Effects of prey turnover on poison frog toxins: using landscape ecology to assess how biotic interactions affect species phenotypes

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Abstract

Ecological studies of species pairs demonstrated that biotic interactions promote phenotypic change and eco-evolutionary feedbacks. However, we have a limited understanding of how phenotypes respond to interactions with multiple taxa. We investigate how interactions with a network of prey species contribute to spatially structured variation in the skin toxins of the Neotropical poison frog *Oophaga pumilio*. Specifically, we assess how beta-diversity of alkaloid-bearing arthropod prey assemblages (68 ant species) and evolutionary divergence among populations (from a neutral genetic marker) contribute to frog poison dissimilarity (toxin profiles composed of 230 different lipophilic alkaloids sampled from 934 frogs at 46 sites). We show that ant assemblage turnover predicts alkaloid turnover and unique toxin combinations across the range of *O. pumilio*. By contrast, evolutionary relatedness is barely correlated with toxin variation. We discuss how the analytical framework proposed here can be extended to other multi-trophic systems, coevolutionary mosaics, microbial assemblages, and ecosystem services.

Introduction

Phenotypic variation within species, an essential component of evolutionary theory, has received increased attention by ecologists (Bolnick et al., 2011; Vildenes and Langangen, 2015). This interest has been chiefly motivated by evidence showing that phenotypic change, both adaptive and plastic, can happen within contemporary time scales and thus has consequences for ecological processes (Shoener, 2011; Hendry, 2015). Changes in trait frequencies can affect survival and reproduction and ultimately determine population density and persistence of a given species. In turn, these demographic changes may influence community-level and ecosystem functions such as nutrient cycling, decomposition, and primary productivity (Miner et al., 2005; Pelletier et al., 2009; Post and Palkovack, 2009). This interplay between evolutionary and ecological processes, or 'eco-evolutionary dynamics', has brought phenotypes to the center of ecological research (Hendry, 2015).

Several studies focusing on interacting species pairs have demonstrated that population-level phenotypic change can originate from biotic interactions, leading to geographic trait variation within species. For instance, different densities of Killifish predators in streams lead to distinct morphological and life history traits in their Trinidadian guppy prey (Endler, 1995). In western North America, levels of tetrodotoxin resistance in garter snake predators can match toxicity levels in local populations of tetrodotoxin-defended newt prey (Brodie et al., 2002). By focusing on the associations between two species, these studies have provided crucial insights into how interactions can lead to trait divergence and potentially shift the evolutionary trajectory of natural populations (Post and Palkovack, 2009; Hendry, 2015). The resulting trait diversity can have broad ecological consequences, altering the role of species in the ecosystem at a local scale (Palkovacs et al., 2009).

55 However, we still have a limited understanding of how interactions among multiple co-
56 distributed organisms contribute to complex phenotypes, particularly when the set of interacting
57 species and their phenotypes vary geographically. An example of a complex phenotype that is
58 shaped by a network of interactions with many species is the chemical defense system of poison
59 frogs (Dendrobatidae). In this clade of Neotropical amphibians, species can exhibit dozens to
60 hundreds of distinct lipophilic alkaloid toxins in their skin, and aposematic color patterns
61 advertise their distastefulness (Saporito et al., 2012; Santos et al., 2016). Poison composition
62 varies spatially within species such that populations closer in geography tend to have alkaloid
63 profiles more similar to one another than to populations farther away (Mebs et al., 2008; Saporito
64 et al., 2006, 2007a; Stuckert et al., 2014). This geographic variation in toxin profiles has been
65 attributed to local differences in prey availability, because poison frogs obtain their defensive
66 alkaloids from dietary arthropods (Daly et al., 2000; Saporito et al., 2004, 2007b, 2009; Jones et
67 al., 2012). For instance, specific toxins in the skin of a frog may match those of the arthropods
68 sampled from its gut (McGugan et al., 2016). However, individual alkaloids may be locally
69 present in frog skins but absent from the arthropods they consume, and vice-versa; thus, the
70 extent to which frog chemical traits reflect arthropods assemblages remains unclear (Daly et al.,
71 2000, 2002; Saporito et al., 2007b; Jones et al., 2012). Population differences may also stem
72 from an effect of shared evolutionary history, because alkaloid sequestration may be partially
73 under genetic control in poison frogs (Daly et al., 2003, 2005, 2009). These drivers of toxin
74 turnover can have broad consequences for the ecology of poison frogs, because alkaloids protect
75 these amphibians from numerous predators (Darst and Cummings, 2006; Gray et al., 2010;
76 Weldon et al., 2013; Murray et al., 2016), ectoparasites (Weldon et al., 2006), and pathogenic
77 microorganisms (Macfoy et al., 2005).

To address the question of how interactions among multiple organisms contribute to spatially structured phenotypes, we investigate how prey assemblage turnover and evolutionary divergence among populations predict the rich spectrum of toxins secreted by poison frogs. We focus on the well-studied toxin profiles of the strawberry poison frog, *Oophaga pumilio*, which exhibits over 230 distinct alkaloids over its Central American range (Daly et al., 1987, 2002; Saporito et al., 2006, 2007a). First, we develop correlative models that approximate the spatial distribution of toxic ants, a crucial source of alkaloids for poison frogs (Saporito et al., 2012; Santos et al., 2016). Then, we apply Generalized Dissimilarity Modeling (GDM) (Ferrier et al., 2007) to assess the contribution of projected ant assemblage turnover to chemical trait dissimilarity among sites that have been screened for amphibian alkaloids, thus treating these toxins as a "community of traits". To examine the effects of evolutionary relatedness, we implement a Multiple Matrix Regression approach (MMRR) (Wang, 2013) that incorporates not only prey turnover but also genetic divergences between *O. pumilio* populations as inferred from a neutral genetic marker.

Material and Methods

Estimating frog poison composition dissimilarity

As poison composition data of *Oophaga pumilio*, we used lipophilic alkaloid profiles derived from gas chromatography coupled with mass spectrometry of 934 frog skins sampled in 53 Central American sites (Daly et al., 1987, 2002; Saporito et al., 2006, 2007a), as compiled by Saporito et al. (2007a). We georeferenced sampled sites based on maps and localities presented by Saporito et al. (2007a) and other studies of *O. pumilio* (Saporito et al., 2006; Wang and

101 Shaffer, 2008; Brown et al., 2010; Hauswaldt et al., 2011; Gehara et al., 2013). Because
102 haphazard sampling may exaggerate poison composition variation in this dataset, and to
103 maximize alkaloid sampling effort, we combined data from different expeditions to each site,
104 pooling data from individuals. As such, we do not focus on potential short-term individual
105 fluctuations in toxin composition (Saporito et al., 2006), but instead on variation tied to spatial
106 gradients. To match the finest resolution available for the data grid predictors (see below), we
107 combined alkaloid data within a 1 km^2 grid cell. The final dataset included 230 alkaloids from 21
108 structural classes in 46 grid cell sites (Fig. 1) (alkaloid and locality data to be presented in the
109 Supplementary Information 1; presentation of raw data pending manuscript acceptance. See
110 Supplementary Information 2 for decisions on alkaloid identity). To estimate matrices of alkaloid
111 composition dissimilarity (pairwise Sorensen's distances) across frog populations, as well as
112 geographic distances between sites, we used the *fossil* package (Vavrek, 2011) in R v. 3.3.3 (R
113 Development Core Team, 2018).
114
115 *Estimating arthropod prey assemblage dissimilarity*
116 To approximate the spatial turnover of prey available to *Oophaga pumilio*, we focused on
117 alkaloid-bearing ants that occur in (but are not necessarily restricted to) Costa Rica, Nicaragua
118 and Panama, where that frog occurs. Ants are *O. pumilio*'s primary prey type, corresponding to
119 more than half of the ingested volume (Donnelly, 1991; Caldwell, 1996; Darst et al., 2004). This
120 frog also eats a large proportion of mites; however, limited occurrence data and taxonomic
121 knowledge for mite species (e.g., McGugan et al., 2016) precluded their inclusion in our spatial
122 analyses. Following a comprehensive literature search of alkaloid occurrence in ant taxa (Ritter
123 et al., 1973; Wheeler, 1981; Jones et al., 1982a,b, 1988, 1996, 1999, 2007, 2012; Daly et al.,

124 1994, 2000, 2005; Schroder et al., 1996; Spande et al., 1999; Leclercq et al., 2000; Saporito et
125 al., 2004, 2009; Clark et al., 2005; Fox et al., 2012; Chen et al., 2013; Adams et al., 2015;
126 Touchard et al., 2016), we estimated ant composition dissimilarity based on species that belong
127 to genera known to harbor alkaloids, as follows: *Acromyrmex, Anochetus*, *Aphaenogaster*, *Atta,*
128 *Brachymyrmex*, *Megalomyrmex*, *Monomorium*, *Nylanderia*, *Solenopsis*, and *Tetramorium* (see
129 Supplementary Information 2 for decisions on alkaloid presence in ant taxa). Georeferenced
130 records were compiled from the Ant Web database as per June 2017 using the *antweb* R package
131 (AntWeb, 2017). The search was restricted to the continental Americas between latitudes 40ºN
132 and 40ºS. We retained only those 68 ant species that had a minimum of five unique occurrence
133 records after spatial rarefaction (see below); the final dataset included a total of 1,417 unique
134 records. Ant locality data to be presented in the Supplementary Information 3 (presentation of
135 raw data pending manuscript acceptance).

136 Because available ant records rarely matched the exact locations where *O. pumilio*
137 alkaloids were characterized, we modeled the distribution of ant species at the landscape level to
138 allow an estimation of prey composition at sites with empirical frog poison data. We created a
139 species distribution model (SDM) for each ant species using MaxEnt (Phillips et al., 2006) based
140 on 19 bioclimatic variables from the Worldclim v. 1.4 database (Hijmans et al., 2005; available
141 at www.worldclim.org). Before modeling, we used the *spThin* R package (Aiello-Lammens,
142 2015) to rarefy ant records and ensure a minimum distance of 5 km between points, thus
143 reducing environmental bias from spatial auto-correlation (Veloz, 2009; Boria et al., 2014). To
144 reduce model over-fitting, we created a minimum-convex polygon defined by a 100 km radius
145 around each species occurrence points, restricting background point selection by the modeling
146 algorithm (Phillips et al., 2009; Anderson and Raza, 2010).

147 To properly parameterize the individual ant SDMs (Shcheglovitova and Anderson, 2013;
148 Boria et al., 2014), we chose the best combination of feature class (shape of the function
149 describing species occurrence vs. environmental predictors) and regularization multiplier (how
150 closely a model fits known occurrence records) using the *ENMeval* R package (Muscarella et al.,
151 2014). For species with 20 or more records, we evaluated models using k-fold cross-validation,
152 which segregates training and testing points in different random bins (in this case, k = 5). For
153 species with less than 20 records, we evaluated model fit using jackknife, a particular case of k-
154 fold cross validation where the number of bins (k) is equal to the total number of points. We
155 evaluated model fit under five combinations of feature classes, as follows: (1) Linear, (2) Linear
156 and Quadratic, (3) Hinge, (4) Linear, Quadratic and Hinge, and (5) Linear, Quadratic, Hinge,
157 Product, and Threshold. As regularization multipliers, we tested values ranging from 0.5 to 5 in
158 0.5 increments (Shcheglovitova and Anderson, 2013; Brown, 2014). For each model, the best
159 parameter combination was selected using AICc. Based on this best combination, we generated a
160 final SDM for each ant species (parameters used in all SDMs are presented in the Supplementary
161 Information 4).

162 To estimate a matrix of prey assemblage turnover based on ant distributions, we
163 converted SDMs to binary maps using the 10th percentile presence threshold. We then extracted
164 presences for each ant species at sites sampled for frog alkaloids using the *raster* R package
165 (Hijmans et al., 2015). Matrices of estimated ant composition dissimilarity (pairwise Sorensen's
166 distances) were calculated with *fossil* in R. To visualize ant species turnover across the range of
167 *O. pumilio*, we reduced the final dissimilarity matrices to three ordination axes by applying
168 multidimensional scaling using the *cmdscale* function in R. Each axis was then assigned a

separate RGB color (red, green, or blue) as per Brown et al. (2014). A distribution layer for *O. pumilio* was obtained from the IUCN database (available at www.iucn.org).

Modeling alkaloid composition turnover as a function of ant species turnover

To test the hypothesis that toxin composition in poison frogs vary geographically as a function of the composition of prey species, we modeled the turnover of *O. pumilio* alkaloids using a Generalized Dissimilarity Modeling (GDM) approach (Ferrier et al., 2007). GDM is an extension of matrix regression developed to model species composition turnover across regions as a function of environmental predictors. Once a GDM model is fitted to available biological data (estimated from species presences at sampled sites), the compositional dissimilarity across unsampled areas can be estimated based on environmental predictors (available for both sampled and unsampled sites). We implemented GDM following the steps of Rosauer et al. (2014) using the *GDM* R package (Manion et al., 2018).

We used the compiled database of alkaloids per site as the base of our GDMs. As predictor variables, we initially included geographic distance and all 68 individual models of ant species distributions at a 1 km^2 resolution. To select the combination of predictors that contribute the most to GDM models while avoiding redundant variables, we implemented a stepwise backward elimination process (Williams et al., 2012), as follows: first, we built a model with all predictor variables; then, those variables that explained less than the arbitrary amount of 0.1% of the data deviance were removed iteratively, until only those variables contributing more than 0.1% were left.

190 To visualize estimated alkaloid turnover on geographic space from GDM outputs, we
191 applied multidimensional scaling on the resulting dissimilarity matrices, following the procedure
192 outlined above for the ant SDMs.
193
194 *Estimating frog population genetic divergence*
195 To evaluate associations between alkaloid composition and shared evolutionary history
196 between frog populations, we used the *cytochrome B* gene dataset of Hauswaldt et al. (2011),
197 who sampled 197 *O. pumilio* individuals from 25 Central American localities. Because most
198 sites sampled for genetic data are geographically close to sites sampled for alkaloids (Saporito et
199 al., 2007a; Hauswaldt et al., 2011), we paired up alkaloid and genetic data based on Voronoi
200 diagrams. A Voronoi diagram is a polygon whose boundaries encompass the area that is closest
201 to a reference point relative to all other points of any other polygon (Aurenhammer, 1991).
202 Specifically, we estimated polygons using the sites sampled for genetic data as references points
203 and paired them with the sites for toxin data contained within each resulting Voronoi diagram.
204 To estimate Voronoi diagrams, we used ArcGIS 10.3 (ESRI, Redlands). To calculate a matrix of
205 average uncorrected pairwise genetic distances among localities, we used Mega 7 (Kumar et al.,
206 2016). Six genetically sampled sites that had no corresponding alkaloid data were excluded from
207 the analyses.
208
209 *Testing associations between toxin composition, prey assemblage dissimilarity, and population*
210 *genetic divergence*
211 To test the effect of population evolutionary divergence on poison composition of *O.*
212 *pumilio*, we implemented a Multiple Matrix Regression with Randomization (MMRR) approach

213 in R (Wang, 2013). To allow comparisons between genetic divergence and prey composition, we
214 also included a matrix of ant assemblage dissimilarity as a predictor variable in MMRR models.
215 As response variables, we focused on two distinct alkaloid datasets, namely individual alkaloids
216 (n = 230) and alkaloid structural classes (n = 21). Additionally, to account for variation in poison
217 composition resulting from ingestion of arthropod sources other than ants (i.e., mites, beetles,
218 millipedes; Saporito et al., 2009), we performed a second set of analyses restricted to alkaloids (n
219 = 125) belonging to alkaloid classes (n = 9) reported to occur in ant taxa. To assess the statistical
220 significance of MMRR models and predictor variables, 10,000 permutations were used.
221 Matrices of alkaloid composition dissimilarity, estimated ant assemblage dissimilarity,
222 genetic distances between *O. pumilio* populations, and geographic distances between sites, as
223 well as Supplementary Information and R scripts used in all analyses, are available online
224 through GitHub (https://github.com/ivanprates/2019_gh_pumilio).
225
226 **Results**
227
228 *Toxin diversity*
229 Compilation of toxin profiles revealed large geographic variation in the number of
230 alkaloids that compose the poison of *Oophaga pumilio*. The richness of individual alkaloids
231 across sites varied between 7 and 48, while the richness of alkaloid structural classes varied
232 between 3 and 17. Alkaloid composition turnover among sites was high; pairwise Sorensen
233 distances ranged from 0.33 to 1 for individual alkaloids, and from 0.08 to 0.86 for alkaloid
234 structural classes (Sorensen distances vary from 0 to 1, with 0 representing identical
235 compositions and 1 representing no composition overlap across sites). A matrix regression

236 framework using MMRR indicated that geographic distances affected the turnover of both
237 individual alkaloids ($p < 0.0001$; $R^2 = 0.16$; $R = 0.4$) and alkaloid structural classes ($p = 0.003$;
238 $R^2 = 0.09$; $R = 0.29$).
239
240 *Ant composition turnover*
241 Distribution modeling of ant species suggested variation of ant richness over the
242 landscape, with a concentration of species in the central portion of the distribution of *O. pumilio*
243 in Costa Rica and southern Nicaragua (Fig. 2). The estimated number of alkaloid-bearing ant
244 species at sites sampled for toxin profiles ranged between 24 and 52. However, ant richness did
245 not have a significant effect on the number of individual alkaloids (linear regression; $p > 0.22$)
246 (Fig. 3a) or alkaloid structural classes ($p > 0.07$).
247 Estimated ant assemblage turnover was low within the distribution of *O. pumilio* (Fig. 2).
248 Pairwise ant Sorensen distances ranged from 0 to 0.47 at sites sampled for frog alkaloids,
249 reflecting the broad ranges inferred for several alkaloid-bearing ant species. MMRR analyses
250 suggested a significant effect of geographic distances between sites on species turnover of
251 alkaloid-bearing ants ($p < 0.0001$; $R^2 = 0.62$; $R = 0.78$).
252 Projection of ant beta-diversity on geographic space suggested that prey assemblages are
253 similar throughout the southern range of *O. pumilio* in Panama and Costa Rica, with a transition
254 in the northern part of the range in inland Nicaragua (Fig. 2). Inner mid-elevations are expected
255 to harbor ant assemblages that are distinct from those in the coastal lowlands.
256
257 *Generalized Dissimilarity Modeling*

258 Dissimilarity modeling supports the idea that ant assemblage turnover affects the spatial
259 variation of poison composition in *O. pumilio*. A GDM model explained 22.9% of the alkaloid
260 composition turnover when the model included geographic distances among sampled sites. A
261 model that did not incorporate geographic distances explained 20% of alkaloid profile
262 dissimilarity. After eliminating those ant distribution models that contributed very little to the
263 GDM (< 0.1%) using a stepwise backward elimination procedure, only nine out of 68 species
264 were retained in the final model, as follows: *Acromyrmex coronatus*, *Anochetus orchidicola*,
265 *Brachymyrmex coactus*, *Brachymyrmex pictus*, *Monomorium ebeninum*, *Solenopsis azteca*,
266 *Solenopsis bicolor*, *Solenopsis pollux*, and an undescribed *Solenopsis* species (sp. "jtl001"). A
267 GDM model not including geographic distances explained 20% of the observed alkaloid beta-
268 diversity also for this reduced ant dataset.
269 Projection of GDM outputs onto geographic space indicates areas expected to have more
270 similar amphibian alkaloid profiles based on ant turnover (Fig. 4). The results suggest latitudinal
271 variation in poison composition, with a gradual transition from the southern part of the
272 distribution of *O. pumilio* (in Panama) through the central and northern parts of the range (in
273 Costa Rica and Nicaragua, respectively) (pink to purple to blue in Fig. 4). Another major
274 transition in poison composition was estimated across the eastern and western parts of the range
275 of *O. pumilio* in Nicaragua (blue to green in Fig. 4).
276
277 *Multiple Matrix Regressions*
278 In agreement with the GDM results, MMRR analyses supported the hypothesis that
279 spatial turnover of *O. pumilio* toxin profiles is affected by prey composition dissimilarity, in spite
280 of the limited variation of ant assemblages. The estimated turnover of alkaloid-bearing ant

281 assemblages had a significant effect on individual alkaloid composition dissimilarity ($p <$
282 0.0001; $R^2 = 0.13$) (Fig. 3b), a result that changed little when considering only those alkaloids
283 from classes known to occur in ants ($p < 0.0001$; $R^2 = 0.11$). Similarly, estimated alkaloid-
284 bearing ant assemblages had a significant yet weak effect on the dissimilarity of alkaloid
285 structural classes among sites ($p = 0.002$; $R^2 = 0.05$), a result that held when considering only
286 alkaloids classes known to occur in ants ($p = 0.007$; $R^2 = 0.03$).
287 Similar to the effect of ant composition, genetic distances between populations of *O.*
288 *pumilio* had a weak yet significant effect on frog alkaloid composition dissimilarity ($p < 0.0001$;
289 $R^2 = 0.09$) (Fig. 3c). A MMRR model incorporating both frog population genetic distances and
290 ant composition dissimilarity explained around 15% of alkaloid composition variation among
291 populations of *O. pumilio* ($p_{ants} < 0.001$; $p_{gen} = 0.05$).
292
293 **Discussion**
294
295 *Effects of prey turnover on trait variation, and its ecological consequences*
296 Based on estimates of the distribution of alkaloid-bearing ants, we found associations
297 between prey composition gradients and spatial turnover in the defensive chemical traits of the
298 strawberry poison frog, *Oophaga pumilio*. Species distribution modeling supported that the pool
299 of alkaloid sources varies in space. Moreover, GDM and MMRR analyses inferred that alkaloid
300 beta diversity in *O. pumilio* covaries with the composition of ant assemblages. These results are
301 consistent with observations that distinct toxins are restricted to specific arthropod taxa (Saporito
302 et al., 2007a, 2012); prey species with limited distributions may contribute to unique defensive
303 phenotypes in different parts of the range of poison frog species. Accordingly, GDM predicted

304 unique alkaloid combinations in the northern range of *O. pumilio* (Nicaragua) relative to central
305 and southern areas (Costa Rica and Panama) (Fig. 4); that northern region remains poorly known
306 in terms of chemical diversity (Mebs et al., 2008), and future surveys may reveal novel toxin
307 combinations in the poison frogs that occur therein. These results are consistent with the view
308 that biotic interactions play a role in phenotypic variation within species and across space and
309 influence functional diversity in ecological communities (Miner et al., 2005; Pelletier et al.,
310 2009; Post and Palkovack, 2009).

311 Our analyses also indicate that different prey species may have disparate contributions to
312 observed chemical trait variation in poison frogs. After eliminating those ants that contributed
313 very little to the GDM (< 0.1%), only nine out of 68 species were retained in the final model.
314 This result may imply that a few prey items contribute disproportionately to the uniqueness of
315 chemical defenses among populations of *O. pumilio*. Alternatively, it may point to redundancy in
316 the alkaloids provided by prey species to poison frogs. Specifically, it is possible that some of the
317 ant species contributed less to alkaloid beta-diversity when in the presence of a co-distributed
318 and potentially functionally equivalent species, therefore being removed during the GDM's
319 stepwise backward elimination procedure.

320 These results have potential implications for the ecology and evolution of the interacting
321 species. For instance, frogs may favor and seek prey types that provide unique chemicals or
322 chemical combinations, increasing survival rates from encounters with their predators. This idea
323 is supported by evidence that alkaloid quantity, type, and richness result in differences in the
324 perceived palatability of poison frogs to predators (Murray et al., 2017; Bolton et al., 2017).
325 Moreover, because alkaloids vary from mildly unpalatable to lethally toxic (Daly et al., 2005;
326 Santos et al., 2016), the composition of amphibian poisons may affect the survival and behavior

327 of their predators following an attack (Darst and Cummings, 2006). From the perspective of
328 arthropod prey, predator feeding preferences and foraging behavior may affect population
329 densities and dynamics, potentially in a predator density-dependent fashion (Bolnick et al., 2011;
330 Pelletier et al., 2009). Finally, functional redundancy among prey types may confer resilience to
331 the defensive phenotypes of poison frogs, ensuring continued protection from predators despite
332 fluctuations in prey availability. Future investigations of these topics will advance our
333 understanding of how the phenotypic outcomes of species interactions affect ecological
334 processes.
335
336 *Evolutionary relatedness and phenotypic similarity*
337 In addition to the contribution of prey assemblages, the results support that phenotypic
338 similarity between populations also varies with evolutionary relatedness. MMRR analyses
339 indicated that population genetic divergence has a significant effect on alkaloid beta diversity in
340 *O. pumilio*, an effect that may reflect physiological or behavioral differences. For instance,
341 feeding experiments support that poison frog species differ in their capacity for lipophilic
342 alkaloid sequestration and that at least a few species can perform metabolic modification of
343 ingested alkaloids (Daly et al., 2003, 2005, 2009). Additionally, an effect of evolutionary
344 divergence on toxin profiles may stem from population differences in foraging strategies or
345 behavioral prey choice (Daly et al., 2000; Saporito et al., 2007a). Importantly, however, MMRR
346 suggested that genetic divergence accounts for less variation in the defensive phenotypes of *O.*
347 *pumilio* than alkaloid-bearing prey.
348 We employed genetic distances from a neutral marker as a proxy for shared evolutionary
349 history, and do not imply that this particular locus contributes to variation in frog physiology. An

350 assessment of how genetic variants influence chemical trait diversity in poison frogs will rely on
351 sampling of functional genomic variation, for which the development of genomic resources (e.g.,
352 Rogers et al., 2018) will be essential. Nevertheless, our approach can be extended to other
353 systems where information on both neutral and functional genetic variation is available. These
354 systems include, for instance, predators where nucleotide substitutions in ion channel genes
355 confer resistance to toxic prey at a local scale (Feldman et al., 2010), and species where variation
356 in major histocompatibility complex loci drives resistance to pathogens locally (Savage and
357 Zamudio, 2016).
358
359 *Other sources of phenotypic variation*
360 Although we show that prey community composition explains part of the spatial variation
361 in frog poisons, this proportion is relatively small. This result may stem from challenges in
362 quantifying turnover of prey species that act as alkaloid sources. Due to restricted taxonomic and
363 distribution information, we estimated species distribution models only for a set of well-sampled
364 ant species and were unable to include other critical sources of dietary alkaloids, particularly
365 mites (Saporito et al., 2012; McGugan et al., 2016). Although not being able to incorporate a
366 substantial fraction of the prey assemblages expected to influence poison composition in *O.*
367 *pumilio*, the GDM was able to explain about 23% of poison variation in this species. This
368 proportion is likely to increase with the inclusion of additional species of ants and other alkaloid-
369 bearing prey taxa, such as mites, beetles, and millipedes (Daly et al., 2000; Dumbacher et al.,
370 2004; Saporito et al., 2003).
371 Limitations in our knowledge of arthropod chemical diversity may also have affected the
372 analyses. Contrary to our expectations, we found a slight decrease in the explanatory value of

373 models that incorporated only alkaloids from structural classes currently known to occur in ants,
374 a result that may reflect an underestimation of ant chemical diversity. For instance, mites and
375 beetles are thought to be the source of tricyclics to Neotropical poison frogs, but the discovery of
376 these alkaloids in African Myrmicinae ants suggests that they may also occur in species from
377 other regions (Schroder et al., 1996). As studies keep describing naturally occurring alkaloids,
378 we are far from a complete picture of chemical trait diversity in both arthropods and amphibians
379 (Saporito et al., 2012).

380 This study demonstrates that incorporating species interactions can provide new insights
381 into the drivers of phenotypic diversity even when other potential sources of variation are not
382 fully understood. It is possible that frog poison composition responds not only to prey
383 availability but also to alkaloid profiles in prey, since toxins may vary within arthropod species
384 (Daly et al., 2002; Saporito et al., 2004, 2007b; Dall'Aglio-Holvorcem et al., 2009; Fox et al.,
385 2012). There is evidence that arthropods synthesize their alkaloids endogenously (Leclercq et al.,
386 1996; Camarano et al., 2012; Haulotte et al., 2012), but they might also sequester toxins from
387 plants, fungi, and symbiotic microorganisms (Saporito et al., 2012; Santos et al., 2016).
388 Accounting for these other causes of variation will be a challenging task to chemical ecologists.
389 An alternative to bypass knowledge gaps on the taxonomy, distribution, and physiology of these
390 potential toxin sources may be to focus on their environmental correlates. A possible extension
391 of our approach is to develop models that incorporate abiotic predictors such as climate and soil
392 variation, similarly to investigations of community composition turnover at the level of species
393 (Ferrier et al., 2007; Zamborlini-Saiter et al., 2016).
394
395 *Eco-evolutionary feedbacks*

396 Associations between prey assemblage composition and predator phenotype provide
397 opportunities for eco-evolutionary feedbacks, the bidirectional effects between ecological and
398 evolutionary processes (Post and Palkovacs, 2009). In the case of poison frogs, there is evidence
399 that toxicity correlates to the conspicuousness of skin color patterns (Maan and Cummings, 2011),
400 which therefore act as aposematic signals for predators (Hegna et al., 2013). However, coloration
401 patterns also mediate assortative mating in poison frogs (Maan and Cummings, 2009; Crothers and
402 Cummings, 2013). When toxicity decreases, the intensity of selection for aposematism also
403 decreases, and mate choice may become a more important driver of color pattern evolution than
404 predation (Summers et al., 1999). Divergence due to sexual selection may happen quickly when
405 effective population sizes are small and population gene flow is limited, which is the case in *O.*
406 *pumilio* (Gehara et al., 2013). By affecting chemical defenses, it may be that spatially structured prey
407 assemblages have contributed to the vast diversity of color patterns seen in poison frogs. On the other
408 hand, our GDM approach inferred similar alkaloid profiles between populations of *O. pumilio*
409 that have distinct coloration patterns (Fig. 4). However, it may be challenging to predict frog
410 toxicity from chemical profiles (Daly et al., 2005), and future studies of this topic will advance
411 our understanding of the evolutionary consequences of poison composition variation.
412
413 *Concluding remarks*
414 Integrative approaches have demonstrated how strong associations between species can
415 lead to tight covariation among species traits, with an iconic example being that of predator and
416 prey species that engage in "coevolutionary arms races" (Thompson, 2005). However, it may be
417 harder to assess the outcomes of weaker interactions among multiple co-distributed organisms
418 (Anderson, 2017). The chemical defense system of poison frogs varies as a function of prey

assemblages composed of tens of arthropod species, each of them providing single or a few toxin molecules (Daly et al., 2005). Not surprisingly, it has been hard to predict the combinations of traits emerging from these interactions (Santos et al., 2016). The landscape ecology framework presented here approaches this problem by incorporating data on biotic interactions throughout the range of a focal species, a strategy that has also improved correlative models of species distributions (Lewis et al., 2017; Sanín and Anderson, 2018). Our framework can be extended to a range of systems that have similar structures, including other multi-trophic interactions (Van der Putten et al., 2001; Del-Claro, 2004; Scherber et al., 2010), geographic coevolutionary mosaics (Greene and McDiarmid, 1981; Mallet et al., 1995; Symula et al., 2001), microbial assemblages (Zomorrodi and Maranas, 2012; Landesman et al., 2014), and ecosystem services (Moorhead and Sinsabaugh, 2006; Allison, 2012; Gossner et al., 2016). Integrative approaches to the problem of how spatially structured biotic interactions contribute to phenotypic diversity will continue to advance our understanding of the interplay between ecological and evolutionary processes.

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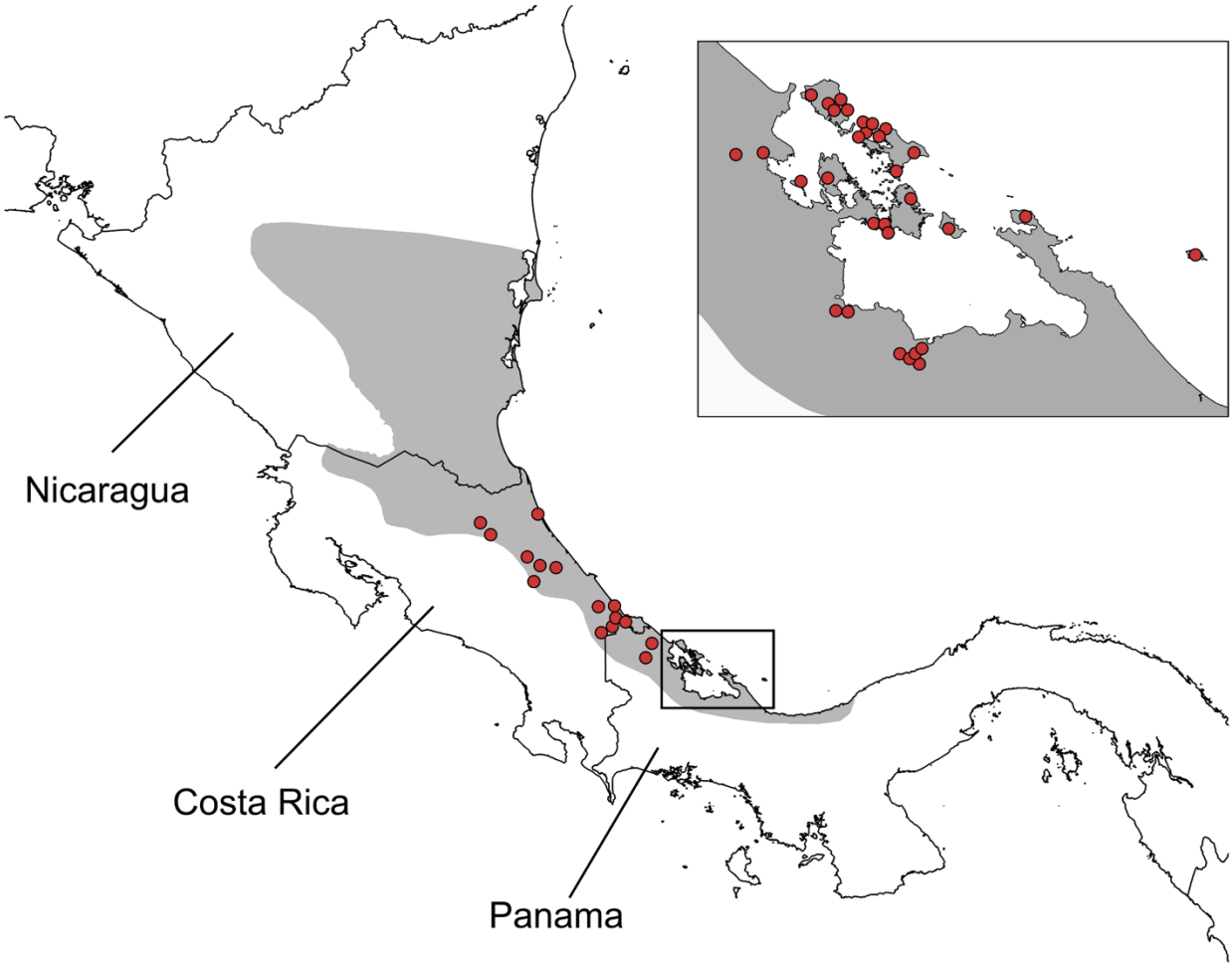
754 **Figure 1.** Sites sampled for skin alkaloid profiles of the strawberry poison frog, *Oophaga*

755 *pumilio*. Each site corresponds to a 1 km^2 grid cell, matching the resolution of environmental

756 predictors. Original alkaloid data compiled by Saporito et al. (2017a). The distribution of *O.*

757 *pumilio* is indicated in dark grey.

758



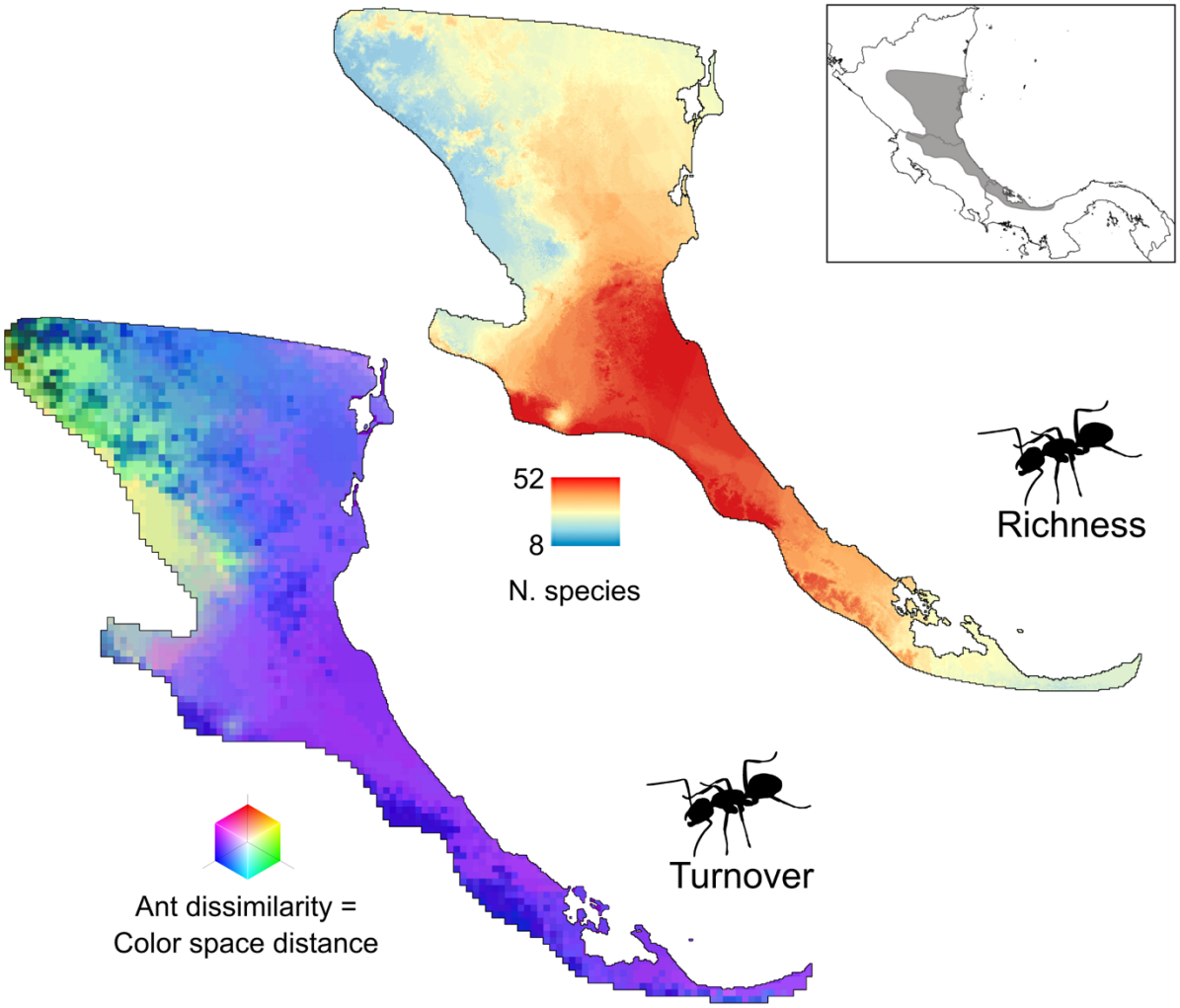
759

760 **Figure 2.** Estimated species turnover (left) and richness of prey assemblages across the range of

761 the poison frog *Oophaga pumilio* based on projected distributions of 68 ant species from

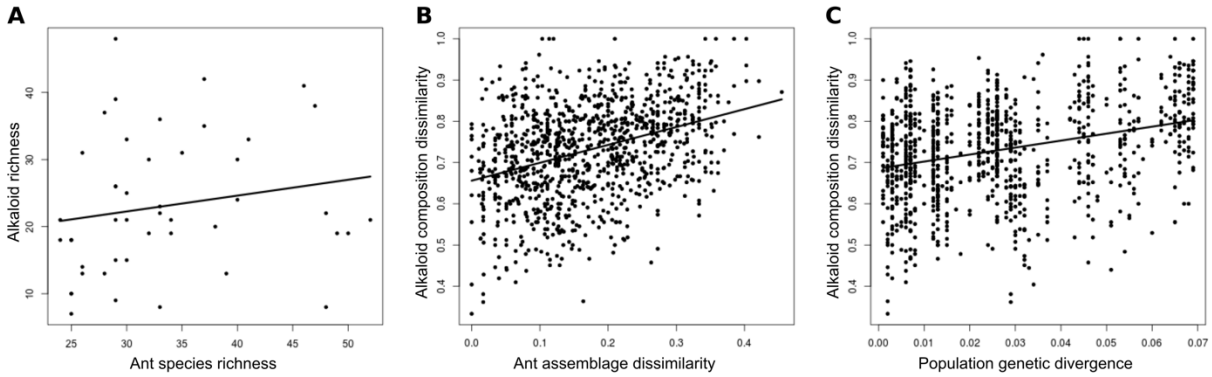
762 alkaloid-bearing genera. Inset indicates the natural range of *O. pumilio* (dark grey).

763



764

765 **Figure 3.** Alkaloid richness in *Oophaga pumilio* as a function of ant assemblage richness across
766 sites (A); alkaloid dissimilarity in *O. pumilio* as a function of ant assemblage dissimilarity across
767 sites (B); and alkaloid dissimilarity in *O. pumilio* as a function of population genetic divergence
768 based on a neutral marker (C). Relationships in B and C are statistically significant; significance
769 was estimated from a Multiple Matrix Regression with Randomization (MMRR) approach (see
770 text).
771



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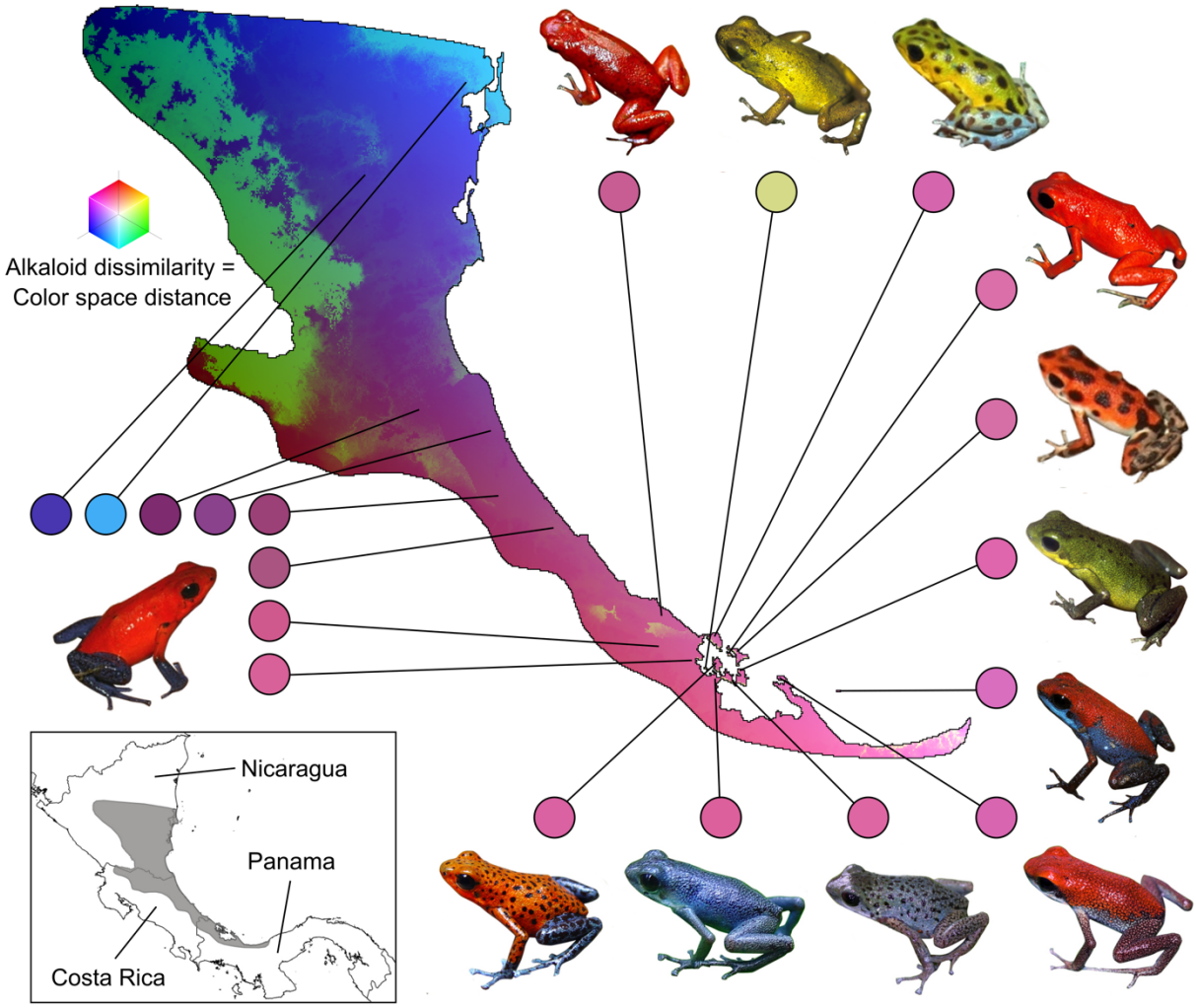
Figure 4. Estimated composition dissimilarity of alkaloid profiles across the range of *Oophaga pumilio* as a function of spatial turnover of alkaloid-bearing ant species, from a Generalized Dissimilarity Modeling approach (GDM). Similar colors on the map indicate similar estimated alkaloid profiles. Pictures indicate dorsal skin coloration patterns in *O. pumilio*. Inset indicates the natural range of *O. pumilio* (dark grey).