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# Chemical similarity and local community assembly in the species rich tropical genus *Piper*

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**Abstract.** Community ecologists have strived to find mechanisms that mediate the assembly of natural communities. Recent evidence suggests that natural enemies could play an important role in the assembly of hyper-diverse tropical plant systems. Classic ecological theory predicts that in order for coexistence to occur, species differences must be maximized across biologically important niche dimensions. For plant–herbivore interactions, it has been recently suggested that, within a particular community, plant species that maximize the difference in chemical defense profiles compared to neighboring taxa will have a relative competitive advantage. Here we tested the hypothesis that plant chemical diversity can affect local community composition in the hyper-diverse genus *Piper* at a lowland wet forest location in Costa Rica. We first characterized the chemical composition of 27 of the most locally abundant species of *Piper*. We then tested whether species with different chemical compositions were more likely to coexist. Finally, we assessed the degree to which *Piper* phylogenetic relationships are related to differences in secondary chemical composition and community assembly. We found that, on average, co-occurring species were more likely to differ in chemical composition than expected by chance. Contrary to expectations, there was no phylogenetic signal for overall secondary chemical composition. In addition we found that species in local communities were, on average, more phylogenetically closely related than expected by chance, suggesting that functional traits other than those measured here also influence local assembly. We propose that selection by herbivores for divergent chemistries between closely related species facilitates the coexistence of a high diversity of congeneric taxa via apparent competition.

**Key words:** *apparent competition; coexistence; Costa Rica; defense; herbivory; La Selva; lowland wet forest; secondary compounds; tropics.*

## INTRODUCTION

A classic goal of ecology has been to understand the processes that determine species community assembly (Weiher and Keddy 1999). Current theory predicts that the assembly of species at the local scale is determined by two major ecological processes: the interaction between a species and its abiotic environment, and interactions among the species themselves (Götzenberger et al. 2012). Within the same trophic level, theory also predicts that species that are more similar to each other will be less likely to coexist than more dissimilar species, due to competition for a limited set of resources (Wright 2002, Götzenberger et al. 2012). This mechanism, generally known as “species-limiting similarity” (Darwin 1859, MacArthur and Levins 1967), has been considered to be one of the major processes responsible for structuring biological communities at the local scale.

Numerous studies have shown that local species composition is constrained to those from the regional species pool that are most phenotypically or ecologically divergent (e.g., Cavender-Bares et al. 2004, Maherali and Klironomos 2007, Cooper et al. 2008, Kraft et al. 2008, Cornwell and Ackerly 2009, Graham et al. 2009, Ingram and Shurin 2009, Kursar et al. 2009, Maherali and Klironomos 2012, Sedio et al. 2012, Coley and Kursar 2014). In the Neotropics, however, one may find numerous closely related species co-occurring. For example, as many as 64 species of *Piper* are recorded to co-occur at a Peruvian lowland location (Marquis 2004). These very diverse local species assemblages from hyper-diverse plant groups (also known as “species swarms,” sensu Gentry [1982]) would seem to challenge the limiting similarity hypothesis. In many of these genera (e.g., *Piper* and *Peperomia* [Piperaceae], *Miconia* and *Clidemia* [Melastomataceae], *Elaphoglossum* [Dryopteridaceae], *Psychotria* [Rubiaceae], *Bursera* and *Protium* [Burseraceae], *Shorea* [Dipterocarpaceae], and *Inga* [Mimosaceae]), there appears to be insufficient morphological and functional differentiation to explain local coexistence (Coley and Kursar 2014).

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Currently, one of the most common strategies to assess species similarity is to describe their functional traits (McGill 2006). This approach measures species characteristics that determine the quantitative and qualitative use of a specific set of available resources, including enemy free space (Swenson 2013). Species with similar values of functional traits are expected to undergo stronger competitive interactions than species that differ more in said traits.

For plants, one of the most important functional traits is secondary chemistry (Hartmann 2007). While other functional traits are related to the use of a single resource, secondary metabolites can be associated with numerous critical functions for plants: protection against abiotic factors (Wahid et al. 2007), reproduction (pollinator and seed disperser attraction; Kessler and Baldwin 2007), competition via allelopathy (Ridenour and Callaway 2001), and plant defense (e.g., direct and indirect defense against herbivores and pathogens; Pusztahelyi et al. 2015). Given that plant secondary chemistry can play a critical role in plant–natural-enemy interactions, it is reasonable to expect that chemical similarity between sympatric taxa can also play an important role in determining species coexistence. The reason for this is that plant species with similar chemistries will share herbivores. As a result, they will suffer higher herbivore attack when co-occurring, i.e., they will experience apparent competition (indirect competition via a shared enemy; Holt and Lawton 1993).

From an evolutionary perspective, trait-based studies of species coexistence also provide a unique opportunity to assess the role of species evolutionary histories on patterns of community assembly, as well as to explore phylogenetic patterns of niche and trait evolution (Webb et al. 2002, Kembel and Hubbell 2006, Losos 2008, Cavender-Bares et al. 2009). An excellent example of this interdisciplinary approach for plant species coexistence is that by Kursar et al. (2009), who focused on secondary compounds as they confer defense against herbivores. They demonstrated that coexisting species of *Inga* (a hyper-diverse tropical taxon) were more different in their secondary chemical defenses than expected by chance. Moreover, they showed a lack of phylogenetic signal in these chemical defenses. Their results suggest the presence of divergent selection on anti-herbivore chemical defenses, and that such divergence is likely to play a pivotal role in structuring community assembly. Here, we address whether or not the results for *Inga* are generalizable to another species-rich genus, *Piper*.

Specifically, we assess the role that (1) plant secondary chemistry and (2) plant phylogeny play for species coexistence of *Piper* in a low land tropical forest. We apply both a species-pair and a community-based approach. We use gas chromatography mass spectroscopy (GC-MS) to assess chemical similarity across a range of secondary metabolite groups. We predicted that (1) local assemblages of *Piper* would consist of species more different in secondary metabolites than expected by chance and (2) chemistry profiles of individual species would be influenced by evolutionary history.

## MATERIALS AND METHODS

### *Site and system*

This study was conducted at the La Selva Biological Station in Costa Rica (operated and owned by the Organization for Tropical Studies [OTS]) between May and August 2007. Located in the Atlantic lowlands of Puerto Viejo de Sarapiquí (Heredia), the station possesses more than 1,600 ha of tropical wet forest and receives approximately 4,000 mm of rainfall annually. Currently, 1,850 species of vascular plants have been cataloged in La Selva, 50 of which are in the genus *Piper*.

*Piper* is a pantropical genus with approximately 1,000 species in the Neotropics (Jaramillo 2006). The natural geographic range of the genus in the New World is from northern Mexico to northern Argentina. *Piper* species are very abundant in low- and mid-elevation forests (but rarely reaching 2,500 m) and are often among the most speciose plant genera in Neotropical forests understories (Gentry 1982, Marquis 2004). Most *Piper* species at La Selva occur in discrete, multi-species patches that can contain up to 21 different species (Salazar et al. 2012). *Piper* secondary metabolite diversity has been extensively studied and there is an important body of published methods for compound isolation, chemical profiling, and artificial synthesis (Parmar et al. 1997, 1998, Kato and Furlan 2007). Furthermore, the effects of their secondary chemistry on herbivores, pathogens, and seed dispersal are well documented (Dyer et al. 2001, 2004, Mikich et al. 2003, Fincher et al. 2008, Marques et al. 2010, Richards et al. 2015).

### *Species coexistence*

To assess *Piper* species coexistence, we sampled 81 natural multi-species patches of *Piper* throughout the primary forest of La Selva. We established transects parallel to the station trails to locate naturally occurring *Piper* patches. All transects were between 50 and 100 m from the trail and a minimum 250 m from each other. To standardize the size of each plot sampled, a 10 m radius plot was set up in every patch. In each plot, we counted the number of individuals of *Piper* 1 cm or greater in diameter at ground level; all *Piper* plants were identified to species.

To assess species coexistence, we used two complementary indices. First, we calculated species co-occurrence *c* score index for all species pairs (based on presence/absence data; Stone and Roberts 1990). Second, to take into account the effect of species abundance, we assessed the degree of species coexistence for all species pairs using a niche overlap approach. Here, plot was considered as the studied niche and we used plot occupancy as a measure of niche use. Occupancy was calculated for all *Piper* species as the relative abundance of each species in every sampled plot. Finally, niche overlap was calculated using Pianka's Index (Pianka 1973) based on 1,000 iterations. Both measures of species coexistence were

calculated using EcoSim 7.1 (Gotelli and Entsminger 2012) and yielded two pair-wise species matrices, one for the co-occurrence *c* score and another for Pianka's niche overlap index.

#### *Chemical similarity*

We collected leaf material from young, fully expanded leaves for all *Piper* species sampled in the plots; samples were obtained from at least four individuals of each species. Additionally, all samples selected had similar herbivore damage (between 5% and 10% leaf area missing, with damage assessed visually). Samples were immediately dried with silica gel after collection and transported to the University of Missouri, St. Louis for chemical analysis. Although it is possible that some high volatility compounds were affected during field collections, all samples were treated identically and therefore, all possible compound loss was likely to be systematic across all samples and species. From each sample, 0.4 g of material was ground under liquid nitrogen. To extract a broad range of secondary metabolites (polar and non-polar), samples were extracted using 1.5 mL of 1:1 methanol-chloroform solution. As an internal standard, 0.1 mg of piperine was added to all samples. Samples were finally filtered (0.2  $\mu$ ) and stored in volatile organic compound (VOC) vials at  $-80^{\circ}\text{C}$  until analysis.

Qualitative chemical analysis of the extracts was performed using GC-MS (HP 5890 coupled with a quadrupole Model 5988A mass detector, Hewlett Packard, USA) with helium as a carrier gas and a HP-5 capillary column (Hewlett Packard, USA) (30 m, 0.32 mm ID, 0.25  $\mu\text{m}$ ; see details for chromatographic conditions in the Supporting Information). It is important to note that because this study focuses only on compounds that are detectable with GC-MS, our chromatographic technique will not detect all secondary compounds that might affect plant herbivore interactions. Nevertheless, a review of more than 3500 records of secondary compounds found in *Piper* from NAPRALERT (Natural Products Alert Database; Loub et al. 1985; data *available online*)<sup>5</sup> showed that GC-MS-detectable compounds account for more than 86% of all *Piper* secondary metabolites (Appendix S1; Fig. S1). Therefore, our approach is likely to capture a significant percentage of the total secondary chemical diversity of *Piper* species. Because the abundance of secondary compounds can vary between individuals due to factors such as induction, genetic variability, and resource availability, we only use presence and absence data of chromatographic features. We assessed chemical similarity between all sampled species by building a mass spectra library containing all chromatographic features for each species (one library per species). The libraries of each species were then cross-referenced across all species using AMDIS (Automated Mass Spectral Deconvolution and Identification System)

to identify common as well as unique features based on mass spectra, molecular weight, and expected retention time (Stein 1999, Stein et al. 2005). It is important to underline that this particular approach can assess chemical similarity among species independently of chemical compound identification (for a proof of the concept of this approach, see Salazar [2013]). This methodology yielded a species-pair matrix of chemical similarity between all sampled *Piper* species.

Using the chemical similarity data from the above methodology, we performed a hierarchical clustering analysis (Ward's algorithm, R package pvclust; Suzuki and Shimodaira 2006, R Core Team 2012) to construct a species chemical dendrogram. Subsequently, we extracted a species-pair matrix of chemical distances from the dendrogram. Additionally, we applied a principal component analysis (JMP 10.0; SAS Institute 2007) to the data on chromatographic features to generate continuous values of chemical diversity for the plant species in order to calculate the phylogenetic signal of overall chemical diversity across our *Piper* species pool (see *Phylogenetic analysis in Materials and methods*).

Finally, the mass spectra of the different compounds in the samples were compared with NIST/EPA/NIH and MassBank Databases (Horai et al. 2010) as well as primary literature. Metabolites that did not have a match from the available mass spectra databases were classified as unknown.

#### *Phylogenetic analysis*

We constructed a phylogenetic tree of all study species to determine the influence of phylogeny on chemical similarity. Samples of leaves were collected to perform sequencing of the *ITS* and the chloroplast intron *psbJ-petA* for phylogenetic analysis (following Jaramillo et al. [2008]). Finally, to (1) control for phylogenetic non-independence of the chemical similarity between the sampled species and (2) to assess the effect of phylogenetic relatedness on species coexistence, we used the Picante R package (Kembel et al. 2010, R Core Team 2012) to generate a species-pair matrix of phylogenetic distances based on branch lengths.

#### *Statistical analysis*

To determine the effect that chemical similarity had on *Piper* species coexistence, we used two complementary approaches. First we used a species-pair approach to ascertain the effect that individual *Piper* species (thus specific species chemical compositions) had on species coexistence. Second, we used a community-based approach to quantify the combinatory or cumulative effect of multiple *Piper* species on local species coexistence.

*Species-pair approach.*—To assess the correlation between species coexistence and chemical similarity, we performed (1) a Mantel test and (2) a partial Mantel test

<sup>5</sup> <https://www.napralert.org/>

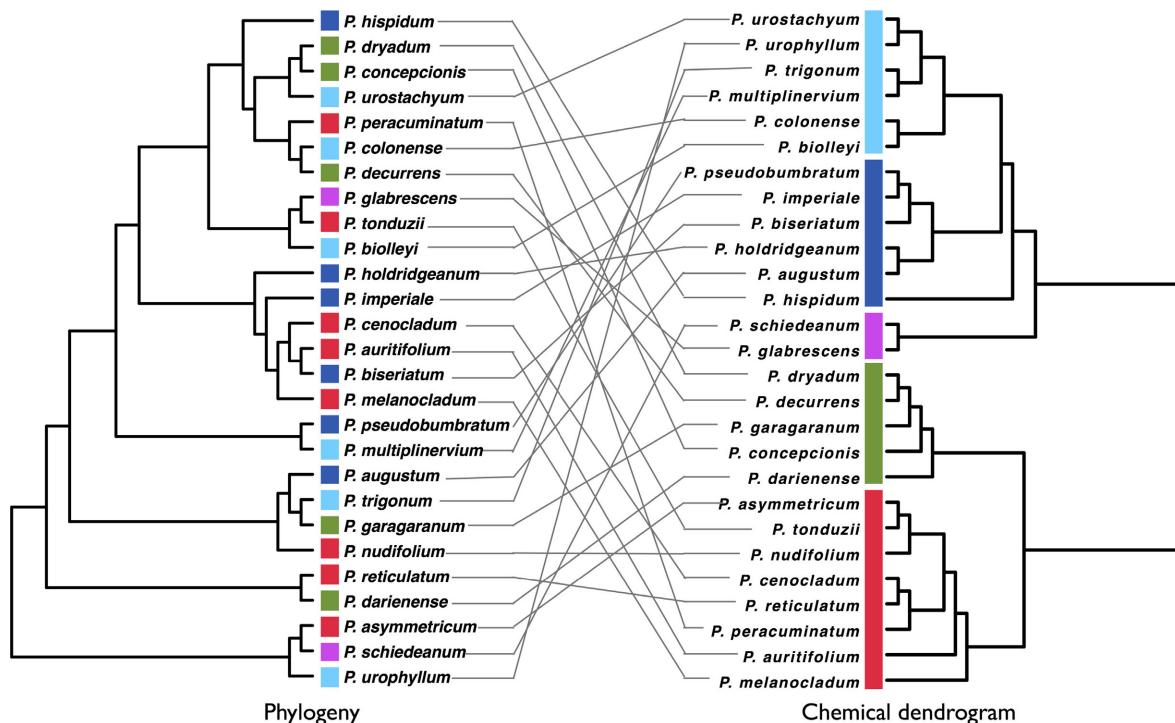


FIG. 1. Comparison of the phylogenetic tree (left) and the chemical dendrogram (right) of the 27 *Piper* species sampled across the 81 natural patches. A description of the chemical characteristics of the five *Piper* chemical clusters is included in the Appendix S1: Fig. S1. [Color figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

controlling for phylogenetic non-independence (10,000 permutations each). Each analysis was conducted with both measures (*c* score index and Pianka's Index) of species coexistence. Additionally, a simple Mantel test between species coexistence and phylogenetic distances was performed to quantify the relationship between phylogenetic similarity and coexistence (10,000 permutations; PASSaGE 2.0; Roseberg and Anderson 2011).

**Community-based approach.**—We quantified the community phylogenetic and chemical over/underdispersion using (1) the Inverse Nearest Relative Index (-NRI), which measures tree-wide patterns of clustering, as well as (2) the Inverse Nearest Taxon Index (-NTI), which assesses clustering independently of deeper node clustering patterns (Webb et al. 2002, Webb and Donoghue 2005, Kembel and Hubbell 2006). To determine community chemical over/underdispersion we used the chemical dendrogram data as input for the analysis. Negative values of -NTI and -NRI indicate that similar species (phylogenetically and also, in this case, chemically) co-occur more than expected by chance; positive values indicate that similar species are not likely to co-occur. The randomization to generate null communities was done by shuffling phylogeny and chemical dendrogram tip labels in order to calculate the standardized effect sizes for -NRI and -NTI (abundance weighted model,  $n = 1,000$  per community; Picante package).

Additionally, we used the first principal component of the PCA derived from the presence/absence chemical data to calculate the Bloomberg's *K* for phylogenetic signal of secondary chemistry over our focal species (Picante package).

## RESULTS

We sampled a total of 2,035 individuals from 27 species of *Piper* across the 81 sampled plots (Appendix S1: Table S1). The number of individuals present per plot was  $25.2 \pm 1.1$  (mean  $\pm$  SE; max–min = 4–51), and the number of *Piper* species per plot was  $5.2 \pm 1.4$  (max–min = 3–11).

The GC-MS analysis yielded more than 1,100 chromatographic features. Approximately 40% of all features were present in all *Piper* species (e.g., phytol, stigmasterol, sitosterol, and tocopherol). Because these shared features were non-informative and most likely related to plant primary chemistry, they were not used for the clustering analysis. Among the remaining features, we found a great diversity of terpenes, phenylpropanoids, some lignans, flavonoids, and alkaloids (Appendix S1: Table S2). The hierarchical clustering showed five discrete chemical clusters (Fig. 1 and Appendix S1: Fig. S2).

Phylogenetic analysis yielded a local species phylogeny that concurs with the current phylogenetic *Piper* data (Appendix S1: Fig. S3). We did not find a strong phylogenetic signal for secondary chemical composition in our

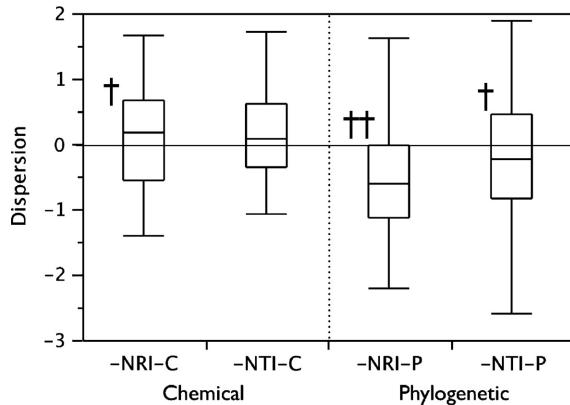


FIG. 2. Standardized chemical and phylogenetic community dispersion measured as Inverse Nearest Relative Index (-NRI) and Inverse Nearest Taxon Index (-NTI). Values above 0 indicate overdispersion and values below 0 indicate underdispersion. Dagggers indicate values significantly different than 0 under a null model (communities assembled at random; † < 0.05, ‡ < 0.005). Box shows 95% confidence intervals, vertical bars show full data range, middle box line shows group average, bar across plot represents grand mean.

focal species ( $K = 0.03$ ). This can be clearly seen in Fig. 1. The small value of  $K$  ( $K < 1$ ) suggests that closely related species are less similar in their secondary chemistry than expected under a Brownian motion model of evolution. Nonetheless, a randomization test showed that  $K$  was not significantly different from 1 ( $Z_{PIC} = -0.51$ ,  $P_{PIC} = 0.40$ ).

For species coexistence, the species-pair approach showed a significant positive relationship between species chemical distance and the likelihood of species co-occurrence for both species presence/absence data (Gotelli's  $c$  score; Mantel test,  $r = 0.20$ ,  $P = 0.0001$ ) and abundance-weighted data (Pianka's index; Mantel test,  $r = 0.17$ ,  $P = 0.0014$ ). Similar results were obtained when controlling for phylogenetic non-independence: presence/absence co-occurrence (partial Mantel test,  $r = 0.20$ ,  $P = 0.002$ ) and abundance-weighted co-occurrence (partial Mantel test,  $r = 0.18$ ,  $P = 0.0015$ ). Both results suggest that chemically distinct species are more likely to co-occur. In contrast, we found a significant negative relationship between species phylogenetic distance and presence/absence co-occurrence ( $r = -0.16$ ,  $P = 0.01$ ). Thus, more closely related species are more likely to co-occur. However, there was no significant relationship between phylogenetic distance and abundance-weighted species co-occurrence ( $r = 0.03$ ,  $P = 0.54$ ).

In our community-based approach, we found that *Piper* species were, on average, more overdispersed with respect to their secondary chemical composition than expected by chance. However, only -NRI was significantly different from zero (-NRI,  $t = 1.83$ ,  $df = 80$ ,  $P = 0.03$ ; -NTI,  $t = 0.77$ ,  $df = 80$ ,  $P = 0.22$ ). In contrast, species composition within the plots was phylogenetically underdispersed. Both -NRI and -NTI were significantly different from zero ( $t = -5.24$ ,  $df = 80$ ,  $P = 0.0001$  and  $t = -2.26$ ,  $df = 80$ ,  $P = 0.01$ , respectively; Fig. 2).

## DISCUSSION

In this study, we found that both chemical and phylogenetic similarity can have significant yet contrasting effects on species coexistence. Results from both of our approaches (species-pair and community-based) showed that *Piper* species with higher secondary chemical similarity were less likely to coexist in the same *Piper* patch than communities assembled at random. In contrast, closely related species were more likely to coexist in the same patch. Furthermore, counter to one of our original predictions, we found that the overall composition in secondary metabolites was not phylogenetically conserved for the 27 studied *Piper* species.

In our species-pair approach, we found that, independent of the measure of coexistence used, chemical similarity had a significant negative effect on species coexistence. This result is consistent with patterns found for two other available studies of Neotropical species-rich genera, *Bursera* (Burseraceae; Becerra 2007) and *Inga* (Mimosaceae; Kursar et al. 2009). As in our case, *Inga* and *Bursera* species were less likely to coexist with conspecifics that had similar secondary metabolite composition. By controlling for phylogenetic non-independence, our results also suggest that the effect that chemical composition has on community assembly is not the result of chemical similarity due to common ancestry.

Phylogenetic distance was also found to be important for *Piper* community assembly. Contrary to the effect of chemical similarity, *Piper* species that were closely related were more likely to coexist in a particular patch. This pattern is likely the result of other unmeasured traits that, unlike chemical similarity, are strongly conserved across the phylogenetic history of our target *Piper* species. One possibility could be a strong environmental niche conservatism in which closely related species are more likely to have similar habitat preferences (Daws et al. 2002, Sedio et al. 2013). Furthermore, given that phylogenetic similarity was only significant for our presence/absence coexistence measures but not for the abundance weighted data, it seems probable that these unknown traits are not necessarily associated with ecological interactions that are density-dependent or that provide *Piper* species a strong competitive advantage. Additional evidence supporting this hypothesis can be found by close examination of the phylogenetic patterns of species abundances. In our data, the most abundant *Piper* species found in our plots were all from different sub-clades of the *Piper* phylogeny (*P. trigonum*, 346 individuals, Peltobryon clade; *P. multiplinervium*, 294 individuals, Pothomorphe clade; *P. urostachyum*, 240 individuals, Radula clade; *P. cenocladum*, 227 individuals, Macrostachys clade). Therefore, given that the most locally abundant *Piper* species are not closely related, these phylogenetically conserved traits are not likely to have a strong impact on interspecific interactions.

Our community-level results concur with those of the species-pair approach. Patches showed a significant overdispersion in terms of *Piper* chemical composition

(positive values of -NRI and -NTI, Fig. 2) suggesting that species within a patch are less chemically similar than expected by chance. Furthermore, these results suggest that secondary metabolite composition is also important for the community assembly of complex multi-species patches. Nevertheless, the fact that this pattern was only found to be significant for -NRI suggests that the effect of chemical composition on community assembly is stronger between *Piper* species from different sub-clusters of the chemical dendrogram. Given that the major sub-clusters in our dendrogram differ mainly in terms of the richness of compounds from specific secondary metabolite groups (e.g., flavonoids, amides, phenylpropanoids; see Appendix S1: Fig. S1), it is likely that the effect of chemical composition on community assembly could be largely driven by differences with respect to representation from these major metabolite groups rather than by the presence or absence of each. It is important to note that, while the average value of -NRI is significantly different from zero, some plots showed chemical underdispersion. Thus, caution should be exercised in their interpretation. Nevertheless, given that the species-pair approach confirmed a general trend, we consider these results to be informative.

Conversely, *Piper* patches showed a significant phylogenetic underdispersion (negative values for -NRI and -NTI, Fig. 2). This suggests that *Piper* species in a patch are more closely related than expected by chance, a pattern that also agrees with the species-pair approach. Nonetheless, phylogenetic underdispersion was significant for both -NRI and -NTI, a result that supports the idea that the effect of phylogeny on community assembly is associated with strongly conserved traits not measured in this study.

Although we did not test the efficacy of secondary metabolites as anti-herbivore and anti-pathogen defenses, we believe that plant-natural-enemy interactions are responsible for a great proportion of the effect that chemical composition has on community assembly (Coley and Kursar 2014). Most of the chemicals found by our analysis are well known to confer direct and indirect anti-herbivore protection to plants (Appendix S1: Fig. S1). Furthermore, a recent paper by Richards et al. (2015) showed that secondary chemical diversity of all species used for this study predicts *Piper* herbivore species richness, leaf damage, and parasitoid attack at the La Selva Biological Station. Although Richards et al. (2015) measured chemical diversity as the diversity of chemical functional groups per *Piper* species, their findings strongly support the link between chemical plant species complexity and plant herbivore interactions.

Our results suggest that *Piper* species that are chemically dissimilar from those already present in a patch would be more likely to colonize and persist within the patch. The colonizing species would have to be sufficiently chemically distinct so as to prevent a potential increase in the number of shared herbivores with other plant hosts already in the community. This successful colonization is likely to be mediated via four distinct

mechanisms. First, the abundance of the colonizing species would initially be low resulting in a low initial abundance of herbivores (the resource concentration hypothesis; Root 1973). Second, the colonizing *Piper* species is less likely to be chemically compatible with the metabolic constraints of the local herbivores. Third, because of the increase in local semiochemical complexity, extant herbivores are likely to suffer chemical disorientation (Zhang and Schlyter 2003). And fourth, if the colonizing species does not share herbivores with plants already present, the novel species would be less likely to be the victim of apparent competition (Holt and Lawton 1993). All advantages acting in concert could facilitate the coexistence of multiple, closely related, yet chemically distinct species. For these mechanisms to function, host chemical similarity should be correlated with host herbivore similarity. In other words, chemically similar plant species must share herbivore species, while chemically distinct plants must have unique herbivore species. Richards et al. (2015) provide some evidence that differences in chemistry among *Piper* species determines the nature each plant species' herbivore fauna.

We acknowledge that, although community chemical similarity is likely to have a significant effect on plant-herbivore interactions, the potential role that plant pathogens could play on mediating coexistence through plant chemical similarity is an interesting possibility that is likely to generate similar patterns and, thus, requires formal testing. Nevertheless, fungal attack of La Selva *Piper*, although not entirely absent, is rare (R. J. Marquis, *personal observation*).

It is important to note that, for our study system, there seem to be two distinct sets of traits that are influencing coexistence in opposite manners. While communities tend to be chemically overdispersed, the same communities are also phylogenetically underdispersed. This is not the only example of species partitioning available niche space, but it has potentially interesting evolutionary outcomes. It is reasonable to expect that taxa under these two intrinsically different evolutionary pressures are likely to engender highly chemically diverse species pools with relatively low phylogenetic diversity.

Although we did not find a strong phylogenetic signal of chemical composition in our local species pool (a pattern that agrees with that of Kursar et al. 2009), phylogeny is likely to have a significant effect on *Piper* secondary chemistry at some, perhaps higher, taxonomic level. For example, although the amide piperine has been found in more than 20 *Piper* species, all of these species belong to the "tropical Asian *Piper* clade" (sensu Jaramillo et al. 2008). A similar pattern can be found for other compounds like piperlonguminine, methysticin, and yangonin (NAPRALERT [see footnote 5, data obtained 2011]; Loub et al. 1985). Finally, compounds like 4-nerolidylcatechol can be found in multiple Neotropical *Piper* species (especially, but not exclusively, in the Pothomorphe clade; NAPRALERT [see footnote 5, data obtained 2011]) but not in species of the Asian *Piper* clades.

*Piper*, like other species-rich plant groups, can have very high local species richness, yet it appears to have very low ecological diversity. Most species in the Neotropics are understory shrubs and small treelets of wet, lowland forest. Pollinated by generalist pollinators (Semple 1974, De Figueiredo and Sazima 2000) and dispersed by similar organisms (mostly a handful of species of the genus *Carollia*, Phyllostomidae; Fleming 1981, 1985, Thies and Kalko 2004), *Piper* belongs to a small but abundant group of taxa that, due to the lack of obvious morphological and functional differentiation, challenge classical notions of ecological interactions and speciation processes (Frodin 2004, Kursar et al. 2009, Sedio et al. 2012). Given our results, we propose that the interaction between *Piper* and its natural enemies (mediated by secondary chemistry) is likely to play a major role in the community assembly and local coexistence of species in this genus. Finally, we put forward that the strong concordance between the finding of Kursar et al. (2009) and the present work could be a glimpse of a more widespread pattern, a pattern in which natural enemies and even multitrophic interactions may perhaps play a key role in the assembly of natural plant communities, as well as the evolutionary processes that have driven tropical plant radiation.

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#### LITERATURE CITED

- Becerra, J. X. 2007. The impact of herbivore–plant coevolution on plant community structure. *Proceedings of the National Academy of Sciences USA* 104:7483–7488.
- Cavender-Bares, J., D. D. Ackerly, D. A. Baum, and F. A. Bazzaz. 2004. Phylogenetic overdispersion in Floridian oak communities. *American Naturalist* 163:823–843.
- Cavender-Bares, J., K. H. Kozak, P. V. A. Fine, and S. W. Kembel. 2009. The merging of community ecology and phylogenetic biology. *Ecology Letters* 12:693–715.
- Coley, P. D., and T. A. Kursar. 2014. On tropical forests and their pests. *Science* 343:35–36.
- Cooper, N., J. Rodríguez, and A. Purvis. 2008. A common tendency for phylogenetic overdispersion in mammalian assemblages. *Proceedings of the Royal Society B* 275: 2031–2037.
- Cornwell, W. K., and D. D. Ackerly. 2009. Community assembly and shifts in plant trait distributions across an environmental gradient in coastal California. *Ecological Monographs* 79:109–126.
- Darwin, C. 1859. *On the origin of species by means of natural selection, or the preservation of favoured races in the struggle for life*. First edition. Murray, London, UK.
- Daws, M. I., D. F. R. P. Burslem, L. M. Crabtree, P. Kirkman, C. E. Mullins, and J. W. Dalling. 2002. Differences in seed germination responses may promote coexistence of four sympatric piper species. *Functional Ecology* 16:258–267.
- De Figueiredo, R. A., and M. Sazima. 2000. Pollination biology of Piperaceae species in southeastern Brazil. *Annals of Botany* 85:455–460.
- Dyer, L. A., C. D. Dodson, J. Beihoffer, and D. K. Letourneau. 2001. Trade-offs in antiherbivore defenses in *Piper cenocladum*: ant mutualists versus plant secondary metabolites. *Journal of Chemical Ecology* 27:581–592.
- Dyer, L. A., D. K. Letourneau, C. D. Dodson, M. A. Tobler, J. O. Stireman III, and A. Hsu. 2004. Ecological causes and consequences of variation in defensive chemistry of a neotropical shrub. *Ecology* 85:2795–2803.
- Fincher, R. M., L. A. Dyer, C. D. Dodson, J. L. Richards, M. A. Tobler, J. Searcy, J. E. Mather, A. J. Reid, J. S. Rolig, and W. Pidcock. 2008. Inter- and intraspecific comparisons of antiherbivore defenses in three species of rainforest understory shrubs. *Journal of Chemical Ecology* 34:558–574.
- Fleming, T. H. 1981. Fecundity, fruiting pattern, and seed dispersal in *Piper amalago* (Piperaceae), a bat-dispersed tropical shrub. *Oecologia* 51:42–46.
- Fleming, T. H. 1985. Coexistence of five sympatric *Piper* (Piperaceae) species in a tropical dry forest. *Ecology* 66:688–700.
- Frodin, D. G. 2004. History and concepts of big plant genera. *Taxon* 53:753–776.
- Gentry, A. H. 1982. Neotropical floristic diversity: Phytogeographical connections between Central and South America, Pleistocene climatic fluctuations, or an accident of the Andean orogeny? *Annals of the Missouri Botanical Garden* 69:557–593.
- Gotelli, N. J., and G. L. Entsminger. 2012. *EcoSim 7.72*. Acquired Intelligence, Jericho, Vermont, USA.
- Götzenberger, L., et al. 2012. Ecological assembly rules in plant communities—approaches, patterns and prospects. *Biological Reviews* 87:111–127.
- Graham, C. H., J. L. Parra, C. Rahbek, and J. A. McGuire. 2009. Phylogenetic structure in tropical hummingbird communities. *Proceedings of the National Academy of Sciences USA* 106:19673–19678.
- Hartmann, T. 2007. From waste products to ecochemicals: fifty years research of plant secondary metabolism. *Phytochemistry* 68:2831–2846.
- Holt, R. D., and J. H. Lawton. 1993. Apparent competition and enemy-free space in insect host–parasitoid communities. *American Naturalist* 142:623–645.
- Horai, H., et al. 2010. MassBank: a public repository for sharing mass spectral data for life sciences. *Journal of Mass Spectrometry* 45:703–714.
- Ingram, T., and J. B. Shurin. 2009. Trait-based assembly and phylogenetic structure in northeast Pacific rockfish assemblages. *Ecology* 90:2444–2453.
- Jaramillo, M. A. 2006. Using *Piper* species diversity to identify conservation priorities in the Chocó Region of Colombia. *Biodiversity and Conservation* 15:1695–1712.
- Jaramillo, M. A., R. Callejas, C. Davidson, J. F. Smith, A. C. Stevens, and E. J. Tepe. 2008. A phylogeny of the tropical genus *Piper* using ITS and the chloroplast intron psbJ-petA. *Systematic Botany* 33:647–660.
- Kato, M. J., and M. Furlan. 2007. Chemistry and evolution of the Piperaceae. *Pure and Applied Chemistry* 79:529–538.
- Kembel, S. W., and S. P. Hubbell. 2006. The phylogenetic structure of a neotropical forest tree community. *Ecology* 87: S86–S99.
- Kembel, S. W., P. D. Cowan, M. R. Helmus, W. K. Cornwell, H. Morlon, D. D. Ackerly, S. P. Blomberg, and C. O. Webb. 2010. Picante: R tools for integrating phylogenies and ecology. *Bioinformatics* 26:1463–1464.
- Kessler, D., and I. T. Baldwin. 2007. Making sense of nectar scents: the effects of nectar secondary metabolites on floral visitors of *Nicotiana attenuata*. *Plant Journal* 49:840–854.
- Kraft, N. J. B., R. Valencia, and D. D. Ackerly. 2008. Functional traits and niche-based tree community assembly in an Amazonian forest. *Science* 322:580–582.

- Kursar, T. A., K. G. Dexter, J. Lokvam, R. T. Pennington, J. E. Richardson, M. G. Weber, E. T. Murakami, C. Drake, R. McGregor, and P. D. Coley. 2009. The evolution of antiherbivore defenses and their contribution to species coexistence in the tropical tree genus *Inga*. *Proceedings of the National Academy of Sciences USA* 106:18073–18078.
- Losos, J. B. 2008. Phylogenetic niche conservatism, phylogenetic signal and the relationship between phylogenetic relatedness and ecological similarity among species. *Ecology Letters* 11:995–1003.
- Loub, W. D., N. R. Farnsworth, D. D. Soejarto, and M. L. Quinn. 1985. NAPRALERT: computer handling of natural product research data. *Journal of Chemical Information and Computer Science* 25:99–103.
- MacArthur, R., and R. Levins. 1967. Limiting similarity convergence and divergence of coexisting species. *American Naturalist* 101:377.
- Maherali, H., and J. N. Klironomos. 2007. Influence of phylogeny on fungal community assembly and ecosystem functioning. *Science* 316:1746–1748.
- Maherali, H., and J. N. Klironomos. 2012. Phylogenetic and trait-based assembly of arbuscular mycorrhizal fungal communities. *PLoS ONE* 7:e36695. doi:10.1371/journal.pone.0036695
- Marques, J. V., A. De Oliveira, L. Raggi, M. C. M. Young, and M. J. Kato. 2010. Antifungal activity of natural and synthetic amides from *Piper* species. *Journal of the Brazilian Chemical Society* 21:1807–1813.
- Marquis, R. J. 2004. The biogeography of Neotropical *Piper*. Pages 78–96 in L. Dyer, editor. *Piper. A model genus for studies of chemistry, ecology, and evolution*. Kluwer Academic Press:78–96.
- McGill, B. J., B. J. Enquist, E. Weiher, and M. Westoby. 2006. Rebuilding community ecology from functional traits. *Trends in Ecology & Evolution* 21:178–185.
- Mikich, S. B., G. V. Bianconi, B. H. L. N. S. Maia, and S. D. Teixeira. 2003. Attraction of the fruit-eating bat *Carollia perspicillata* to *Piper gaudichaudianum* essential oil. *Journal of Chemical Ecology* 29:2379–2383.
- Parmar, V. S., et al. 1997. Phytochemistry of the genus *Piper*. *Phytochemistry* 46:597–673.
- Parmar, V. S., et al. 1998. Polyphenols and alkaloids from *Piper* species. *Phytochemistry* 49:1069–1078.
- Pianka, E. M. 1973. The structure of lizard communities. *Annual Review of Ecology and Systematics* 4:53–74.
- Pusztahelyi, T., I. J. Holb, and I. Pócsi. 2015. Secondary metabolites in fungus–plant interactions. *Frontiers in Plant Science* 6:573.
- R Core Team. 2012. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <https://cran.r-project.org>
- Richards, L. A., L. A. Dyer, M. L. Forister, A. M. Smilanich, C. D. Dodson, M. D. Leonard, and C. S. Jeffrey. 2015. Phytochemical diversity drives plant–insect community diversity. *Proceedings of the National Academy of Sciences USA* 112:10973–10978.
- Ridenour, W. M., and R. M. Callaway. 2001. The relative importance of allelopathy in interference: the effects of an invasive weed on a native bunchgrass. *Oecologia* 126:444–450.
- Root, R. B. 1973. Organization of a plant–arthropod association in simple and diverse habitats: the fauna of collards (*Brassica oleracea*). *Ecological Monographs* 43:95–124.
- Roseberg, M., and C. D. Anderson. 2011. PASSaGE: pattern analysis, spatial statistics and geographic exegesis. Version 2.0. *Methods in Ecology and Evolution* 2:229–232.
- Salazar, D. 2013. Exploring the local and regional effects of plant diversity on plant herbivore interactions. Thesis. University of Missouri–St Louis. Department of Biology, Saint Louis, Missouri, USA. <https://apps.umsl.edu/webapps/weboffice/ETD/query.cfm?id=r9241>
- Salazar, D., H. K. Detlev, and R. J. Marquis. 2012. Directed seed dispersal of *Piper* by *Carollia perspicillata* and its effect on understory plant diversity and folivory. *Ecology* 94:2444–2453.
- SAS Institute. 2007. JMP 10.0. SAS Institute, Cary, North Carolina, USA.
- Sedio, B. E., S. J. Wright, and C. W. Dick. 2012. Trait evolution and the coexistence of a species swarm in the tropical forest understory. *Journal of Ecology* 100:1183–1193.
- Sedio, B. E., J. R. Paul, C. M. Taylor, and C. W. Dick. 2013. Fine-scale niche structure of Neotropical forests reflects a legacy of the Great American Biotic Interchange. *Nature Communications* 4:2317.
- Seiple, K. S. 1974. Pollination in Piperaceae. *Annals of the Missouri Botanical Garden* 61:868–871.
- Stein, S. E. 1999. An integrated method for spectrum extraction and compound identification from gas chromatography/mass spectrometry data. *Journal of the American Society for Mass Spectrometry* 10:770–781.
- Stein, S., Y. Mirokhin, D. Tchekhovskoi, and G. Mallard. 2005. The NIST mass spectral search program for the NIST/EPA/NIH mass spectral library. Scientific Instrument Services, Ringoes, New Jersey, USA.
- Stone, L., and A. Roberts. 1990. The checkerboard score and species distributions. *Oecologia* 85:74–79.
- Suzuki, R., and H. Shimodaira. 2006. Pvcust: an R package for assessing the uncertainty in hierarchical clustering. *Bioinformatics* 22:1540–1542.
- Swenson, N. G. 2013. The assembly of tropical tree communities—the advances and shortcomings of phylogenetic and functional trait analyses. *Ecography* 36:264–276.
- Thies, W., and E. K. V. Kalko. 2004. Phenology of neotropical pepper plants (Piperaceae) and their association with their main dispersers, two short-tailed fruit bats, *Carollia perspicillata* and *C. castanea* (Phyllostomidae). *Oikos* 104:362–376.
- Wahid, A., S. Gelani, M. Ashraf, and M. R. Foolad. 2007. Heat tolerance in plants: an overview. *Environmental and Experimental Botany* 61:199–223.
- Webb, C. O., and M. J. Donoghue. 2005. Phylomatic: tree assembly for applied phylogenetics. *Molecular Ecology Notes* 5:181–183.
- Webb, C. O., D. D. Ackerly, M. A. McPeck, and M. J. Donoghue. 2002. Phylogenies and community ecology. *Annual Review of Ecology and Systematics* 33:475–505.
- Weiher, E., and P. Keddy. 1999. *Ecological assembly rules: perspectives, advances, retreats*. Cambridge University Press, Cambridge, UK.
- Wright, S. J. 2002. Plant diversity in tropical forests: a review of mechanisms of species coexistence. *Oecologia* 130:1–14.
- Zhang, Q. H., and F. Schlyter. 2003. Redundancy, synergism, and active inhibitory range of non-host volatiles in reducing pheromone attraction in European spruce bark beetle *Ips typographus*. *Oikos* 101:299–310.

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