloid classes particularly abundant across diablito populations, including histrionicotoxins, decahydroquinolines, 3,5-disubstituted indolizidines and pyrrolidines (Blum et al., 1980; Jones et al., 1982, 1999; McGugan et al., 2016;

Moskowitz et al., 2020; Spande et al., 1999). Dietary selectivity in *O. sylvatica* may suggest a preference for specific ant prey based on their alkaloid content, or it may simply reflect that these frogs inhabit microhabitats where alkaloid-rich ants are abundant, leading to incidental consumption without active behavioural preference, as previously observed in *O. pumilio* (Donnelly, 1991). Further behavioural assays are required to distinguish between these competing hypotheses. Additionally, given the varied but overall high number of mites recovered from stomach contents across localities, our data suggest that mites are also an important defensive alkaloid source for *O. sylvatica*, probably of 5,8-disubstituted and 5,6,8-trisubstituted indolizidines and pumiliotoxins, as previously found in *O. pumilio* (Saporito et al., 2006; Saporito, Donnelly, Jain, et al., 2007). Mite taxonomy and chemistry are drastically understudied compared to ants and future studies should also make efforts to include mites in their analyses (but see Saporito, Donnelly, Jain, et al., 2007; Saporito et al., 2011, 2015).

We found that *H. infraguttatus* selected *Pheidole* ants, which were also selected by *O. sylvatica* from the 'Cristobal Colón' locality within this study. Previous alkaloid sampling of *O. sylvatica* and *Pheidole* ants found evidence of overlapping alkaloids within the same sampling location in both frogs and ants (Moskowitz et al., 2020). Our data suggest that selection for alkaloid-rich ants is not exclusive to aposematic species. Recent evidence found that *Pheidole* ants constituted the largest portion of the diet in the cryptic *Allobates femoralis* frog, although they were not actively selected (Sanches et al., 2023). It is unclear whether *H. infraguttatus* frogs have fewer alkaloids because their diet is less ant and mite rich or if they lack the physiological mechanisms required for alkaloid sequestration in higher concentrations, as has been noted recently in other cryptic species (Tarvin et al., 2024). For example, in laboratory toxin feeding trials, *A. femoralis* was able to uptake alkaloids as effectively as *O. sylvatica*, but exhibited signs of physiological distress (Coty et al., 2025). Further captive feeding experiments would be helpful to fully understand the implications of dietary selectivity for the evolution of acquired chemical defences among poison frogs.

Poison frogs may selectively consume certain ant genera based on their alkaloid content, reflecting underlying feeding preferences. However, dietary selectivity can also arise from factors beyond behavioural preference. For instance, *Solenopsis* and *Pheidole* ants may be consumed more frequently, regardless of their abundance, because they are smaller and require less handling effort than bigger, more aggressive ant genera, as observed in the chemically defended frog, *Chiasmocleis leucosticta* (Meurer et al., 2021). In contrast, the Neotropical toad, *Rhinella alata*, selectively consumed larger, more conspicuous ants despite their rarity, while avoiding the more abundant but smaller *Solenopsis* ants (McElroy & Donoso, 2019). Also, as we limited our genus-level analysis to ants here, we cannot determine whether frogs select ants over other prey categories that may offer different metabolic benefits or higher nutritional value. For example, when prey availability is controlled under lab conditions, lab-raised *Dendrobates tinctorius* frogs gravitate to the most nutritious

prey (larvae), regardless of size and other prey options including ants (Moskowitz et al., 2022). A careful comparison of frog diet with all available arthropods in the leaf litter through metabarcoding could be useful to determine whether behavioural selectivity is consistent across prey categories. Although we found solid evidence on the relationship between alkaloid defences, frog diets and arthropod availability, it is worth noting that alkaloid profiles in poison frogs reflect long-term accumulation, whereas dietary composition and prey availability are measured at a single time point, potentially capturing only a subset of the frogs' long-term foraging patterns. Finally, we collected leaf litter samples with Winkler sacs, which often capture abundant small, specialist myrmecines compared to pitfall traps, which are better at capturing bigger scavengers like *Anochetus* or *Hypoconera* ants (Olson, 1991; Sabu & Shiju, 2010). Integrating multiple sampling methods may provide a more ecologically realistic view of the arthropod community available to poison frogs, including variation in foraging patterns that could underestimate prey availability.

Our study demonstrates that dietary selectivity and environmental prey availability shape poison frog chemical defences. *Oophaga sylvatica* showed alkaloid profiles that varied with local leaf litter ant availability, while the sympatric *Hyloxalus infraguttatus*, traditionally considered 'undefended', carried small but detectable alkaloid levels. Both species selectively consumed certain small ant genera, suggesting toxic prey selection is not exclusive to aposematic species. These findings highlight dietary selectivity as a key factor in the evolution of chemical defences. Future studies should replicate these findings in both natural and controlled settings, compare environmental arthropod chemistry to better understand prey availability and use captive feeding experiments to test how diet influences alkaloid accumulation.

AUTHOR CONTRIBUTIONS

Nora A. Martin and Lauren A. O'Connell conceived the ideas; Nora A. Martin, Lauren A. O'Connell, David A. Donoso and Aurora Alvarez-Buylla designed the methodology. Nora A. Martin, Aurora Alvarez-Buylla and Camilo Rodríguez performed formal analyses. Nora A. Martin, Aurora Alvarez-Buylla, Adolfo Chamba-Carrillo, Janet Rentería and David A. Donoso conducted the investigation and collected data. Lauren A. O'Connell and David A. Donoso provided resources. Nora A. Martin, Aurora Alvarez-Buylla, Colin R. Morrison, David A. Donoso, Ana B. García-Ruilova and Camilo Rodríguez curated the data. Nora A. Martin, Lauren A. O'Connell, Aurora Alvarez-Buylla and David A. Donoso prepared the original manuscript draft and Nora A. Martin, Lauren A. O'Connell, Aurora Alvarez-Buylla, David A. Donoso, Elicio E. Tapia, Adolfo Chamba-Carrillo, Janet Rentería, Luis A. Coloma, Ana B. García-Ruilova, Camilo Rodríguez and Katherine Fiocca reviewed and edited subsequent drafts. Nora A. Martin, Lauren A. O'Connell and Camilo Rodríguez created visualizations. Lauren A. O'Connell supervised the project, managed project administration and acquired funding. All authors contributed critically to the drafts and gave final approval for publication.

ACKNOWLEDGEMENTS

We thank María Dolores Guarderas (Wikiri) for logistical and resource support in Ecuador. We are thankful to the Peter Dorrestein lab at The University of California at San Diego for creating the GNPS environment that allows us to perform the metabolomic analysis of poison frog alkaloids. We would also like to thank Mabel Gonzalez, who was a visiting researcher in the Dorrestein Lab and helped us implement GNPS in our lab. LAC acknowledges the support of the Saint Louis Zoo. We acknowledge that our work at Stanford University takes place on the ancestral and unceded land of the Muwekma Ohlone tribe.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest directly or indirectly related to the content of this manuscript.

DATA AVAILABILITY STATEMENT

Data and code are archived in the Zenodo Digital Repository: <https://doi.org/10.5281/zenodo.17065449> (Martin et al., 2025). Repository URL: <https://github.com/laurenconnelllab/Poison-frog-ant-diet-and-alkaloid-defense>.

ETHICAL PERMITS

Remaining tissues were deposited in the amphibian collection of Centro Jambatu de Investigación y Conservación de Anfibios in Quito, Ecuador. Collections and exportation of specimens were done under permits (Collection permit: No. 0013-18 IC-FAU-DNB/MA; Export permit: No. 214-2019-EXP-CM-FAU-DNB/MA; CITES export permit No. 19EC000036/V5) issued by the Ministerio de Ambiente de Ecuador. The Administrative Panel on Laboratory Animal Care of Stanford University approved all frog-related procedures (Protocol #34153).

STATEMENT OF INCLUSION

Our research team includes collaborators from multiple international institutions as well as local researchers from the country where the study took place. All authors actively participated in study planning and design, ensuring diverse viewpoints were integrated into the research approach. Additionally, we have intentionally cited relevant studies from regional scientists.

ORCID

Camilo Rodríguez  <https://orcid.org/0000-0002-9748-1773>

Colin R. Morrison  <https://orcid.org/0000-0003-2370-3617>

David A. Donoso  <https://orcid.org/0000-0002-3408-1457>

REFERENCES

- Agrawal, A. A., Petschenka, G., Bingham, R. A., Weber, M. G., & Rasmann, S. (2012). Toxic cardenolides: Chemical ecology and coevolution of specialized plant–herbivore interactions. *New Phytologist*, 194(1), 28–45. <https://doi.org/10.1111/j.1469-8137.2011.04049.x>
- Aksenov, A. A., Laponogov, I., Zhang, Z., Doran, S. L. F., Belluomo, I., Veselkov, D., Bittremieux, W., Nothias, L. F., Nothias-Espósito, M., Maloney, K. N., Misra, B. B., Melnik, A. V., Smirnov, A., Du, X., Jones,

- K. L., Dorrestein, K., Panitchpakdi, M., Ernst, M., Van Der Hooft, J. J. J., ... Veselkov, K. (2021). Auto-deconvolution and molecular networking of gas chromatography-mass spectrometry data. *Nature Biotechnology*, 39(2), 169–173. <https://doi.org/10.1038/s41587-020-0700-3>
- Alvarez-Buylla, A., Fischer, M.-T., Moya Garzon, M. D., Rangel, A. E., Tapia, E. E., Tanzo, J. T., Soh, H. T., Coloma, L. A., Long, J. Z., & O'Connell, L. A. (2023). Binding and sequestration of poison frog alkaloids by a plasma globulin. *eLife*, 12, e85096. <https://doi.org/10.7554/eLife.85096>
- AmphibiaWeb Database Search. (2025). <https://amphibiaweb.org/search/index.html>
- Basset, Y., Butterill, P. T., Donoso, D. A., Lamarre, G. P. A., Souto-Vilarós, D., Perez, F., Bobadilla, R., Lopez, Y., Alejandro Ramírez Silva, J., & Barrios, H. (2023). Abundance, occurrence and time series: Long-term monitoring of social insects in a tropical rainforest. *Ecological Indicators*, 150, 110243. <https://doi.org/10.1016/j.ecolind.2023.110243>
- Beran, F., & Petschenka, G. (2022). Sequestration of plant defense compounds by insects: From mechanisms to insect-plant coevolution. *Annual Review of Entomology*, 67, 163–180. <https://doi.org/10.1146/annurev-ento-062821-062319>
- Blum, M. S., Jones, T. H., Hölldobler, B., Fales, H. M., & Jaouni, T. (1980). Alkaloidal venom mace: Offensive use by a thief ant. *Naturwissenschaften*, 67(3), 144–145. <https://doi.org/10.1007/BF01073620>
- Brooks, M. E., Kristensen, K., van Benthem, K. J., Magnusson, A., Berg, C. W., Nielsen, A., Skaug, H. J., Mächler, M., & Bolker, B. M. (2017). glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. *The R Journal*, 9(2), 378–400. <https://doi.org/10.32614/RJ-2017-066>
- Brühl, C. A., Mohamed, M., & Linsenmair, K. E. (1999). Altitudinal distribution of leaf litter ants along a transect in primary forests on Mount Kinabalu, Sabah, Malaysia. *Journal of Tropical Ecology*, 15(3), 265–277. <https://doi.org/10.1017/S0266467499000802>
- Caldwell, J. P. (1996). The evolution of myrmecophagy and its correlates in poison frogs (Family Dendrobatidae). *Journal of Zoology*, 240(1), 75–101. <https://doi.org/10.1111/j.1469-7998.1996.tb05487.x>
- Catý, S. N., Alvarez-Buylla, A., Vasek, C., Tapia, E. E., Martin, N. A., McLaughlin, T., Golde, C. L., Weber, P. K., Mayali, X., Coloma, L. A., Morris, M. M., & O'Connell, L. A. (2025). Alkaloids are associated with increased microbial diversity and metabolic function in poison frogs. *Current Biology*, 35(1), 187–197.e8. <https://doi.org/10.1016/j.cub.2024.10.069>
- Daly, J. W., Secunda, S. I., Garraffo, H. M., Spande, T. F., Wisnieski, A., & Cover, J. F. (1994). An uptake system for dietary alkaloids in poison frogs (Dendrobatidae). *Toxicon*, 32(6), 657–663. [https://doi.org/10.1016/0041-0101\(94\)90335-2](https://doi.org/10.1016/0041-0101(94)90335-2)
- Daly, J. W., Spande, T. F., & Garraffo, H. M. (2005). Alkaloids from amphibian skin: A tabulation of over eight-hundred compounds. *Journal of Natural Products*, 68(10), 1556–1575. <https://doi.org/10.1021/np0580560>
- Daly, J. W., Wilham, J. M., Spande, T. F., Garraffo, H. M., Gil, R. R., Silva, G. L., & Vaira, M. (2007). Alkaloids in bufonid toads (Melanophryniscus): Temporal and geographic determinants for two Argentinian species. *Journal of Chemical Ecology*, 33(4), 871–887. <https://doi.org/10.1007/s10886-007-9261-x>
- Darst, C. R., Menéndez-Guerrero, P. A., Coloma, L. A., & Cannatella, D. C. (2005). Evolution of dietary specialization and chemical defense in poison frogs (Dendrobatidae): A comparative analysis. *The American Naturalist*, 165(1), 56–69. <https://doi.org/10.1086/426599>
- Deslippe, R. J., & Guo, Y.-J. (2000). Venom alkaloids of fire ants in relation to worker size and age. *Toxicon*, 38, 223–232.
- Donnelly, M. A. (1991). Feeding patterns of the strawberry poison frog, *Dendrobates pumilio* (Anura: Dendrobatidae). *Copeia*, 1991(3), 723. <https://doi.org/10.2307/1446399>
- Donoso, D. A. (2017). Tropical ant communities are in long-term equilibrium. *Ecological Indicators*, 83, 515–523. <https://doi.org/10.1016/j.ecolind.2017.03.022>
- Donoso, D. A., & Ramón, G. (2009). Composition of a high diversity leaf litter ant community (Hymenoptera: Formicidae) from an Ecuadorian pre-montane rainforest. *Annales De La Société Entomologique De France (N.S.)*, 45(4), 487–499. <https://doi.org/10.1080/00379271.2009.10697631>
- Dray, S., & Dufour, A.-B. (2007). The ade4 package: Implementing the duality diagram for ecologists. *Journal of Statistical Software*, 22, 1–20. <https://doi.org/10.18637/jss.v022.i04>
- Fick, S. E., & Hijmans, R. J. (2017). WorldClim 2: New 1-km spatial resolution climate surfaces for global land areas. *International Journal of Climatology*, 37(12), 4302–4315. <https://doi.org/10.1002/joc.5086>
- Funkhouser, J. W. (1956). New frogs from Ecuador and southwestern Colombia. *Zoologica: Scientific Contributions of the New York Zoological Society*, 41(9), 73–80. <https://doi.org/10.5962/p.190356>
- Gibb, H., Sanders, N. J., Dunn, R. R., Watson, S., Photakis, M., Abril, S., Andersen, A. N., Angulo, E., Armbrrecht, I., Arnan, X., Baccaro, F. B., Bishop, T. R., Boulay, R., Castracani, C., Del Toro, I., Delsinne, T., Diaz, M., Donoso, D. A., Enriquez, M. L., ... Parr, C. L. (2015). Climate mediates the effects of disturbance on ant assemblage structure. *Proceedings of the Royal Society B: Biological Sciences*, 282(1808), 20150418. <https://doi.org/10.1098/rspb.2015.0418>
- Hoelen, P. O., Donoso, D. A., Argoti, A., Staab, M., von Beeren, C., & Blüthgen, N. (2022). Rapid ant community reassembly in a neotropical forest: Recovery dynamics and land-use legacy. *Ecological Applications*, 32(4), e2559. <https://doi.org/10.1002/eap.2559>
- Jeckel, A. M., Kocheff, S., Saporito, R. A., & Grant, T. (2019). Geographically separated orange and blue populations of the Amazonian poison frog *Adelphobates galactonotus* (Anura, Dendrobatidae) do not differ in alkaloid composition or palatability. *Chemoecology*, 29(5–6), 225–234. <https://doi.org/10.1007/s00049-019-00291-3>
- Jones, T. H., Blum, M. S., & Fales, H. M. (1982). Ant venom alkaloids from *Solenopsis* and *Monorium* species. *Tetrahedron*, 38(13), 1949–1958. [https://doi.org/10.1016/0040-4020\(82\)80044-6](https://doi.org/10.1016/0040-4020(82)80044-6)
- Jones, T. H., Gorman, J. S. T., Snelling, R. R., Delabie, J. H. C., Blum, M. S., Garraffo, H. M., Jain, P., Daly, J. W., & Spande, T. F. (1999). Further alkaloids common to ants and frogs: Decahydroquinolines and a quinolizidine. *Journal of Chemical Ecology*, 25(5), 1179–1193. <https://doi.org/10.1023/A:1020898229304>
- Lawrence, J. P., Rojas, B., Blanchette, A., Saporito, R. A., Mappes, J., Fouquet, A., & Noonan, B. P. (2023). Linking predator responses to alkaloid variability in poison frogs. *Journal of Chemical Ecology*, 49(3–4), 195–204. <https://doi.org/10.1007/s10886-023-01412-7>
- Lenth, R. V. (2023). emmeans: Estimated marginal means, aka least-squares means. <https://CRAN.R-project.org/package=emmeans>
- Mackay, W. P., Silva, S., Lightfoot, D. C., Pagani, M. I., & Whitford, W. G. (1986). Effect of increased soil moisture and reduced soil temperature on a desert soil arthropod community. *American Midland Naturalist*, 116(1), 45. <https://doi.org/10.2307/2425936>
- Martin, N. A., Rodríguez, C., Alvarez-Buylla, A., Fiocca, K., Morrison, C. R., Chamba-Carrillo, A., García-Ruilova, A. B., Rentería, J., Tapia, E. E., Coloma, L. A., Donoso, D. A., & O'Connell, L. A. (2025). Data from: Poison frog chemical defenses are influenced by environmental availability and dietary selectivity for ants. *Zenodo Digital Repository*. <https://doi.org/10.5281/zenodo.17065449>
- McElroy, M. T., & Donoso, D. A. (2019). Ant morphology mediates diet preference in a neotropical toad (*Rhinella alata*). *Copeia*, 107(3), 430. <https://doi.org/10.1643/CH-18-162>
- McGugan, J. R., Byrd, G. D., Roland, A. B., Catý, S. N., Kabir, N., Tapia, E. E., Trauger, S. A., Coloma, L. A., & O'Connell, L. A. (2016). Ant and mite diversity drives toxin variation in the little devil poison frog. *Journal of Chemical Ecology*, 42(6), 537–551. <https://doi.org/10.1007/s10886-016-0715-x>

- Mebs, D. (2002). *Venomous and poisonous animals: A handbook for biologists, toxicologists and toxinologists, physicians and pharmacists*. CRC Press.
- Meurer, W., Gonçalves, F. G., Bovendorp, R. S., Percequillo, A. R., & Bertoluci, J. (2021). Diet electivity and preferences for food resources in *Chiasmocleis leucosticta* (Anura: Microhylidae). *Journal of Herpetology*, 55(4), 325–329. <https://doi.org/10.1670/20-106>
- Moses, J., Fayle, T. M., Novotny, V., & Klimes, P. (2021). Elevation and leaf litter interact in determining the structure of ant communities on a tropical mountain. *Biotropica*, 53(3), 906–919. <https://doi.org/10.1111/btp.12914>
- Moskowitz, N. A., D'Agui, R., Alvarez-Buylla, A., Fiocca, K., & O'Connell, L. A. (2022). Poison frog dietary preference depends on prey type and alkaloid load. *PLoS One*, 17(12), e0276331. <https://doi.org/10.1371/journal.pone.0276331>
- Moskowitz, N. A., Dorritie, B., Fay, T., Nieves, O. C., Vidoudez, C., Cambridge Rindge Latin 2017 Biology Class, Masconomet 2017 Biotechnology Class, Fischer, E. K., Trauger, S. A., Coloma, L. A., Donoso, D. A., & O'Connell, L. A. (2020). Land use impacts poison frog chemical defenses through changes in leaf litter ant communities. *Neotropical Biodiversity*, 6(1), 75–87. <https://doi.org/10.1080/23766808.2020.1744957>
- Moskowitz, N. A., Roland, A. B., Fischer, E. K., Ranaivorazo, N., Vidoudez, C., Aguilar, M. T., Caldera, S. M., Chea, J., Cristus, M. G., Crowdis, J. P., DeMessie, B., desJardins-Park, C. R., Effenberger, A. H., Flores, F., Giles, M., He, E. Y., Izmaylov, N. S., Lee, C. C., Pagel, N. A., ... O'Connell, L. A. (2018). Seasonal changes in diet and chemical defense in the Climbing Mantella frog (*Mantella laevigata*). *PLoS One*, 13(12), e0207940. <https://doi.org/10.1371/journal.pone.0207940>
- Myers, C. W., & Daly, J. W. (1976). Preliminary evaluation of skin toxins and vocalizations in taxonomic and evolutionary studies of poison-dart frogs (Dendrobatidae). *Bulletin of the AMNH*, 157, Article 3 <http://hdl.handle.net/2246/622>
- Nayik, G. A., & Kour, J. (Eds.). (2022). *Handbook of plant and animal toxins in food: Occurrence, toxicity, and prevention*. CRC Press. <https://doi.org/10.1201/9781003178446>
- Oksanen, J., Simpson, G. L., Blanchet, F. G., Kindt, R., Legendre, P., Minchin, P. R., O'Hara, R. B., Solymos, P., Stevens, M. H. H., Szoecs, E., Wagner, H., Barbour, M., Bedward, M., Bolker, B., Borcard, D., Carvalho, G., Chirico, M., De Caceres, M., Durand, S., & Weedon, J. (2022). vegan: Community ecology package. <https://CRAN.R-project.org/package=vegan>
- Olson, D. M. (1991). A comparison of the efficacy of litter sifting and pitfall traps for sampling leaf litter ants (Hymenoptera, Formicidae) in a tropical wet forest, Costa Rica. *Biotropica*, 23(2), 166. <https://doi.org/10.2307/2388302>
- Parr, C. L., Dunn, R. R., Sanders, N. J., Weiser, M. D., Photakis, M., Bishop, T. R., Fitzpatrick, M. C., Arnan, X., Baccaro, F., Brandão, C. R. F., Chick, L., Donoso, D. A., Fayle, T. M., Gómez, C., Grossman, B., Munyai, T. C., Pacheco, R., Retana, J., Robinson, A., ... Gibb, H. (2017). GlobalAnts: A new database on the geography of ant traits (Hymenoptera: Formicidae). *Insect Conservation and Diversity*, 10(1), 5–20. <https://doi.org/10.1111/icad.12211>
- Prates, I., Paz, A., Brown, J. L., & Carnaval, A. C. (2019). Links between prey assemblages and poison frog toxins: A landscape ecology approach to assess how biotic interactions affect species phenotypes. *Ecology and Evolution*, 9(24), 14317–14329. <https://doi.org/10.1002/ece3.5867>
- Roberts, M. F., & Wink, M. (Eds.). (1998). *Alkaloids: Biochemistry, ecology, and medicinal applications*. Springer US. <https://doi.org/10.1007/978-1-4757-2905-4>
- Sabu, T. K., & Shiju, R. T. (2010). Efficacy of pitfall trapping, Winkler and Berlese extraction methods for measuring ground-dwelling arthropods in moistdeciduous forests in the Western Ghats. *Journal of Insect Science*, 10(1), 98. <https://doi.org/10.1673/031.010.9801>
- Salazar, F., Reyes-Bueno, F., Sanmartin, D., & Donoso, D. A. (2015). Mapping continental Ecuadorian ant species. *Sociobiology*, 62(2), 132–162. <https://doi.org/10.13102/sociobiology.v62i2.132-162>
- Sanches, P. R., Santos-Guerra, L. E., Pedroso-Santos, F., Kaefer, I. L., & Costa-Campos, C. E. (2023). What do Co-mimics eat? Trophic ecology of *Ameerega pulchripecta* (Anura, Dendrobatidae) and *Allobates femoralis* (Anura, Aromobatidae) in eastern Brazilian Amazonia. *Journal of Herpetology*, 57(4), 408–417. <https://doi.org/10.1670/22-074>
- Sánchez Loja, S., Donoso, D. A., & Paez Vacas, M. I. (2023). Conspicuous and cryptic poison frogs are picky and prefer different meals in synopy. *Evolutionary Ecology*, 38, 679–691. <https://doi.org/10.1007/s10682-023-10282-0>
- Santos, J. C., Coloma, L. A., & Cannatella, D. C. (2003). Multiple, recurring origins of aposematism and diet specialization in poison frogs. *Proceedings of the National Academy of Sciences of the United States of America*, 100(22), 12792–12797. <https://doi.org/10.1073/pnas.2133521100>
- Santos, J. C., Tarvin, R. D., & O'Connell, L. A. (2016). A review of chemical defense in poison frogs (Dendrobatidae): Ecology, pharmacokinetics, and autoresistance. In B. A. Schulte, T. E. Goodwin, & M. H. Ferkin (Eds.), *Chemical signals in vertebrates* (Vol. 13, pp. 305–337). Springer International Publishing. https://doi.org/10.1007/978-3-319-22026-0_21
- Saporito, R. A., Donnelly, M. A., Garraffo, H. M., Spande, T. F., & Daly, J. W. (2006). Geographic and seasonal variation in alkaloid-based chemical defenses of *Dendrobates pumilio* from Bocas del Toro, Panama. *Journal of Chemical Ecology*, 32(4), 795–814. <https://doi.org/10.1007/s10886-006-9034-y>
- Saporito, R. A., Donnelly, M. A., Jain, P., Martin Garraffo, H., Spande, T. F., & Daly, J. W. (2007). Spatial and temporal patterns of alkaloid variation in the poison frog *Oophaga pumilio* in Costa Rica and Panama over 30 years. *Toxicon*, 50(6), 757–778. <https://doi.org/10.1016/j.toxicon.2007.06.022>
- Saporito, R. A., Donnelly, M. A., Madden, A. A., Garraffo, H. M., & Spande, T. F. (2010). Sex-related differences in alkaloid chemical defenses of the dendrobatid frog *Oophaga pumilio* from Cayo Nancy, Bocas del Toro, Panama. *Journal of Natural Products*, 73(3), 317–321. <https://doi.org/10.1021/np900702d>
- Saporito, R. A., Donnelly, M. A., Norton, R. A., Garraffo, H. M., Spande, T. F., & Daly, J. W. (2007). Oribatid mites as a major dietary source for alkaloids in poison frogs. *Proceedings of the National Academy of Sciences*, 104(21), 8885–8890. <https://doi.org/10.1073/pnas.0702851104>
- Saporito, R. A., Garraffo, H. M., Donnelly, M. A., Edwards, A. L., Longino, J. T., & Daly, J. W. (2004). Formicine ants: An arthropod source for the pumiliotoxin alkaloids of dendrobatid poison frogs. *Proceedings of the National Academy of Sciences of the United States of America*, 101(21), 8045–8050. <https://doi.org/10.1073/pnas.0402365101>
- Saporito, R. A., Norton, R. A., Andriamaharavo, N. R., Garraffo, H. M., & Spande, T. F. (2011). Alkaloids in the mite *Scheloribates laevigatus*: Further alkaloids common to oribatid mites and poison frogs. *Journal of Chemical Ecology*, 37(2), 213–218. <https://doi.org/10.1007/s10886-011-9914-7>
- Saporito, R. A., Norton, R. A., Garraffo, H. M., & Spande, T. F. (2015). Taxonomic distribution of defensive alkaloids in Nearctic oribatid mites (Acari, Oribatida). *Experimental and Applied Acarology*, 67(3), 317–333. <https://doi.org/10.1007/s10493-015-9962-8>
- Savitzky, A. H., Mori, A., Hutchinson, D. A., Saporito, R. A., Burghardt, G. M., Lillywhite, H. B., & Meinwald, J. (2012). Sequestered defensive toxins in tetrapod vertebrates: Principles, patterns, and prospects for future studies. *Chemoeology*, 22(3), 141–158. <https://doi.org/10.1007/s00049-012-0112-z>
- Silva, R. R., & Brandão, C. R. F. (2014). Ecosystem-wide morphological structure of leaf-litter ant communities along a tropical latitudinal

- gradient. *PLoS One*, 9(3), e93049. <https://doi.org/10.1371/journal.pone.0093049>
- Spande, T. F., Jain, P., Garraffo, H. M., Pannell, L. K., Yeh, H. J. C., Daly, J. W., Fukumoto, S., Imamura, K., Tokuyama, T., Torres, J. A., Snelling, R. R., & Jones, T. H. (1999). Occurrence and significance of decahydroquinolines from dendrobatid poison frogs and a Myrmicine ant: Use of ^1H and ^{13}C NMR in their conformational analysis. *Journal of Natural Products*, 62(1), 5–21. <https://doi.org/10.1021/np980298v>
- Strauss, R. E. (1979). Reliability estimates for Ivlev's electivity index, the forage ratio, and a proposed linear index of food selection. *Transactions of the American Fisheries Society*, 108(4), 344–352. [https://doi.org/10.1577/1548-8659\(1979\)108<344:REFIEI>2.0.CO;2](https://doi.org/10.1577/1548-8659(1979)108<344:REFIEI>2.0.CO;2)
- Stuckert, A. M., Saporito, R. A., Venegas, P. J., & Summers, K. (2014). Alkaloid defenses of co-mimics in a putative Müllerian mimetic radiation. *BMC Evolutionary Biology*, 14(1), 76. <https://doi.org/10.1186/1471-2148-14-76>
- Tarvin, R. D., Coleman, J. L., Donoso, D. A., Betancourth-Cundar, M., López-Hervas, K., Gleason, K. S., Sanders, J. R., Smith, J. M., Ron, S. R., Santos, J. C., Sedio, B. E., Cannatella, D. C., & Fitch, R. (2024). Passive accumulation of alkaloids in inconspicuously colored frogs refines the evolutionary paradigm of acquired chemical defenses. *eLife*, 13, RP100011. <https://doi.org/10.7554/eLife.100011.2>
- Tiede, Y., Schlautmann, J., Donoso, D. A., Wallis, C. I. B., Bendix, J., Brandl, R., & Farwig, N. (2017). Ants as indicators of environmental change and ecosystem processes. *Ecological Indicators*, 83, 527–537. <https://doi.org/10.1016/j.ecolind.2017.01.029>
- Toft, C. A. (1995). Evolution of diet specialization in poison-dart frogs (Dendrobatidae). *Herpetologica*, 51(2), 202–216.
- Venables, W. N., & Ripley, B. D. (2002). *Modern applied statistics with S*. <https://www.stats.ox.ac.uk/pub/MASS4/>
- Virjamo, V., & Julkunen-Tiitto, R. (2016). Variation in piperidine alkaloid chemistry of Norway spruce (*Picea abies*) foliage in diverse geographic origins grown in the same area. *Canadian Journal of Forest Research*, 46(4), 456–460. <https://doi.org/10.1139/cjfr-2015-0388>
- Walsh, C. T., & Tang, Y. (2017). *Natural product biosynthesis: Chemical logic and enzymatic machinery*. Royal Society of Chemistry.
- Wang, M., Carver, J. J., Phelan, V. V., Sanchez, L. M., Garg, N., Peng, Y., Nguyen, D. D., Watrous, J., Kapon, C. A., Luzzatto-Knaan, T., Porto, C., Bouslimani, A., Melnik, A. V., Meehan, M. J., Liu, W.-T., Crusemann, M., Boudreau, P. D., Esquenazi, E., Sandoval-Calderón, M., ... Bandeira, N. (2016). Sharing and community curation of mass spectrometry data with global natural products social molecular networking. *Nature Biotechnology*, 34(8), 828–837. <https://doi.org/10.1038/nbt.3597>
- Weldon, P. J. (2017). Poison frogs, defensive alkaloids, and sleepless mice: Critique of a toxicity bioassay. *Chemoecology*, 27(4), 123–126. <https://doi.org/10.1007/s00049-017-0238-0>

- Wise, D. H., & Lensing, J. R. (2019). Impacts of rainfall extremes predicted by climate-change models on major trophic groups in the leaf litter arthropod community. *Journal of Animal Ecology*, 88(10), 1486–1497. <https://doi.org/10.1111/1365-2656.13046>

SUPPORTING INFORMATION

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

Table S1. *p*-values of pairwise Wilcoxon test on differences in summed alkaloids between frog populations.

Table S2. Summary of the results of pairwise comparisons of a PERMANOVA in alkaloid composition between *O. sylvatica* populations.

Table S3. Summarized results of estimated marginal means between frog species and prey items. *p*-values were adjusted using Tukey's method.

Table S4. Summary of the results of pairwise correlations between each alkaloid class and ant & mite abundance.

Table S5. Summary of the results of pairwise comparisons of a PERMANOVA in leaf litter ant composition between study sites. *p*-values were adjusted using Tukey's method.

Table S6. Summary of the results of pairwise comparisons of a negative binomial regression comparing ant abundance between *O. sylvatica* populations. *p*-values were adjusted using Tukey's method.

Table S7. *p*-values of pairwise Wilcoxon test on differences in principal components between linear selectivity categories.

How to cite this article: Martin, N. A., Rodríguez, C., Alvarez-Buylla, A., Fiocca, K., Morrison, C. R., Chamba-Carrillo, A., García-Ruilova, A. B., Rentería, J., Tapia, E. E., Coloma, L. A., Donoso, D. A., & O'Connell, L. A. (2025). Poison frog chemical defences are influenced by environmental availability and dietary selectivity for ants. *Journal of Animal Ecology*, 00, 1–15. <https://doi.org/10.1111/1365-2656.70142>