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Exploring the local and regional effects of plant diversity on plant herbivore interactions

By

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Doctor of Philosophy in Biology

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Dissertation Abstract:

The study of biological diversity and its effects on ecosystem functioning and species interactions has always been a fundamental part of biology. The accelerating loss of species in conjunction with an increasing change in the natural environment has underlined the importance of the role that biodiversity has on the evolutionary and ecological dynamics of natural systems. In this dissertation I explore the effect of local and regional patterns of plant diversity in plant-herbivore interactions. Furthermore, this work goes beyond the classical concepts of taxonomical diversity and investigates the role of phylogenetic and chemical diversity on plant-herbivore interactions.

To accomplish this work I have chosen as study system the Pantropical plant genus *Piper* (Piperaceae). With more that 1000 species in the Neotropics, a natural range that expands from northern Mexico to northern Argentina, and are often among the top ten most speciose genera in Neotropical forests *Piper* represents an ideal system to tackle both local a regional questions in plant-herbivore interactions.

In chapter one I explore the patters of herbivore diversity along latitudinal gradient by following to widely distributed *Piper* species from Mexico to Bolivia. Here I show how that changes in herbivore diversity along this latitudinal gradient are likely changing the nature and intensity of the evolutionary herbivore pressures experienced by plants.

In chapter two I examine how non-random patterns of seed dispersal by bats are increasing local understory *Piper* diversity. Furthermore, I show how these changes in local diversity are also reducing Piper herbivore damage due to possible resource dilution effects. This represents the first evidence of a direct link between seed dispersal and plant herbivore interactions.

In chapter three I explore the relationship between inter-specific chemical diversity and intra-specific chemical variation. Here I put forward the potential association between the number of dominant secondary compounds present in a particular *Piper* species and the relative ecological value that said compounds have.

Finally, in chapters four and five I use a metabolomic approach to investigate the role that *Piper* chemical diversity at the community has on species coexistence and community assembly. Here I show how natural *Piper* communities are more chemically diverse than expected by chance. This section also shows that Piper communities with higher chemical diversity have less herbivore damage.

As a unit, this work provides strong evidence of the importance of taxonomical and chemical diversity for plant herbivore interaction.

Acknowledgments

After 42 months of fieldwork spread over 5 different countries it will be impossible to give credit to all the people that deserve it. Thus, I will not attempt to give an exhaustive section of acknowledgement instead; I will just try to underline the people that played a fundamental role in the development, execution, and crystallization of this work. Nevertheless, to all of you that, in one-way or another have helped me achieve my goals, my greatest appreciation is due.

Counter clockwise:

I will like to thank my advisor Robert Marquis (hereafter, Bob), how had: (1) the braveness to accept a student with dyslexia and paid with countless hours of frustrations for it, (2) the imagination to see in each of my crazy ideas a wordy research project, and (3) the patience to see me grow as a professional.

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Chapter 1

Herbivore Pressure Increases Towards the Equator

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Increases in species diversity and density from higher to lower latitudes are well-documented. Nevertheless, the consequences of these changes in diversity for structuring ecological communities and influencing biotic evolution are largely unknown. It is widely believed that this increase in species diversity is associated with increased intensity of ecological interactions closer to the equator. For plant-herbivore interactions in particular, the predictions are that, at lower latitudes, plants will be attacked by more individual herbivores, more herbivore species, and more specialized herbivores, and therefore, will suffer greater damage. We used a large-scale latitudinal transect from Mexico to Bolivia to quantify changes in leaf damage, diversity, and abundance of lepidopteran larvae on two widely distributed

host species of the genus *Piper* (Piperaceae). We show that both density and species richness of herbivores were highest at the Equator and decreased with increasing latitude, both northward and southward. Contrary to expectation, however, this increase in herbivore diversity was due to the addition of generalist not specialist species. Finally, and again contrary to expectation, the increase in herbivore density with decreasing latitude did not produce a corresponding damage gradient. We propose that the lack of a latitudinal concordance between increases in herbivore density and diversity with decreasing latitude, and the resulting herbivore damage, supports the hypothesis of better plant anti-herbivore defenses at lower latitudes. Furthermore, the changes in the relative abundance of generalist versus specialist suggest that the nature of the selective pressure is intrinsically different between higher and lower latitudes.

Introduction

The vast body of qualitative and quantitative evidence on the changes in species diversity across the latitudinal gradient has raised much speculation on the possible ecological and evolutionary consequences of this pattern. A longstanding hypothesis in community ecology predicts that, as species diversity and species abundance increase, the intensity and frequency of ecological interactions between species increase (1). Therefore, competition, predation, and parasitism are all thought to be more intense at lower latitudes (1, 2). Similarly, current theory predicts that this increase in species interactions at lower latitudes imposes strong natural selection for mechanisms that will reduce the intensity or frequency of negative interactions. As a result, species at lower latitudes are likely to have higher levels of specialization to lessen competition, and greater defenses to reduce predation (2). For plant-herbivore interactions in particular, it is expected that at lower (tropical) latitudes, abundance and species richness of insect herbivores will be higher and therefore, lower latitudes will also have more specialized herbivores and plants will suffer greater biomass loss to herbivory (3-5). Consequently, this herbivore species latitudinal gradient is likely to impose stronger herbivore pressure on plants at lower latitudes (6-8).

Herbivore pressure can be defined as the strength of natural selection imposed by herbivores for plant phenotypes that have greater fitness in the presence of those herbivores. Herbivore pressure is expected to increase with greater tissue loss when damage reduces growth and reproduction (quantitatively) (9) but also with changes in the identity of the herbivore species (qualitatively) (7). Increased number of species attacking a given plant host should result in a qualitatively different set of selective pressures compared to attack by fewer species because each herbivore reacts idiosyncratically to the defensive arsenal of a plant and may therefore influence plant fitness in different ways (10-13). From the plant's point of view, each combination of traits embodied by a specific herbivore species may represent an additional and potentially unique evolutionary hurdle that the plant may need to overcome, and will be manifested as tradeoffs in defense allocation.

Furthermore, to accurately assess qualitative changes in herbivore pressure, it is not only critical to document the taxonomic identity of the herbivores, but also to examine patterns of host use by those herbivores (e.g. whether the herbivores are specialists or generalists) (14). Because specialists are likely to be better adapted to specific qualitative defense compound types such as alkaloids, and generalists are expected to be better equipped to overcome quantitative digestibility-reducing compounds like polyphenols, current theory predicts that specialist and generalist herbivores will exert different and perhaps contrasting selective pressures (14-16).

Herbivore pressure traditionally has been measured as the amount of tissue loss to herbivores, overlooking the identity of the herbivores causing that damage. We argue that neither by itself is sufficient to characterize the selection pressure imposed by herbivores; measuring total damage alone does not reveal the identity of the causative agents, and simply identifying the herbivore fauna is insufficient because total tissue loss is not always related to the number of insect species causing that damage (17). Moreover, different herbivore species often create different patterns of damage due to species-specific differences in size, phenology, and feeding behaviors resulting in differential effects on growth and reproduction (18, 19).

Despite an abundance of theoretical work, few studies have systematically explored the consequences of the latitudinal diversity gradient on plant-herbivore interactions and none has quantified both quantitative and qualitative changes in herbivore pressure within the tropical realm (6). Finally, no studies on terrestrial systems have included sites from both sides of the Equator (20).

Here we used a large-scale latitudinal transect from Mexico to Bolivia to quantify changes in diversity and abundance of lepidopteran larvae on two widely distributed host species, *Piper aduncum* and *P. aequale* (a pioneer and a primary forest species respectively). *Piper* (Piperaceae) is a genus of shrubs, vines, and small trees represented by some 1500 species in the New World (21). *Piper* represents a common and diverse component of Neotropical wet forest understories, with up to 64 species in a single lowland forest location (22).

Each host plant species was sampled in five locations distributed along the latitudinal gradient (Los Tuxtlas, Mexico, 18° north; La Tirimbina, Costa Rica, 10° north; Jatun Sacha, Ecuador, 0°; Yanamono, Peru, 3° south, Madidi, Bolivia, 15° south). Herbivores were collected, reared to adults, and then classified into three categories depending on their diet: true specialists: herbivore species that feed on only one host plant species (in this case *P. aduncum* or *P. aequale*); genus specialists: herbivores that feed on two or more plant species from the genus *Piper*; and generalists: herbivores that feed on two or more host species from different plant families. In addition, we assessed the amount of leaf area removed by herbivores on each sampled *Piper* plant. Finally we quantified their average leaf toughness as a measure of leaf mechanical defenses.

Results and Discussion

We found that total herbivore species richness per m^2 of foliage sampled increased significantly with decreasing latitude ($F = 12.81$, $p < 0.0001$ for *P. aequale*; $F = 8.35$, $p = 0.0001$ for *P. aduncum*) (**Fig. 1, Table S1**). Additionally, herbivore diversity was additive approaching the Equator, as most species of herbivores found at higher latitudes were also found at lower latitude sites, but not vice versa. Thus patterns of increasing insect herbivore species at lower latitude, previously documented at the community level (4), are mirrored by those at the individual plant level for these two species of *Piper*.

The three diet categories of herbivores showed different latitudinal patterns in species richness. Contrary to expectation, categories with a wider taxonomical diet breadth had a higher rate of increase towards the equator than did specialists (**Fig. S1, Table S1**). True specialist herbivore species richness did not change with latitude. Only one true specialist herbivore species (*Eois*; Geometridae) was found per host plant and both of these herbivore species were found at all sites. Genus specialists increased with decreasing latitude; however, most differences in total species richness between sites were due to an increase in generalist herbivore diversity between the high latitude sites (Mexico and Bolivia) and Ecuador. Notably, the gradients of herbivore richness held true for both sides of the Equator (**Fig. 2**).

Density of lepidopteran larvae per square meter of leaf area increased with decreasing latitude ($F = 4.76$, $p = 0.0046$ for *P. aduncum*; $F = 9.17$, $p < 0.0001$ for *P. aequale*). This latitudinal trend held true for all categories of herbivores except the *P. aduncum* specialists, which showed a non-significant decrease in density at lower latitudes (**Fig. 2, S2 Table S1**).

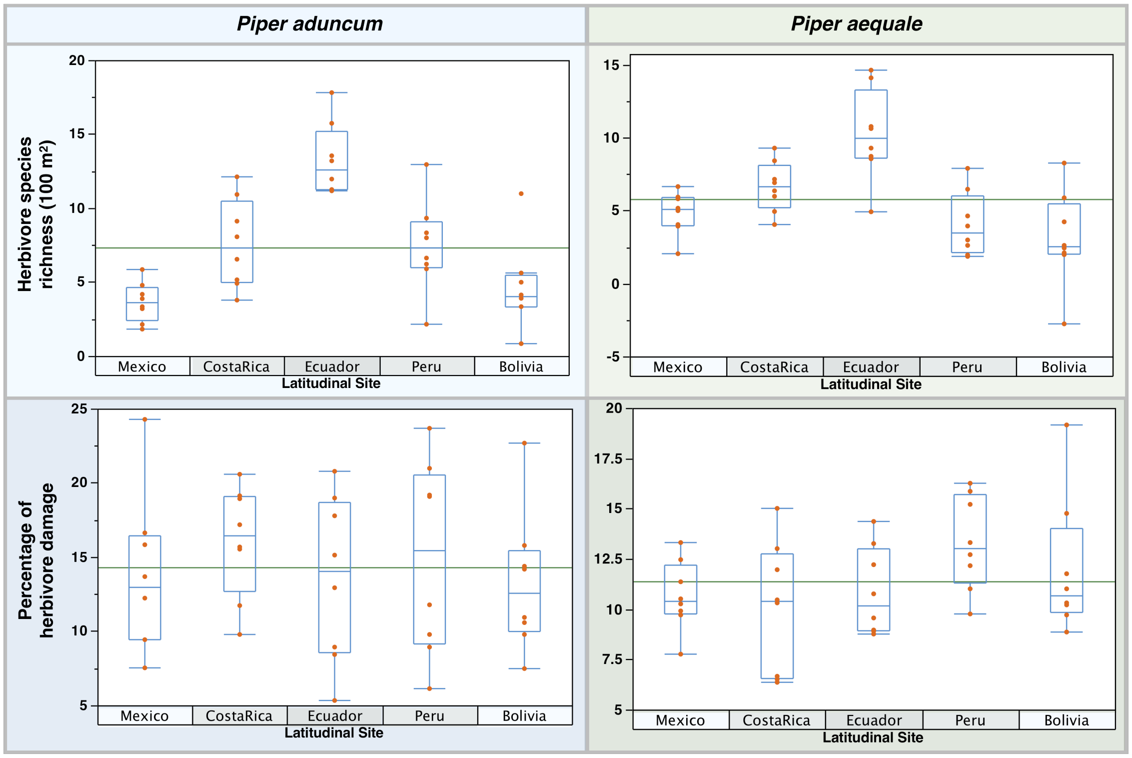

Figure 1: Total herbivore species richness and percentage of leaf herbivore damage per latitudinal site of *Piper aduncum* and *P. aequale*. Species richness was measured as the number of species of lepidopteran larvae present per 100 m^2 leaf area. Leaf herbivory was measured as the percentage of leaf area removed by lepidopteran herbivores. Points in the graph represent each of the eight sampled populations per latitudinal site.

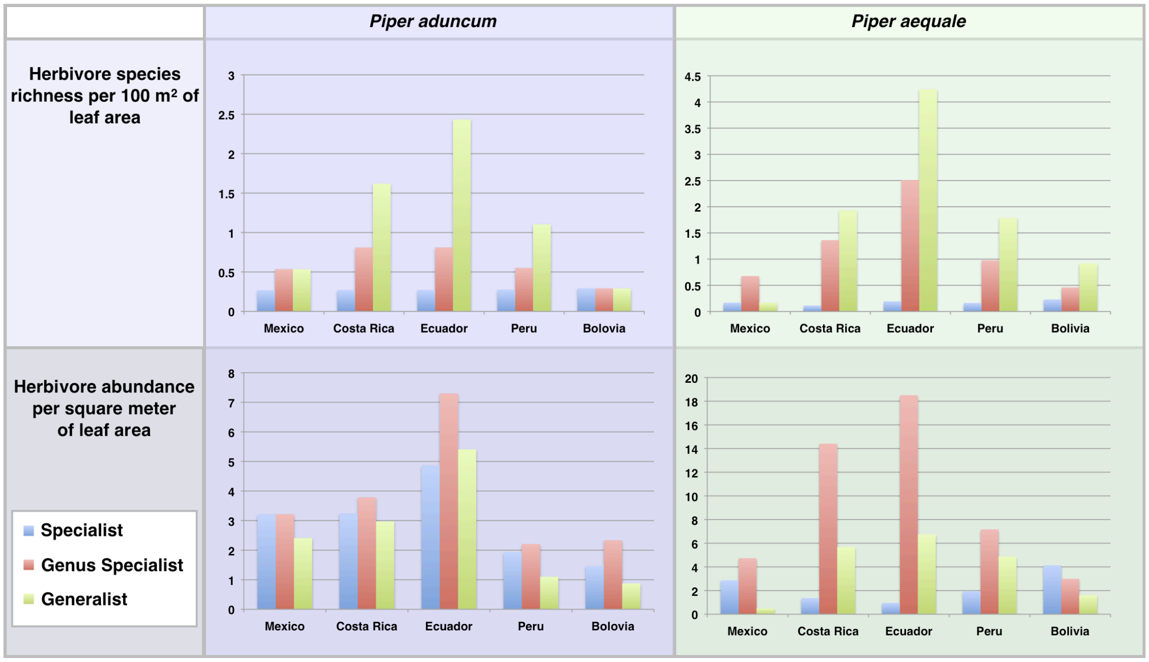

Figure 2: Herbivore species richness and abundance per square meter of leaf area at each latitudinal site. Herbivores are subdivided by diet breadth. Species richness was measured as the total number of species of lepidopteran herbivores. Herbivore abundance was measured as the total number individuals per 100 m^2 leaf area at each latitudinal site.

Total herbivore density was 200% higher at the Equator for *P. aequale* and 400% higher for *P. aduncum* when compared to the high latitude sites. Our data concur with a recent study of temperate saltmarsh plants that showed an increase of 1-2 species of herbivores with decreasing latitude and this increase was also attributed to generalists (7).

With the exception of *P. aequale*'s specialist herbivores, the three diet categories of herbivores showed a pattern of increased species densities at lower latitudes. For both *Piper* species, genus specialists and generalist herbivores showed a significant increase in species densities at lower latitudes (**Table S1)**. However, in contrast to the pattern found for species richness, genus specialists showed the greatest increase in species density with decreasing latitude (**Fig. 2**). All gradients of herbivore abundance also held true for both sides of the Equator (**Fig. 2**). These strong latitudinal patterns of herbivore numbers, herbivore diversity, and changes in the average diet breadth of the herbivore community (specialist vs. generalists) are consistent with the existence of stronger qualitative herbivore pressure at lower latitudes within the tropical realm.

To test the general relationship between herbivore abundance and leaf damage we first analyzed the data independently of latitude (pooling together all data from all populations). After a correlation analysis we found that herbivore abundance significantly explained 37% of the variation in herbivore damage for *P. aduncum* ($n = 40$, $p = 0.0001$) and 14% for *P. aequale* ($n = 40$, $p = 0.019$). Furthermore, when analyzed independently, each one of our latitudinal sites also showed a significant positive relationship between herbivore density and herbivore damage. Nevertheless, sites at lower latitudes had smaller regression slopes than sites at higher latitudes (**Fig. S3, Table S2**). An identical pattern was found for herbivore diversity (**Fig. S3, Table S2**). However, we did not find significant differences in herbivore damage per site for neither *Piper* species hosts despite of the changes in herbivore richness and density across the latitudinal gradient **(F = 0.33, p = 0.85 for *P. aduncum,* and F = 1.48, p= 0.23 for *P. aequale*, Fig. 1, 3, Table S1)**. This latitudinal trend in damage, coupled with greater density of herbivores at lower latitudes, suggest that the contribution each individual herbivore made to total leaf area damage was smaller at lower latitudes.

Although several studies have shown differences in herbivore leaf damage between high and low latitudes (6), most report small differences, between 0.5 and 7 percentage points. These previous comparisons have been made without controlling for herbivore or plant taxa, leaving uncertain the relative importance of site (latitude) versus phylogeny for damage estimates. In addition, a recent meta-analysis (20) found no significant relationship between latitude and herbivore damage for 38 latitudinal comparisons. When we controlled for phylogeny (by using the same widespread species of host plants), we found no differences in damage levels either north or south of the equator (**Fig. 1**).

Despite the fact that our data show no significant changes in quantitative herbivore pressure (leaf damage), it is likely that the higher herbivore species richness and the additional number of taxonomic families of lepidopteran herbivores found at lower latitudes (**Fig. 3, Table S1, S3**) translate into greater qualitative herbivore pressure closer to the equator. The distinct evolutionary makeup of each additional herbivore lineage is likely to influence the response of that particular taxon to the mechanical and chemical defenses of its host (11). *Piper aequale* in Ecuador was attacked by caterpillars from four families not found in either Mexico or Bolivia, and *P. aduncum* by seven additional families (**Fig. 3, Table S3**). Furthermore, the increment in the abundance and diversity of generalist species suggests that the nature and evolutionary direction of the herbivore pressure at lower latitudes is significantly different from that at higher latitudes. Within this context, it is possible that the additional herbivore pressure imposed by generalists could increase the relative abundance, diversity, and efficiency of qualitative defense compound types such as piperamides (23) at lower latitudes. If this holds true, this could help explain the reduction of the relative effect that herbivores have on leaf damage at lower latitudes also found in this study.

We argue that the lack of concordance between the latitudinal changes in herbivore density and diversity and the resulting herbivore damage supports the long-standing hypothesis of better plant anti-herbivore defenses at lower latitudes (1, 6, 8, 24). These changes in palatability could be the result of latitudinal variations in leaf quality (6), or specifically, local plant host adaptation in terms of (a) higher concentration of secondary compounds, (b) locally endemic secondary chemicals, (c) increased indirect defenses (e.g., ant-plant mutualisms), or (d) greater structural defenses.

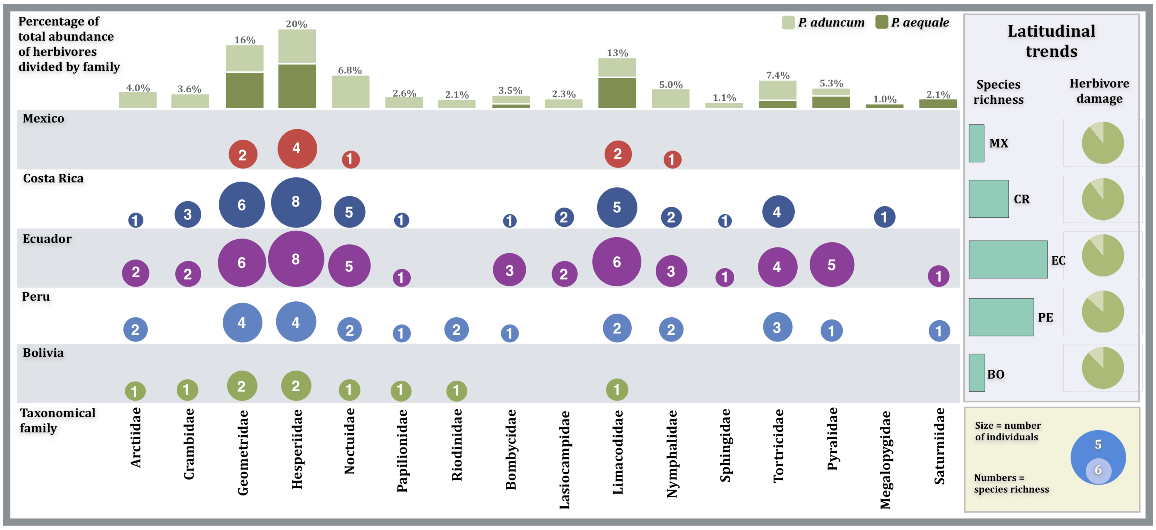

Figure 3: Visualization of the latitudinal changes in species diversity of the most common lepidopteran herbivores found feeding on *P. aduncum* and *P. aequale*. Bubble size represents the abundance of herbivores of a particular family at each latitudinal site (herbivore individuals per m^2 of leaf area). Numbers inside bubbles show number of species of a particular family at each site. Vertical bars above represent the percentage of the total abundance of herbivores accounted for by each taxonomic family per plant host (light green: *P. aduncum*, dark green: *P. aequale*). In the "latitudinal trends" box, horizontal bars represent changes in total herbivore species richness across the latitudinal gradient. The pie charts show the mean percentage of herbivore damage at each latitudinal site (dark green = undamaged leaf area; light green = leaf area removed by lepidopteran herbivore).

Average leaf toughness also did not differ among sites, suggesting that possible differences in leaf palatability are likely associated with leaf chemistry (*P. aduncum:* F = 1.1417, p = 0.3531; *P. aequale*: F = 0.2247, p = 0.92; **Fig. S4**). Nevertheless, changes in leaf palatability do not successfully explain the apparent paradox of higher herbivore abundances without higher leaf damage.

A potential explanation for this paradox is a decrease in the herbivore feeding performance (25, 26) across the latitudinal gradient due to higher parasitism rates. Previous attempts to find latitudinal gradients in herbivore parasitism have met with mixed results (2). In our study, percent parasitism of reared caterpillars increased from 3.20% in Mexico to 8.05% in Costa Rica, and then again to 9.93% in Ecuador (n = 125, 298, and 292, respectively), lending support to this hypothesis. Another possible explanation is a latitudinal increase in host plant leaf turnover. With a higher per plant leaf turnover at lower latitudes, the observed estimates of herbivore damage would underestimate the actual amount of leaf area removed. However, recent studies have shown that when leaf longevity is taken in account when comparing herbivory rates between low and high latitudes, leaf damage is equal or more intense at higher latitudes (27-29). Finally, this pattern could be the result of reduced body size, and therefore reduced lifetime leaf tissue consumption per herbivore, at lower latitudes (e.g., Bergmann's rule; 30, 31).

Contrary to expectations, our findings do not support the hypothesis that a particular host-plant species will have a more abundant or a more diverse array of specialist herbivores at lower latitudes. Instead we found that increases in herbivore species richness at lower latitudes were due to the addition of generalist herbivore species. It is likely that as plant species diversity increases towards the Equator, the relative abundance of any given host plant decreases. This scenario will likely make it difficult for specialist herbivores to find their specific host, while giving an ecological and evolutionary advantage to an herbivorous insect with a generalized diet (32). Nevertheless, we recognize the existence of an evolutionary conundrum for herbivores at lower latitudes. While increasing host plant diversity likely makes a generalist diet beneficial, the increasing diversity and abundance of herbivores will generate strong selection for narrower diet breadths in order to reduce intra-guild competition.

Although we found that generalist and not specialist herbivores contributed to higher herbivore species richness at lower latitudes, we cannot draw any clear conclusions about possible patterns of herbivore specialization across the latitudinal gradient (3,5). Our study was not designed to exhaustively measure herbivore diet breadth. Thus, it is possible that generalist herbivores are more "specialized" at lower latitudes in terms of the number of host plant species on which they feed (3). We did find a significant correlation between richness of genus specialist species and the number of local *Piper* species ($r^2 = 0.7$, $p < 0.002$), lending support to the hypothesis that latitudinal changes in herbivore species richness mirror changes in plant host diversity (5).

We acknowledge that any changes in plant-herbivore interactions across the latitudinal gradient are likely due to multiple evolutionary and ecological factors, including changes in predator abundance (33) and in the surrounding plant community structure (32, 34). However, our analysis is based on herbivores found feeding on their host. Thus, processes that could change the encounter rate between herbivore and host will not have a major effect on our results.

Conclusions

Overall, we found support for the hypothesis that plants suffer greater herbivore pressure at lower latitudes. Despite the lack of significant differences in quantitative herbivore pressure across latitudinal sites, the increase in herbivore diversity and abundance are likely to generate a comparable increase in the selective pressure that herbivores inflict on their host-plants. Furthermore, changes in the relative abundance of generalist versus specialist herbivores suggest that the selective pressure imposed by insect herbivores on plants is likely to be significantly different between higher and lower latitudes.

Methods

Data Collection: At each site we selected 8 populations of each *Piper* species, at least 5 km apart (See **Text S1** for description of the field sites and target *Piper* species, **Fig. S5**). At each population we randomly choose 20 adult plants (reproductive), all of similar size (no smaller than 1.5 m tall). Every leaf and every branch of each plant were carefully searched for lepidopteron larvae. When a larva was found, it was collected, identified, and cataloged (**Text S1**). All herbivore specimens were photographed in order to compare the species between populations and sites (photographs of herbivores are available upon request). Additionally, all other *Piper* and most common non-*Piper* species at each site were also explored for herbivores in order to assist in the determination of diet breadth of the herbivores feeding on *P. aduncum* and *P. aequale*. In order to standardize herbivore abundances and species diversities, we counted the total number of leaves present on each *Piper* plant of the herbivore census. Finally, we also assessed the average leaf area of each *Piper* population.

Herbivore Data: All herbivores collected during the census were placed in plastic bags (30 x 30 cm) with fresh plant material from either *P. aduncum* or *P. aequale.* The bags were placed in improvised rearing facilities at ambient temperature and protected from direct sunlight (**Fig S6**). Bags were checked daily to remove frass and to add new leaf material as needed. All bags with pupated larvae were marked and followed. Once emerged, butterflies and moths were photographed for later identification.

Herbivore diet breadth was assessed using three main strategies. First, if specimens of herbivore species were abundant at the site, no-choice feeding trials were implemented in the field. Herbivores where placed in plastic bags with the two most abundant *Piper* species (excluding the two target species) and the two most abundant non-*Piper* plant species at the site. Successful feeding was recorded when herbivores fed on the new host as larvae completed pupation. Secondly, general herbivore censuses were carried out with 30 m transects along trails at each latitudinal site. No less than seven transects were performed at each site. At each transect all plants within 3 m of the trail were searched and all caterpillars found were collected and compared to the one found feeding from the two target species. Finally, to confirm the results of the two previous strategies and to assess the diet breadth of the abundant caterpillar herbivores, we carried out an extensive literature and data base review (**Text S1).**

Plant Data: Herbivore damage on the two *Piper* species was assessed visually. To reduce possible error on the assessment of herbivory, a training period was carried out before the first herbivore census. Visual assessments of herbivory followed by actual measurements of leaf area (using image processing and analysis software ImageJ - http://rsbweb.nih.gov/ij/-) allowed us to compare, correct, and standardize visual assessments. Additionally, extensive research has been done on the patterns of herbivore damage on *Piper* species (35). This research allowed us to discriminate most of the herbivore leaf damage caused by lepidopteran herbivores from the damage caused by non-lepidopteran herbivores and leaf pathogens. All herbivory estimates on this study are limited to damage caused by lepidopteran larvae, as much as possible (**Fig. S7**).

To assess changes in leaf toughness between *Piper* populations and latitudinal sites we randomly collected 30 fully expanded leaves of both *Piper* species from every population. Only fully expanded and the most distal leaves on a growing branch were assessed. To estimate toughness we used a Wagner Force Dial (FDK 32, Wagner Instruments, USA) to measure the amount of grams of force needed to pierce a 0.5 cm diameter hole at the center of the leaf but never through a primary or secondary vein. Parasitism levels of caterpillars at the different latitudinal sites were estimated by calculating the percentage of parasitism found across all larval rearing at each improvised rearing lab. Parasitism was confirmed by the presence of at least one larva, pupa or adult parasitoid inside the rearing bag. Dead caterpillars without signs of parasitism were left in their rearing bags for eight days to allow possible parasitoids to emerge. If no parasitoid emerged before the eighth day, the caterpillar was considered to be free of parasites.

Statistical Analysis: We used oneway ANOVAs to compare herbivore species abundances, species richness, herbivore damage, and leaf toughness between all sites. To contrast the effect of herbivore density and herbivore species richness on leaf herbivory between the latitudinal sites we used linear regression through origin. **Full methods** and any associated references are available in the online version of the paper.

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Supplementary material for chapter 1

Appendix A: Supporting Methods

Text S1:

Site Descriptions

Sites for the present study were selected based on two main criteria: (1) similar biotic and abiotic factors, and (2) the presence of both focal *Piper* species.

Mexico, Veracruz, Los Tuxtlas (Fig S5)**:** Los Tuxtlas is a tropical rainforest in the southeastern part of Veracruz, Mexico. This site is considered to be the "northern limit" of the lowland tropical rainforest ecosystem of the Neotropics (1, 2). We carried out the present study within the Los Tuxtlas Biological Research Station (UNAM; 95 W, 18 N), and the surrounding area. The reserve has 650 ha of forest with an average temperature of 26° C, ranging from 20°– 28° C, and a mean annual rainfall of 4500 mm (1). All sampling at this site was performed between 100 and 600 meters above sea level.

Costa Rica, Heredia, La Tirimbina (Fig S5)**:** La Tirimbina Biological Reserve is located in the Atlantic lowlands of Heredia, Costa Rica (3). The reserve includes 350 ha of lowland tropical rainforest but forms part of a network of private and national reserves that include the La Selva Biological Station (Organization of Tropical Studies) and Braulio Carrillo National Park. The reserve has a mean annual temperature of 25.3° C (ranging from 20.2° to 30.0° C) and 3800 mm of annual precipitation (10°82 N, 84°87 W). All sampling at this site was performed between 150 and 300 meters above sea level.

Ecuador, Napo, Jatun Sacha (Fig S5)**:** Jatun Sacha Biological Station and Reserve are located in eastern Ecuador where the upper Amazon Basin meets the base of the eastern Andes. The reserve includes 1700 ha of tropical lowland rainforest (00° 59'S; 77° 36'W) with a mean annual temperature or 25.0° C and 3700 mm of annual precipitation. All sampling at this site were performed between 400 and 500 meters above sea level (4).

Yanamono, Loreto, Peru (Fig S5)**:** The Yanamono region is located 80 km northeast of Iquitos. It includes a 200 ha island in the Amazon River and riverside reserve of more than 1600 ha of lowland tropical rainforest that is owned by the local community (03°25 S, 72°45W). It has an annual precipitation of 3600 mm and a mean temperature of 26° C, ranging from 20.8° to 31.7° C (5). All sampling at this site was performed between 100 and 150 meters above sea level.

Chalalan, Madidi, La Paz, Bolivia (Fig S5)**:** The Chalalan Natural Reserve is located within the Madidi National Park, in the department of La Paz, Bolivia (14°25 S, 67°55 W). The park includes 12,700 km^2 of tropical lowland rainforest with a mean annual temperature or 26.0° C (ranging between 18° to 32° C) and 3200 mm of annual precipitation (6, 7). All sampling at this site were performed between 400 and 500 meters above sea level.

Plant host species details

***Piper aduncum* L.** (Species Plantarum. Editio sexta 1: 29. 1753.) is one of the most widely known species of *Piper* in the Neotropics). It is a very common pioneer and secondary forest small tree species that can grow up to 12 m of height. It is commonly found in medium to full sunlight, forest edges, clearings, river shores, and urban and agricultural settings (8). It has been extensively studied for its complex secondary chemistry (9-11). However, more recently, this species has gained more attention because of its invasive status in the Pacific islands of Southeast Asia (8, 12). In its native range the species is more common at low elevations (0-1000 meters above sea level) and normally grows in multi-*Piper* species patches. Leaves are relatively tough and coarse, covered by small stiff trichomes.

***Piper aequale* Vahl.** (Eclogae Americanae 1: 4, t. 3. 1797.) is a relatively unknown, exclusively neotropical species. This small shrub is a "primary forest" species that can grow up to 3 m of height. It is commonly found in the forest understory under closed canopy (13). Like *P. aduncum*, this species has been studied for its secondary chemistry although, not in much detail (10, 14). In its range the species is found mainly at low elevations (0-1300 meters above sea level) and normally grows in small multi-*Piper* species patches. Leaves are relatively soft, smooth, and glabrous.

Herbivore diet breadth assessment:

After the identification of the caterpillars, databases were used to compile a list of plants that have been reported as hosts for the each particular caterpillar. In a very few cases where (a) caterpillars of a particular species were not abundant enough to complete feeding trials in the field, and (b) the particular species was not found in any of the databases or bibliographical sources consulted, we extrapolated their diet breadth from the predominant diet breadth of close relatives (species from the same genus present at the same locality).

List of sources used to confirm and assess herbivores diet breadth:

Database: Dynamic database for an inventory of the macrocaterpillar fauna, and its food plants and parasitoids, of the Area de Conservacíon Guanacaste (ACG), northwestern Costa Rica. Accessed: 2009-2010 (15). http://janzen.sas.upenn.edu/caterpillars/database.lasso

Database: Caterpillars and parasitoids of a tropical lowland wet forest. Accessed: 2009-2010 (16). http://caterpillars.unr.edu/lsacat/index_frames.htm

Database: Caterpillars and parasitoids of the Eastern Andes in Ecuador. Accessed: 2009-2010 (17). http://caterpillars.unr.edu/lsacat/ecuador/index.htm

Database: Sistema Costarricense de Información sobre Biodiversidad. Accessed: 2009-2010 (18). http://crbio.cr/portalCRBio/welcome.htm

Database: Butterflies and Moths of North America. Accessed: 2010 (19). http://www.butterfliesandmoths.org/

Database: Papillon Poitou Charentes. Accessed: 2010 (20). http://www.papillon-poitou-charentes.org/

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Database: Biodiversity and Environmental Resource Data System of Belize. Accessed: 2010 (22). http://www.biodiversity.bz

Database: Catalogo de insectos de Nicaragua (Bio-Nica). Accessed: 2010 (23). http://www.bio-nica.info/insectos/index.html

Database: HOSTS - A Database of the World's Lepidopteran Hostplants . Accessed: 2010 (24). http://www.nhm.ac.uk/hosts

Article: The Butterflies (Lepidoptera) of the Tuxtlas Mts., Veracruz, Mexico, Revisited: Species-Richness and Habitat Disturbance (25).

Article: A Survey of the Butterfly Fauna of Jatun Sacha, Ecuador (Lepidoptera: Hesperioidea and Papilionoidea)(4).

Book: Mariposas de Costa Rica (26).

Appendix B: Supporting Tables

Species	Response Variables	Sig.	Source	df	F	P
P. aduncum	Total Herbivore Species Richness	*	Latitudinal Site	4	12.81	<0.0001
		~	Population	7	0.37	0.9079
P. aequale		*	Latitudinal Site	4	8.35	0.0001
		*	Population	7	4.45	0.002
P. aduncum	Specialist Herbivore Species Richness	~	Latitudinal Site	4	1.25	0.3118
		~	Population	7	2.00	0.0909
P. aequale		~	Latitudinal Site	4	1.48	0.2334
		~	Population	7	1.07	0.4045
P. aduncum	Genus Herbivore Species Richness	*	Latitudinal Site	4	5.35	0.0025
		~	Population	7	0.50	0.8257
P. aequale		~	Latitudinal Site	4	2.29	0.0840
		~	Population	7	2.24	0.0604
P. aduncum	Generalist Herbivore Species Richness	*	Latitudinal Site	4	6.40	0.0009
		~	Population	7	0.21	0.9783
P. aequale		*	Latitudinal Site	4	4.64	0.0053
		~	Population	7	1.92	0.1026
P. aduncum	Total Herbivore Density	*	Latitudinal Site	4	4.76	0.0046
		~	Population	7	0.4146	0.8850
P. aequale		*	Latitudinal Site	4	9.17	<0.0001
		*	Population	7	2.77	0.0252
P. aduncum	Specialist Herbivore Density	~	Latitudinal Site	4	1.22	0.3240
		~	Population	7	1.40	0.2441
P. aequale		~	Latitudinal Site	4	1.13	0.3584
		~	Population	7	0.98	0.4605
P. aduncum	Genus Herbivore Density	*	Latitudinal Site	4	5.10	0.0032
		~	Population	7	0.24	0.9691
P. aequale		*	Latitudinal Site	4	4.16	0.009
		~	Population	7	1.86	0.1137
P. aduncum	Generalist Herbivore Density	*	Latitudinal Site	4	9.33	<0.0001
		~	Population	7	0.26	0.9635
P. aequale		*	Latitudinal Site	4	5.31	0.0026
		~	Population	7	1.81	0.1229
P. aduncum	Herbivory percentage	~	Latitudinal Site	4	0.33	0.8553
P. aequale		~	Latitudinal Site	4	1.48	0.2335

Table S1. Results for the ANOVAs test for the effect of latitudinal site and population on herbivore density, species richness, and plant damage. All values were normalized by total leaf area. Variables with asterisks indicate a significant difference among sites or populations.

Latitudinal Site	R2*	Adjusted R2	Std. Error**	df	F	p
Piper aduncum; Percentage of herbivory vs. Total herbivore abundance						
Mexico	0.902	0.888	4.93499	8	64.379	0.0001
Costa Rica	0.884	0.868	5.99812	8	53.454	0.0001
Ecuador	0.918	0.906	4.38692	8	78.48	0.0001
Peru	0.932	0.922	4.48672	8	95.704	0.0001
Bolivia	0.936	0.927	3.83755	8	102.142	0.0001
Piper aequale; Percentage of herbivory vs. Total herbivore abundance						
Mexico	0.837	0.813	4.766	8	35.822	0.001
Costa Rica	0.981	0.978	1.57183	8	361.5	0.0001
Ecuador	0.927	0.916	3.20069	8	88.436	0.0001
Peru	0.774	0.742	6.90461	8	24.001	0.002
Bolivia	0.808	0.78	5.81336	8	29.383	0.001
Piper aduncum; Percentage of herbivory vs. Herbivore species richness						
Mexico	0.94	0.932	3.84506	8	110.581	0.0001
Costa Rica	0.934	0.924	4.53973	8	98.536	0.0001
Ecuador	0.928	0.917	4.12246	8	89.799	0.0001
Peru	0.895	0.88	5.56708	8	59.71	0.0001
Bolivia	0.813	0.786	6.55284	8	30.432	0.001
Piper aequale; Percentage of herbivory vs. Herbivore species richness						
Mexico	0.913	0.901	3.4678	8	73.884	0.0001
Costa Rica	0.911	0.898	3.40091	8	71.715	0.0001
Ecuador	0.887	0.87	3.97923	8	54.745	0.0001
Peru	0.668	0.621	8.37194	8	14.086	0.007
Bolivia	0.833	0.809	5.41765	8	34.892	0.001

Table S2: Regression through the origin of **percentage of herbivory** with **total herbivore abundance** and **herbivore species richness** for *P. aduncum* and *P. aequale*. Herbivore abundance is measured as the number of lepidopteran larvae present per 100 m^2 of leaf area. Species richness is calculated as the number of species of lepidopteran larvae present per 100 m^2 of leaf area.

* For regression through the origin (no-intercept model), R Square measures the proportion of the variability in the dependent variable about the origin explained by regression. This CANNOT be compared to R Square for models that include an intercept.

** Of the estimate.

Piper aduncum

Piper aequale

Mexico, Veracruz, Los Tuxtlas Biological Station

Family	Number of Species	Number of individuals
Geometridae	1	17
Hesperiidae	2	15
Limacodidae	1	3
Noctuidae	1	5
Nymphalidae	1	8

Family	Number of Species	Number of individuals
Limacodidae	1	9
Geometridae	1	9
Hesperiidae	2	12

**Costa Rica, Heredia,
Tirimbina Biological Station**

Arctiidae	1	1
Bombycidae	1	7
Crambidae	3	10
Geometridae	3	9
Hesperiidae	5	45
Lasiocampidae	2	2
Limacodidae	3	6
Noctuidae	5	49
Nymphalidae	2	33
Papilionidae	1	14
Sphingidae	1	3
Tortricidae	3	5

Megalopygidae	1	2
Tortricidae	1	3
Limacodidae	2	8
Geometridae	3	12
Hesperiidae	3	14

Ecuador, Napo, Jatun Sacha Biological Station

Arctiidae	2	3
Bombycidae	2	2
Crambidae	2	3
Geometridae	4	9
Hesperiidae	5	20
Lasiocampidae	2	4
Limacodidae	3	3
Noctuidae	5	21
Nymphalidae	3	29
Papilionidae	1	9
Sphingidae	1	3
Tortricidae	3	3
Pyralidae	3	5

Geometridae	2	18
Hesperiidae	3	27
Limacodidae	3	12
Tortricidae	1	3
Saturniidae	1	1
Bombycidae	1	2
Pyralidae	2	2

Peru, Iquitos, Yanamono Island

Arctiidae	2	2
Bombycidae	1	2
Geometridae	2	12
Hesperiidae	2	28
Limacodidae	1	2
Noctuidae	2	10
Riodinidae	2	3
Nymphalidae	2	6
Papilionidae	1	18
Tortricidae	3	3

Limacodidae	1	2
Geometridae	2	7
Saturniidae	1	1
Pyralidae	1	1
Hesperiidae	2	8

Bolivia, La Paz, Chalalan Ecological Reserve

Geometridae	1	18
Hesperiidae	1	8
Crambidae	1	2
Noctuidae	1	5
Riodinidae	1	2
Papilionidae	1	2
Arctiidae	1	1

Limacodidae	1	3
Geometridae	1	5
Hesperiidae	1	8

Table S3: Number of species and individuals of lepidopteran larvae by family collected at the five latitudinal sites.

Appendix B: Supporting Figures

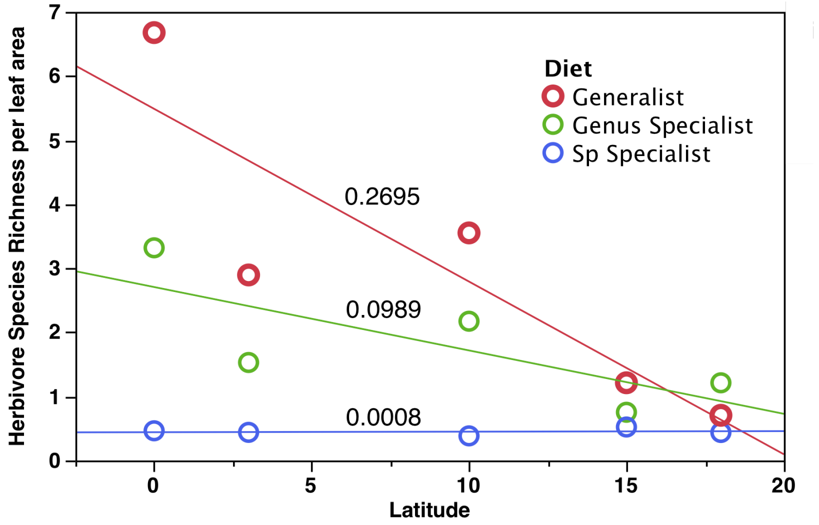
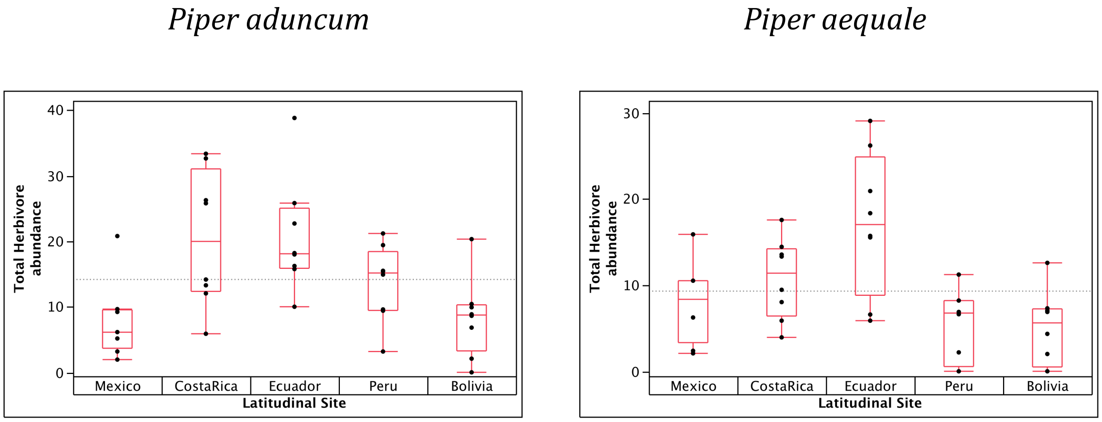


Figure S1: Simple linear regressions for the diet breadth categories of herbivores across the latitudinal gradient. The numbers above the regression lines are the slopes for each regression. Generalists: $r^2 = 0.7653$, F = 9.784, p = 0.0521 (Red); Genus specialists: $r^2 = 0.5807$, F = 4.154, p = 0.1343 (Green); Specialists: $r^2 = 0.01662$, F = 0.0507, p = 0.8363 (Blue).


Piper aduncum

Piper aequale

Figure S2: Total abundance per latitudinal site of *Piper aduncum* and *P. aequale*. Herbivore abundance is measured as the number of lepidopteran larvae present per 100 m^2 leaf area. Points on the graph represent each of the eight sampled populations per latitudinal site. ANOVAs showed a significant greater herbivore abundance at lower latitudes (*P. aduncum*: F = 4.76, df = 4, p = 0.0046; *P. aequale*: F = 9.17, df = 4, $p < 0.0001$).

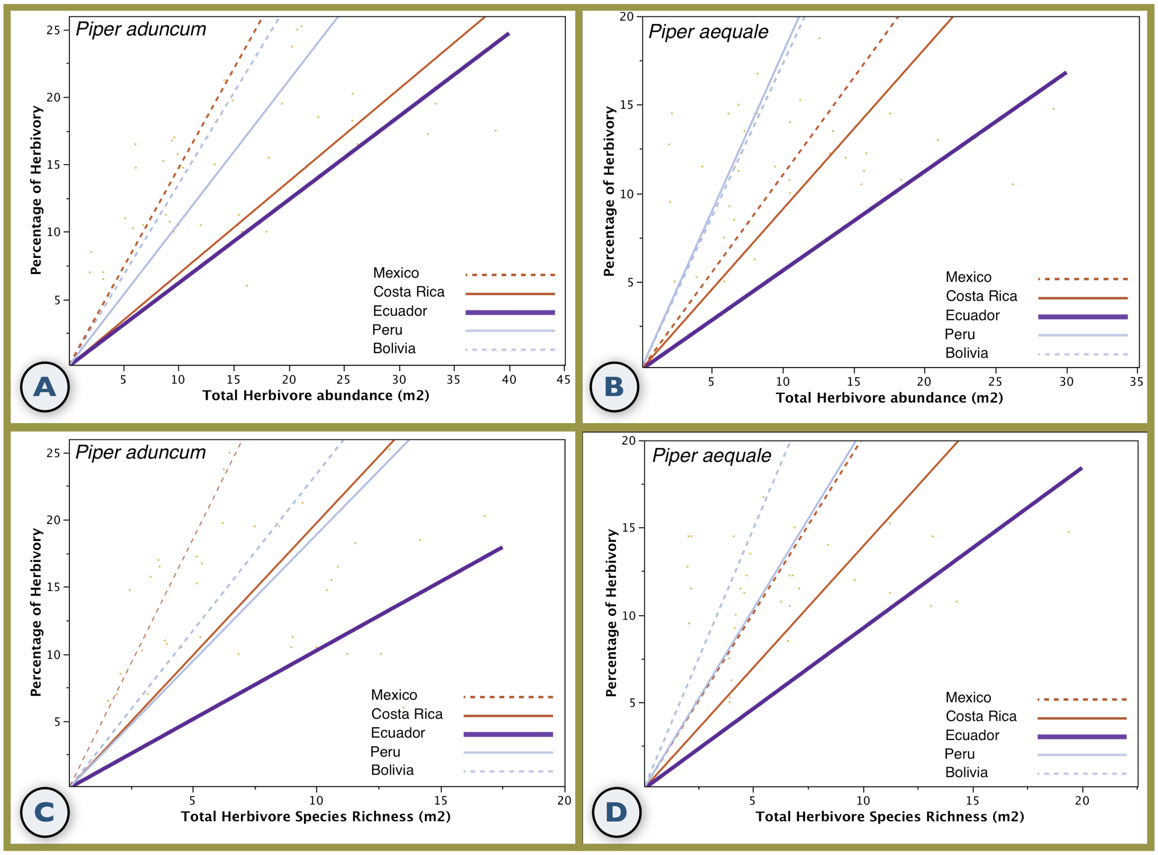
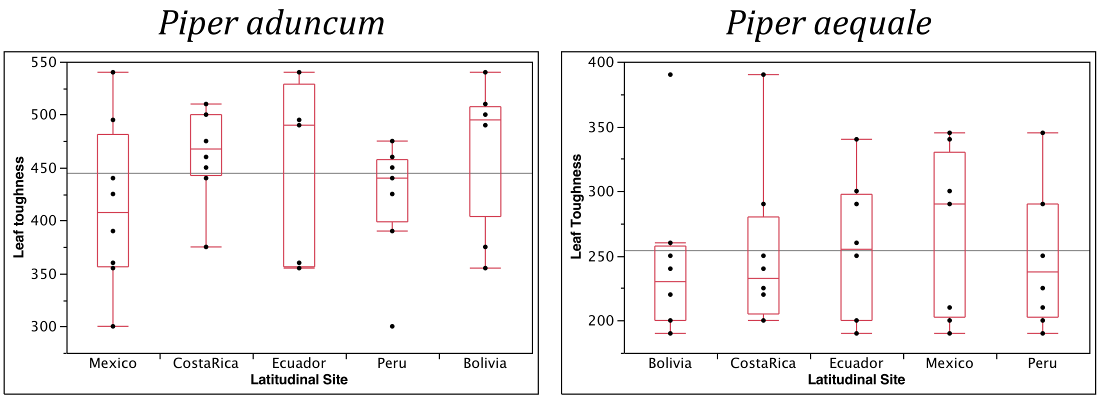


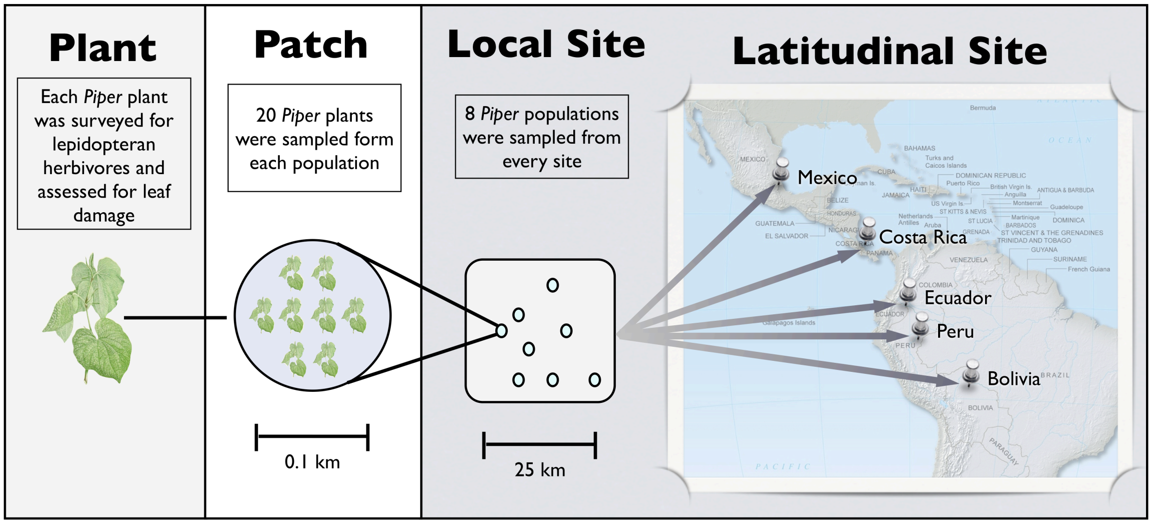
Figure S3: Linear regression through the origin for **percentage of herbivory** versus **herbivore abundance** (A and B), and **total herbivore species richness** (C and D) latitudinal site of *Piper aduncum* and *P. aequale*. **Dashed lines** represent the study sites at higher latitudes (Bolivia and Mexico), **thin solid lines** represent the "medium" latitude sites (Costa Rica and Peru), and the **thick solid line** represents the latitude 0 site (Ecuador). **Red lines** are sites north of the equator and **blue lines** are sites south from the equator. Specifics on the regression analysis are in Table S2 and S3.



Piper aduncum

Piper aequale

Figure S4: Leaf toughness per latitudinal site of *Piper aduncum* and *P. aequale*. All leaves were collected from the tip of the branches. Toughness was measured as the amount of grams of force needed to pierce a 0.5 cm diameter hole at the center of the leaf. Points in the graph represent the average toughness of 30 leaves from the 8 sampled populations per latitudinal site. ANOVAs showed no significant differences in leaf toughness across the five latitudinal sites (*P. aduncum*: F = 1.1417, df = 4, p = 0.3531; *P. aequale*: F= 0.2247, df = 4, p = 0.92).



Each *Piper* plant was surveyed for lepidopteran herbivores and assessed for leaf damage

20 *Piper* plants were sampled form each population

8 *Piper* populations were sampled from every site

Figure S5: Diagram of the herbivore and herbivore damage census sampling design.

Figure S6: On-site improvised rearing labs.

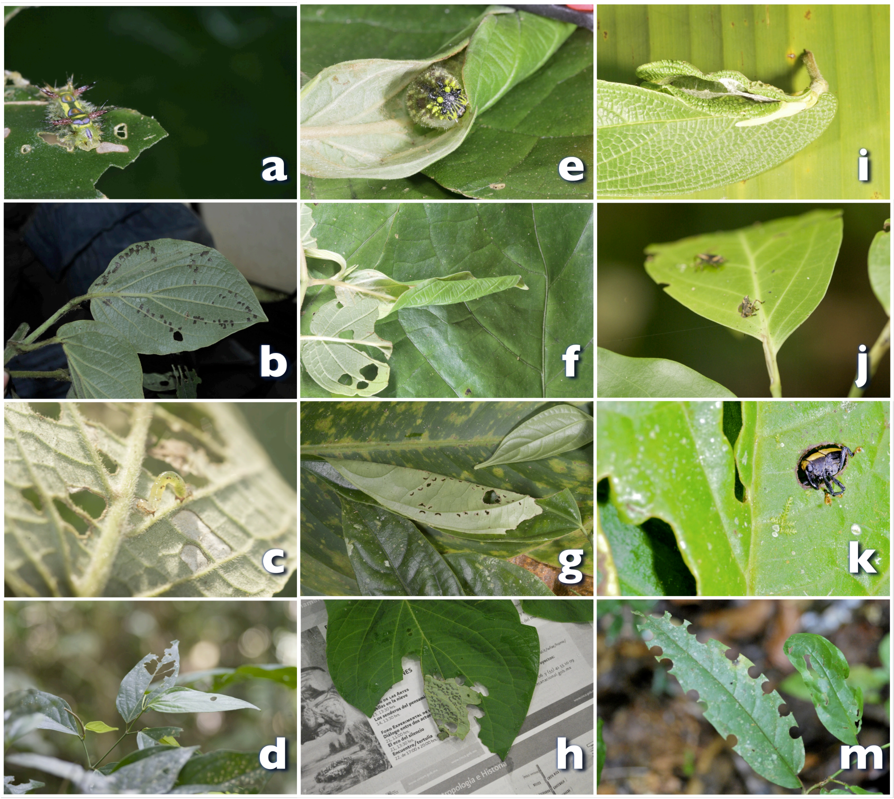


Figure S7: Examples of types of herbivore damage common in neotropical *Piper* species. **a:** Generalist lepidopteran larva (Limacodidae). **b, c:** Specialist geometrids (*Eois* spp. - Geometridae). **d:** Specialist *Eois* spp. on *P. aequale*. **e, f:** Genus specialist leaf roller (*Consul fabius* – Nymphalidae). **g, h:** Genus specialist leaf folder (*Quadrus cerialis* – Hesperiidae). **i:** Generalist leaf roller. **j, k:** Weevil damage (Curculionidae)**.** **m:** Leafcutter bee damage (Megachilidae).

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Chapter 2

Directed seed dispersal of *Piper* by *Carollia perspicillata* and its effect on understory plant diversity and folivory

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Directed dispersal occurs when seeds are differentially deposited to sites where offspring survivorship is higher than at randomly chosen sites. Traditionally, characteristics of the dispersal target sites that could increase survivorship of the dispersed plants are thought to be intrinsic to the sites. If directed dispersal is constant over extended periods of time, however, it is likely that non-random patterns of dispersal could modify the ecological characteristics of the target site in ways than could increase survivorship and fitness of the dispersed plants. Here we

report patterns of *Piper* diversity (richness, equitability and similarity) and *Piper* folivory within plots near natural or artificial roosts of *Carollia perspicillata* versus similar plots without bat roosts. Plots with bat roosts, both natural and artificial, had significantly higher *Piper* species diversity. Additionally, we found that plots with a higher *Piper* species diversity showed less specialist folivory, higher generalist folivory, and lower total herbivore leaf damage than plots with low *Piper* diversity. Finally, plots with bat roosts also showed less specialist folivory, lower generalist folivory, and lower total folivory when compared to plots without roosts. We propose that long lasting non-random patterns of seed dispersal can change the local ecological characteristics of target sites via changes in plant diversity, and that these changes are likely to reduce the local rates of folivory and therefore, increase seed and adult plant survivorship.

Introduction

Seed dispersal is an important ecosystem service that can influence the fitness of both the dispersed offspring and the dispersing adult (Hubbell 1979, Dalling and Hubbell 2002, Hooper et al. 2004). The characteristics of the site to which a propagule is dispersed can determine the probability of its survival (Howe and Smallwood 1982, Howe and Miriti 2000, Dalling and Hubbell 2002, Hooper et al. 2002, Hooper et al. 2004, Howe and Miriti 2004). Two mechanisms that mediate this effect are: (1) colonization of sites whose abiotic conditions are suitable for germination, growth, and reproduction, and (2) escape from the negative influence of biotic forces, including natural enemies and intra- and inter-specific competition (Janzen 1970, Howe and Smallwood 1982). Despite this general conceptual framework, the potential effect of dispersal on plant fitness is thought to be highly unpredictable due to the apparent stochastic nature of dispersal events (Wenny 2001).

Directed dispersal occurs when seeds are differentially deposited by their vectors to sites where the probability that a seed will germinate, survive, and reproduce will be higher relative to randomly chosen sites (Howe and Smallwood 1982, Wenny and Levey 1998, Wenny 2001). Directed dispersal is common in myrmecochory, found to occur for a few bird species, and important for the demography of some temperate and tropical plant species (Wenny and Levey 1998, Wenny 2001, Pearson and Theimer 2004, Briggs et al. 2009). Studies of directed dispersal by agents other than ants and birds are few (Wehncke et al. 2003). In addition, study of the effects of directed dispersal has been restricted to seeds and seedlings (seed germination rate and secondary predation, seedling competition and mortality due to density dependent factors, pathogens, and natural enemies). To the best of our knowledge no study has assessed the impact of directed dispersal on adult plants and its potential effect on community processes.

Traditionally, characteristics of the directed dispersal target sites that could increase survivorship and fitness of the dispersed plants are thought to be unaffected by the dispersal event itself (Howe and Smallwood 1982, Wenny 2001). However, if the magnitude of dispersal to a non-random site (number of seeds dispersed per unit time) is high and constant over extended periods, it is likely that such non-random dispersal could change the ecological characteristics of the target site in ways that would eventually change the survivorship and fitness of the dispersed plants (see below). Given enough time, patterns of non-random dispersal could change the local community structure of the target site, and thereby generate changes in the community dynamics that could go beyond the increased survivorship of the dispersed plants.

One possible mechanism by which non-random dispersal patterns could affect community dynamics is by changing local plant species richness and diversity. Non-random patterns of seed dispersal could have opposing effects on local site diversity depending on the nature of the relationship between the dispersal agent and the dispersed species. If the dispersal agent is a generalist (dispersing a diverse assemble of plant taxa), local diversity at the dispersal target site will increase with directed dispersal. In contrast, if the dispersal agent is a specialist, then local plant diversity at the target site should decrease.

One community process affected by plant diversity is herbivory (Murdoch et al. 1972, Brown and Ewel 1987, Lewinsohn et al. 2005, Scherber et al. 2006, Unsicker et al. 2006). Theory and empirical data suggest that biomass lost to herbivores will be lower at sites with higher plant species diversity (Andow 1991, Pacala and Crawley 1992). When this is the case, we would expect that non-random patterns of seed dispersal by generalist dispersers would result in lower levels of herbivory. Given the typically strong relationship between herbivory and plant survival, distribution, and fitness (Marquis 1992, Dyer et al. 2004, Fine et al. 2004, Marquis 2004), it is reasonable to expect that plants dispersed by generalists to nonrandom sites will have an advantage over conspecific plants that are dispersed randomly. The most commonly cited hypothesis to explain the relationship between plant diversity and herbivore damage is the "resource concentration hypothesis" (Root 1973). Root's hypothesis states that herbivore damage suffered by a given plant in a community will depend on the encounter rate between the plant and its herbivores. In more complex (more diverse) plant assemblages, a particular resource (plant host) will have a lower relative abundance than in a more simple (less diverse) community. Consequently, this reduction in the relative abundance of a plant hosts constitutes a "dilution effect" that translates into a similar reduction in the encounter rate between that plant host and its potential herbivores. Furthermore, the hypothesis predicts that generalist and specialist herbivores are likely to interact differently with plant host diversity. Herbivores that have wider diet breadths (generalist herbivores) will be less affected by increasing diversity than herbivores with narrow diet breadths (specialist herbivores). For this reason it is critical to differentiate between these two kinds of herbivore damage when trying to associate insect herbivory with plant host diversity.

Frugivorous bats are important seed dispersers in tropical ecosystems (Whittaker and Jones 1994, Corlett 1998, Galindo-Gonzalez et al. 2000, Henry and Jouard 2007, Muscarella and Fleming 2007, Kelm et al. 2008b). Among neotropical frugivorous bats, *Carollia perspicillata* (Phyllostomidae) is both one of the most locally common species and one of the most widely distributed species, occurring from Mexico to southern Brazil. This species includes a large variety of fruits in its diet, but often with a strong preference for the infructescenses of *Piper* (Piperaceae), which may represent as much as 65 percent of the diet of these bats (Fleming 1988, Charles-Dominique 1991, Cloutier and Thomas 1992, Bizerril and Raw 1997, Lopez and Vaughan 2007). *Carollia perspicillata* is considered to be a *Piper*-specialist, but with a generalist behavior feeding on a wide variety of *Piper* species (Bizerril and Raw 1997, Thies and Kalko 2004, Kelm et al. 2008a).

Carollia perspicillata normally consumes fruits at feeding roosts away from the fruiting tree (Fleming 1988, Charles-Dominique 1991). In lowland forests, these feeding roosts are in mostly medium to large cavities in tree trunks to which *C. perspicillata* shows roost fidelity (Lewis 1995). Frugivorous bats have been found to be effective seed dispersers, which may defecate in flight or often shortly before engaging in flight, presumably to reduce body weight (Gorchov et al. 1993, Corlett 1998, Reiter et al. 2006, Muscarella and Fleming 2007). These behavioral traits increase the likelihood of seeds being dispersed near roosts, resulting in a *Piper* seed shadow associated with the roosts.

The objective of this study was to test three hypotheses. First, we tested the hypothesis that the diversity of *Piper* species near *Carollia perspicillata* roosts would be higher than at sites away from the roost due to non-random defecation patterns by the bats. The second hypothesis tested was that levels of folivory would change with changes in *Piper* diversity due to a resource dilution effect (a reduction in the relative abundance of any one *Piper* species). Finally, our third tested hypothesis was that levels of folivory on *Piper* individuals near bat roosts would be lower compared to the rest of the understory due to locally higher *Piper* species diversity produced by seed dispersal by the bats. Lower levels of folivory on *Piper* individuals found at these sites would suggest that increased local species diversity is likely to have a long-term effect on the site's community dynamics. We quantified *Piper* species richness and folivory (total damage, and amount due to specialist and generalist insect species separately) in the presence and absence of *Carollia* natural and artificial roosts in a lowland wet forest of Costa Rica. By doing so, we were able to confirm experimentally the effect of *Carollia* presence on *Piper* community structure and the consequences for community-level patterns of folivory. Nonrandom patterns of *Piper* seed dispersal to locations of higher *Piper* diversity, resulting in lower folivory, would constitute evidence for directed dispersal to "safe sites" for *Piper* species. Furthermore, if all three hypotheses hold true, these "safe sites" with reduced folivory will not only increase the survival of individuals in the early life stages, but also be more suitable sites to grow and reproduce. As a result, the long-term survivorship and fitness of the *Piper* plants present at the site might be increased.

Materials and Methods

Study site

The study was conducted at the La Selva Biological Station of the Organization for Tropical Studies (OTS) in the Atlantic lowlands of Costa Rica in July-August 2007 (10°26'N, 83°59'W, 4000 mm annual rainfall). This field station is located near the town of Puerto Viejo de Sarapiquí (Heredia), comprising ca. 1700 ha of tropical wet forest in various states of regeneration (75% of primary forest). To date, 1850 species of plants have been found in La Selva, 50 of which are *Piper* (2.7% of all neotropical *Piper* species and around 50% of all *Piper* species present in Costa Rica (TROPICOS 2010)).

Data Collection

To assess the effect of directed dispersal by *Carollia perspicillata* on local *Piper* communities, we established triplets of 42 circular plots, 10 m in radius, around focal trees, each member of a triplet representing a different treatment. In the first member of a triplet (NR, "natural roost" plots, N = 14), the plot was centered on a tree with a natural *Carollia perspicillata* roost (Fig. A1, A2). At the time of the study, natural roosts had been occupied by *C. perspicillata* for at least seven years. All focal trees for these plots were mature individuals with a diameter at breast height (DBH) larger than 100 cm. In the second member (SP, "same species" plots, N = 14), the center of the plot was the nearest tree of the same species as the NR but with no bat roost; all focal trees for these plots had a minimum DBH of 100 cm. These plots controlled for the effect of tree species identity on *Piper* species richness and insect folivory. The mean distance between SP and NR plots was 161 m (range: 43-232m). The third member of a triplet (SD, "same diameter" plots, N = 14) used as its center the nearest tree of the same DBH (within 20 cm) as NR regardless of identity but with no bat roost. These plots controlled for the potential effect of tree size. The mean distance between SD and NR plots was 87 m (range: 34-140 m). We also established eight additional plots (AR, "artificial roost" plots), 10 m in radius, centered on an artificial bat roost (Fig. D2), each occupied by *C. perspicillata* for at least five years (Kelm et al. 2008b). The spatial distribution of the AR plots was unrelated to that of the triplets. The mean distance between AR and NR plots was 389 m (range: 80-1050 m). Most AR plots were located in a different section of the forest than the triplets.

In each plot we counted the number of individuals of *Piper* 1 cm or greater in diameter at ground level, identifying each to species. For each *Piper* present in the plot we visually assessed the percentage of specialist and generalist folivory, measured as the percentage of leaf area removed. Every plant was given a value between 0% and 100%, in increments of 5%, for each folivory type. Specialist and generalist folivory in all *Piper* plants were easily distinguishable due to the characteristic skeletonization of leaves by *Piper* specialist herbivores from the genus *Eois*, Geometridae (Connahs et al. 2009, Dyer et al. 2010). *Eois* caterpillars are highly specialized and feed on one (rarely on two) species of *Piper* (Appendix B). Mature leaves are skeletonized from below, leaving pale, oval "windows", comprised of only the upper epidermis. All other damage was assigned to generalist herbivores (Dyer et al. 2010). Additionally, given that plant defenses (and therefore herbivory) can change with plant ontogeny (Boege and Marquis 2005), plant size data for each *Piper* individual were also collected. Plants were classified into one of three size categories; size 1 (smaller than 20 cm), size 2 (between 20 and 60 cm), and size 3 (taller than 60 cm).

To assess the effect of *C. perspicillata* on patterns of diversity of non-*Piper* plants, and the effect of surrounding plant diversity could have on *Piper* folivory (neighborhood effects), we also counted and identified all plants present within all plots (hereafter, non-*Piper* diversity). Finally, to test for the potential effect of light availability on folivory, we measured canopy openness for each plot using hemispherical photography (hereafter: Light (Frazer et al. 1999)). Light availability can influence leaf quality and leaf palatability, in so doing, potentially affecting levels of leaf herbivory

(Angulo-Sandoval and Aide 2000, Blundell and Peart 2001, Takafumi et al. 2010, Salgado-Luarte and Gianoli 2011).

Statistical analysis

To better represent the relative abundances of *Piper* species, as well as the probability of a plant-herbivore encounter, we computed the Gini-Simpson's Index (also known as Hurlbert's probability of interspecific encounter: PIE, (Hurlbert 1971)) for each plot as a measure of equitability. This index controls for the effect of different abundances among the *Piper* species present within the plots. An identical procedure was followed for non-*Piper* diversity. Given that there were fewer AR plots than those of the other treatments, we calculated sample-based rarefaction curves to accurately assess changes in *Piper* species richness among treatments (Software Estimate S, v7.5 (Colwell 2005)). Furthermore, to measure the *Piper* species similarity between the plot treatments we used the Horn-Morisita Index using R statistical software (v. 2.15.2) and the Vegetarian package (v.1.2) (Horn 1966, Jost 2007, Charney and Record 2009, R Core Team 2012). Given that most of the data collected was percentage data (folivory, Gini-Simpson's Index, canopy openness), we transformed the data using the *logit* function to achieve normality (Warton and Hui 2011). Normality was then confirmed by visual inspection and the Shapiro-Wilk test (Software JMP 8).

We analyzed the data using generalized linear mixed effects models (GLMM). To address hypothesis 1 we assessed the effect of treatment, light, and non-*Piper* diversity (as fixed effects) on *Piper* diversity in each plot (Gini-Simpson's Index) with triplet block as a random effect. Given the strong effect that light can have on herbivore damage; we included this variable as a fixed effect in order to contrast the effect of diversity and light on leaf damage. Similarly, to explore the potential impact that bat roosts could have on non-*Piper* plants as well as neighborhood effects we assessed the effect of treatment, light, and *Piper* diversity (all fixed effects) on non-*Piper* diversity in each plot. In these first two models the experimental unit was the plot. AR plots were coded to belong to separate spatial blocks. To address hypothesis 2 we assessed the effect of both *Piper* and non-*Piper* diversity on specialist, generalist, and total folivory. Here we used the Gini-Simpson's Index, light, plant size, and non-*Piper* diversity as fixed effects. Because different species of *Piper* are likely to have different average levels of folivory, we used the species identity of each plant and triplet block as crossed random effects. Finally, to test hypothesis 3, we assessed the effect of plot treatment, light, non-*Piper* diversity, and plant size (fixed effects) on the three types of folivory using triplet block and *Piper* species as crossed random effects. Plant size was included as a fixed effect to evaluate the effect of the treatments on different plant life stages. In these latter two sets of models, the experimental units were the *Piper* individuals within each plot. Model fit was evaluated via AIC's and maximum likelihood tests (see Appendix B for details on model construction and model selection).

In addition, to complement the GLMM analysis and allow a clearer interpretation of the effects of the different treatments, we calculated orthogonal contrasts between (1) treatments with and without roosts (AR/NR vs SD/SP), (2) treatments without roosts (SD vs SP), and (3) treatments with roosts (AR vs NR). This analysis was performed using a multiple linear hypothesis approach (Bretz et al. 2010) with the multcomp package (v1.2) for R (Horthorn et al. 2013).

Results

Bat roosts and Piper Diversity

The observed number of *Piper* species was higher on plots that had bat roosts at their center than those without roosts (means [standard error]: AR = 10.2 [1.2], NR = 12.1 [1.2], SD=4.2 [3.2], SP = 4.1 [2.6]). After standardizing the sampling effort among the treatments using sample-based rarefaction curves (samples = 8), *Piper* species richness estimates for plots with bats roosts were two times higher that plots without roosts (AR = 29, NR = 25, SP = 14, SD = 13; **Fig. 1**). *Piper* abundance (total number of *Piper* individuals) was also higher in the plots with bat roosts than the other two treatments (AR = 27.1 [2.6], NR = 24.0 [2.0], SD = 12.4 [2.0], SP = 13.6 [2.0]). The generalized linear mixed model showed that *Piper* diversity (Gini-Simpson Index) was significantly higher in plots with roosts (AR = 0.85 [0.03], NR = 0.89 [0.01], SD=0.54 [0.08], SP = 0.62 [0.07]; **Fig. 2**, **Tables 1.A, 2.A, C1**). There was no effect of bat roosts on non-*Piper* diversity (**Table C2, Fig. D1**). In addition to the increased *Piper* diversity, plots with bat roost were similar in *Piper* species composition and relative abundances (**Table 3**). In contrast, plots without roosts showed relatively low similarity to each other. Finally, the similarity between plots with and without roosts was variable, depending on the comparison (**Table 3**). Light did not affect *Piper* and non-*Piper* diversity in our plots (**Table C2**).

Table 1: Results of the generalized linear mixed model analysis showing the optimal models.

A. Hypothesis 1 (Effect of bat roosts on *Piper* diversity) [a]			
Piper Diversity = Plot Treatment + (Block) (n = 50)			
	df	F	P
Intercept	1	32.96	<0.0001
Treatment	3	7.96	<0.0001
B. Hypothesis 2 (Effect of *Piper* diversity on folivory) [ß]			
Specialist Folivory =*Piper* Diversity + Light + *Piper* Size + (Block) + (*Piper* Species) (n = 917)			
	df	F	P
Piper Diversity*	1	1026.6	<0.0001
Light †	1	76.7	<0.0001
Piper Size	2	7.3	0.0007
Generalist Folivory ~Piper Diversity + Light + Non-Piper Diversity + (Block) + (*Piper* Species) n=917			
	df	F	p
Piper Diversity*	1	48.9	<0.0001
Light †	1	286.8	<0.0001
Non-*Piper* Diversity*	1	59.8	<0.0001
Total Folivory ~*Piper* Diversity + Light + Non-*Piper* Diversity + (Block) + (*Piper* Species) n=917			
	df	F	p
Piper Diversity*	1	478.6	<0.0001
Light †	1	296.2	<0.0001
Piper Size	2	7.6	<0.0001
Non-Piper Diversity*	1	16.8	<0.0001
C. Hypothesis 3 (Effect of bat roosts on folivory) [ß]			
Specialist Folivory ~Plot Treatment + Light + *Piper* Size + Non-*Piper* Diversity + (Block) + (*Piper* Species) n=917			
	df	F	p
Treatment	3	613.2	<0.0001
Light †	1	129.6	<0.0001
Non-*Piper* Diversity	1	57.6	<0.0001
Generalist Folivory ~Plot Treatment + Light + *Piper* Size + Non-*Piper* Diversity + (Block) + (*Piper* Species) n=917			
	df	F	p
Treatment	3	151.1	<0.0001
Light †	1	138.5	<0.0001
Piper Size	2	4.45	0.011
Non-*Piper* Diversity *	1	23.1	<0.0001
Total Folivory ~Plot Treatment + Light + *Piper* Size + Non-*Piper* Diversity + (Block) + (*Piper* Species) n=917			
	df	F	p
Treatment	3	320.7	<0.0001
Light †	1	208.7	<0.0001
Piper Size	2	3.33	0.036
Non-*Piper* Diversity *	1	53.5	<0.0001

Notes: * *Piper* diversity and non-*Piper* diversity were calculated using the Gini-Simpson index. † Light is measured as the percentage of canopy openness. Folivory, diversity, and light are logit function transformed. [a] Model A was analyzed in R using the nlme package. [ß] Models B and C were analyzed using the lme4 package and p values where calculated using the lmerTest package. Additional information on model selection, methods and Markov Chain Monte Carlo analysis is available in the appendix.

Table 2: Results from the orthogonal contrasts analysis (multiple linear hypothesis approach) comparing treatment effects associated with hypotheses 1 and 3.

A. Hypothesis 1 (Effect of bat roosts on *Piper* diversity)

***Piper* Diversity~Plot Treatment + (Block) n=50**

Contrast	Estimate (SE)	z	p
NR/AR vs SP/SD	-6.06 (1.16)	-5.19	<0.0001
NR vs AR	-2.01 (1.04)	-1.93	0.13
SP vs SD	0.6 (0.53)	-1.19	0.5

C. Hypothesis 3 (Effect of bat roosts on folivory)

Specialist Folivory ~Plot Treatment + Light + Non-*Piper* Diversity + (Block) + (*Piper* Species)

Contrast	Estimate (SE)	z	p
NR/AR vs SP/SD	1.77 (0.04)	42.25	<0.0001
NR vs AR	0.32 (0.02)	8.84	0.06
SP vs SD	-0.05 (0.03)	-1.85	0.17

Generalist Folivory ~Plot Treatment + Light + *Piper* Size + Non-Piper Diversity + (Block) + (*Piper* Species)

Contrast	Estimate (SE)	z	p
NR/AR vs SP/SD	-0.01 (0.04)	-0.38	0.97
NR vs AR	0.55 (0.02)	20.82	<0.0001
SP vs SD	-0.07 (0.03)	-2.3	0.07

Total Folivory ~Plot Treatment + Light + *Piper* Size + Non-*Piper* Diversity + (Block) + (*Piper* Species)

Contrast	Estimate (SE)	z	p
NR/AR vs SP/SD	1.18 (0.04)	27.65	<0.0001
NR vs AR	0.50 (0.02)	18.1	<0.0001
SP vs SD	-0.08 (0.03)	-1.58	0.6

Notes: Each analysis contains three contrasts: (1) plots with roosts versus plots without roosts (NR/AR vs SP/SD), (2) same diameter tree plots versus same species tree plots (SP vs SD), and (3) artificial bat roost plots versus natural bat roost plots, (NR vs AR). The null hypothesis test on each contrast is that both factors in the contrast are equal. Model A was analyzed in R using the nlme package and multcomp package. Models B and C were analyzed using the lme4 package and multcomp package.

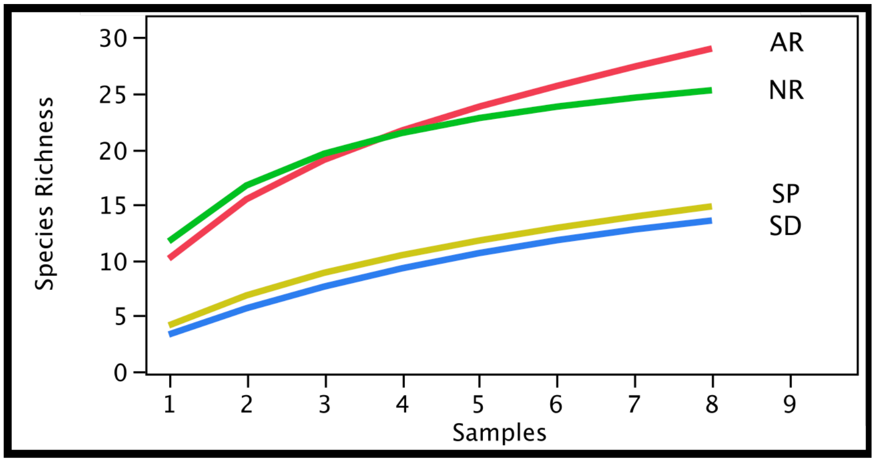

Figure 1: Sample based rarefactions (at eight samples) for the plot treatments. Species richness: AR = 29 species, (computed number of individuals = 217, CI 95%= 21.63, 36.37); NR = 25.26 species (computed number of individuals = 190.86, CI 95%= 20.57, 29.95); SP = 14.82 species (computed number of individuals = 106.86, CI 95%= 9.77, 19.88); SD = 13.55 species (computed number of individuals = 83.14, CI 95%= 8.66, 18.44). CI = confidence intervals.

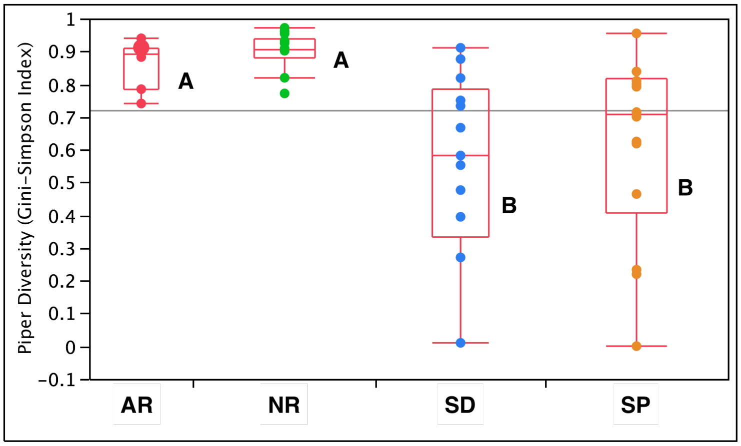

Figure 2: Species diversity of *Piper* (Gini-Simpson Index) for each of the four different plot types. Treatments sharing the same letter are not significantly different. Each point represents a sampled plot. AR: artificial bat roost plots (n=8), NR: natural bat roost plots (n=14), SD: same diameter tree plots and (n=14), SP: same species tree plots (n=14).

Piper diversity and folivory

Levels of folivory were relatively high with an average of 26.5% (SE = 1.4) leaf area missing, with approximately 10% (SE = 0.58) generalist versus 16% (SE = 1.1) specialist folivory. *Piper* diversity had a significant negative effect on specialist folivory and had the strongest effect on specialist herbivore damage of all included fixed variables (**Tables 1.B, C1**). Similarly, *Piper* diversity had a significant negative effect on total folivory but a contrasting significantly positive effect on generalist leaf damage (**Table 1.B, C2**). Non-*Piper* diversity varied between 0.95 to 0.70 (Gini-Simpson Index) and had a significantly negative effect on generalist and total folivory (**Tables 1.B, C2**) but not on specialist folivory (**Table C2, Fig. D1**). Light had a significant positive effect on all measures of folivory, with the strongest effect on generalist folivory (**Tables 1.B, C1**). Finally, plant size was found to have a small but significant effect on specialist leaf damage. Being smaller (sizes 1 and 2) had a positive effect on specialist folivory. Being larger (size 3) had no effect on folivory (**Table C1**).

Bat roosts and folivory

Plots with natural and artificial bat roosts showed significantly smaller amounts of specialist and total leaf damage compared to the other two treatments, while generalist folivory was higher in plots with natural roosts (**Table 2.C, Fig. 3**). There was no significant difference in specialist herbivory between plots with natural and artificial roost (**Tables 2.C, C1**). However, plots with natural roosts showed significantly higher amounts of total and generalist folivory when compared to plots with artificial roosts

(**Tables 2.C, C1, Fig. 3**). Plots lacking bat roosts were not significantly different in any of the three measures of folivory (**Table 2.C**).

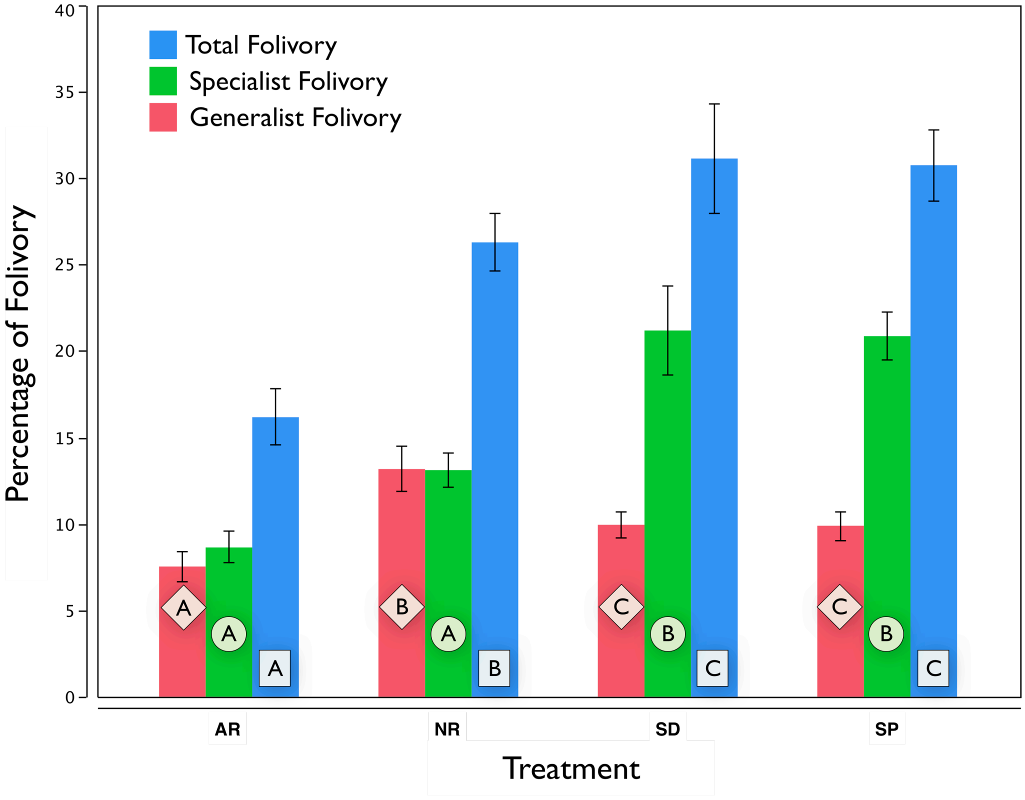

Figure 3: Mean percentage of total, specialist and generalist folivory by plot types. Letters on the bars show significant differences between plot types; categories of folivory from two plot types not sharing the same letter are significantly different (see Table 2 and S1 for details). Error bars show one standard error from the mean. AR: artificial bat roost plots (n=8), NR: natural bat roost plots (n=14), SD: same diameter tree plots and (n=14), SP: same species tree plots (n=14).

Table 3: Results of the community similarity analysis (Horn-Morisita Index) between for the different plot treatments.

Treatments	NR	SD	SP
AR	0.94	0.64	0.84
NR	…	0.56	0.76
SD	…	…	0.66

Notes : Numbers closer to 1 indicate high similarity in terms of species composition and species equitability. Treatments: AR: artificial bat roost plots (n=8), NR: natural bat roost plots (n=14), SD: same diameter tree plots and (n=14), SP: same species tree plots (n=14).

Discussion

In this study we combined an experiment with correlative data to explore the potential role of *Carolia perspicillata* on the directed dispersal of plants of the genus *Piper*. Our results show that the presence of day roosts of *Carollia* perspicillata significantly increases the local diversity of *Piper* communities (hypothesis 1). The results also show that local *Piper* diversity can have a strong effect on the level of leaf damage caused by insect herbivores (hypothesis 2). Finally, the combination of non-random patterns of seed dispersal by *Carollia perspicillata* and the relationship between *Piper* diversity and *Piper* folivory, significantly reduce the levels of insect damage on individuals arising from seeds dispersed near *Carollia* roosts (hypothesis 3).

Bat roosts and Piper species richness

The patterns we describe here for *Piper* species richness, abundance, and diversity among plots with different focal tree types suggest that there is non-random dispersal of this plant genus by bats, most of which are likely to be *Carollia perspicillata*. Plots around natural bat roosts had higher *Piper* species richness, higher *Piper* abundance, and higher *Piper* diversity than those around trees without roosts, both of the same tree species and around trees of the same diameter but of a different species. Moreover, *Piper* diversity around artificial roosts in place for approximately five years was equivalent to that around natural roosts. Despite the lack of formal data on the age of the natural roosts, many of these roosts are well known by bat researchers and naturalist guides working in La Selva for at least 10 years (Pineda 2011).

Despite their relatively young age, the artificial roost already exhibited a significantly higher *Piper* species richness, abundance, and diversity than the non-roost plots. This is not surprising considering the effectiveness of bats as seed dispersers. Single fecal samples of *C. perspicillata* in the research area have been found to contain 1.7 ± 0.8 different seed species and 275 ± 358 single seeds, mostly of *Piper* (Kelm et al. 2008b). To fuel the daily energy expenditure of *C. perspicillata* [c. 80 kJ, (Delorme and Thomas 1996)], these bats would need to ingest at least 4-7 infructescences of *P. hispidum* (Kelm et al. 2008b), and with 2200 seeds per infructescense (Greig 1993), this would result in a minimum 7000 to 12000 seeds dispersed per bat per night. The heavy seed rain expected from each bat in combination with multiple bats sharing the same roost (Kelm et al. 2008b), bats defecating before or soon after flight (Reiter et al. 2006), and bats feeding on a wide assemble of *Piper* species (Lopez and Vaughan 2007), would represent a high input of seeds close to roosts.

The relatively small difference in *Piper* species richness and diversity between the natural and artificial roost plots, despite their age difference, suggests the possibility of a species carrying capacity or a species richness saturation point that would represent an upper limit to the number of *Piper* species that can coexist in a small area. Spiegel and Nathan (2010) have contrasted the effects of directed dispersal pressure and density dependent processes at the directed dispersal target site (also known as the Directed Dispersal Paradox). Their models suggest that sites that experience strong non-random dispersal are likely to reach a saturation point where the potential benefits of non-random dispersal are balanced by the negative effects of density dependent mortality.

Another possibility that could explain this species richness plateau is that local species richness is limited by the regional species pool. However, La Selva has an estimate regional pool of 50 *Piper* species and we only found total of 32 *Piper* species within our plots (**Table C4**). The per plot species richness apparent limit is more likely due to density dependent, intraspecific negative interactions (e.g., competition) and limited resources (similar to a lottery model *sensu* Iwasa et al. (1995)).

The influence of Piper species diversity on folivory

Our results also supported our second hypothesis that changes in *Piper* species diversity would likely change levels of herbivore leaf damage. Specialist herbivory was negatively correlated with species diversity, a pattern often found in other tropical and temperate studies (Andow 1991, Pacala and Crawley 1992, Haddad et al. 2001, Loreau et al. 2001, Scherber et al. 2006, Unsicker et al. 2006). Generalist folivory showed a significant positive correlation with the number of *Piper* species present in the plots. As stated in the introduction, one mechanism that could explain this pattern of species diversity on plant folivory is Root's "resource concentration" hypothesis (Root 1973). It is expected that the relative diversity of host plants in a community will affect the encounter rate between a specific herbivore and its plant host, and as a result, the amount of folivory (Brown and Ewel 1987, Andow 1991, Otway et al. 2005, Scherber et al. 2006). In the case of *Piper*, specialist herbivores responsible for leaf skeletonization are mainly highly specialized caterpillars of the genus *Eois* (Geometridae, Appendix B). Because each species of *Eois* is likely to feed only on one (and rarely two) species of *Piper* (Connahs et al. 2009, Dyer et al. 2010, Strutzenberger et al. 2010), an increase in local *Piper* species diversity is likely to decrease their encounter rate with their specific plant hosts thus, reducing the amount of specialist leaf damage.

In contrast, generalists are not expected to be affected by an increase in plant species diversity of a particular taxon. Although many of the generalist herbivores that feed on *Piper* also feed on taxa from other plant families, there is a small but abundant group of generalists that will eat many species of *Piper* but only *Piper* species. Species like *Quadrus cerialis* (Hesperiidae) and some species of *Gonodonta* (Noctuidae) and *Consul* (Nymphalidae), among others, are very rarely found feeding on non-*Piper* plants (Marquis 1992, Dyer and Gentry 2002, Janzen and Hallwachs 2009). For these "generalists", an increase in species diversity and species abundance of *Piper* is likely to increase their encounter rate with potential hosts, thereby boosting the amount of generalist damage. Given the natural history of *Piper* herbivores and the patterns we found in our data, we believe that our results are consistent with Root's hypothesis.

Canopy openness and plant size

Canopy openness was found to have a significant positive effect on all measures of folivory. This effect is likely due to increased leaf quality with higher levels of understory light (Chazdon 1992). Plant size showed a significant positive effect on folivory. Our data suggest that smaller plants tend to suffer higher levels of herbivory

(Table C1), which in turn suggests ontogenetic changes in resistance or leaf quality (Boege and Marquis 2005, Barton and Koricheva 2010, Quintero and Bowers 2012).

The influence of bat seed dispersal on Piper leaf area loss

Finally, our data support our third hypothesis linking the presence of bat roosts with changes in leaf area loss to herbivores. Levels of total and specialist folivory were significantly lower in both plot types with bat roosts. This result suggests that non-random patterns of *Piper* seed dispersal by bats have a significant effect on the amount of folivory among local *Piper* communities. Furthermore, due to reduced folivory, *Piper* plants at these high diversity spots have a significant advantage over individuals away from such dispersal sites. Marquis (1987) showed that *Piper arieianum* plants with lower rates of folivory show a significant increase in growth and seed production (see also Dyer et al. 2004a).

An alternative mechanism by which bats may influence the surrounding vegetation could be through the positive effect of guano deposition. If this were the case, we would expect higher diversity and higher abundance of all plant species around roosts compared to non-roost trees, not just *Piper* species. Neither expectation held true.

We propose that sites where *C. perspicillata* roosts are established do not initially have any intrinsic advantages for *Piper* plants. As non-random patterns of *Piper* dispersal persist, however, it is likely that these sites eventually develop characteristics that could improve the chances of survival and reproduction of individuals dispersed to the site. With the constant addition of new *Piper* species, the increase in local species richness will likely reduce the relative abundance of any particular *Piper* species, thus reducing the encounter rate between the *Piper* host and its herbivores. Finally, the resulting reduction in herbivore pressure will translate into a significant advantage for subsequent seeds dispersed to these sites and the plants arising from those seeds. Given that directed dispersal occurs when seeds are differentially deposited to sites that grant a fitness advantage relative to randomly chosen sites, we believe that, at least for our study site, *C. perspicillata* plays the role of a directed dispersal agent for *Piper* species. These effects may persist long after the bat roost is abandoned.

We acknowledge the fact that herbivory and diversity can change across time. Therefore we suggest caution in generalizing or extrapolating our results. Furthermore, it is important to note that the effect of herbivory in plant fitness may vary among species.

There is no indication of any direct advantage of these patterns of dispersal for the bats; other systems of directed dispersal show a similar unidirectional benefit for the plants (Wenny 2001). In such systems directed dispersal is not the result of a co-evolutionary adaptation of the dispersal vector but a “side effect” of foraging behaviors and habitat preferences (Wenny 2001). Nonetheless, the potential increase in plant fitness derived from this pattern of seed dispersal by bats is important for understanding the evolution and conservatism of reproductive structures in *Piper* (a spike of tightly packed flowers, and subsequently, tightly packed fruits; e.g., Burger 1972)). The potential advantage of non-random dispersal to sites of low herbivore pressure could have played an important role in the evolution of the close relationship between *Carollia* bats and *Piper* plants.

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Supplementary material for chapter 2

Appendix A: Supplementary figures of the studied bat roosts, experimental design.

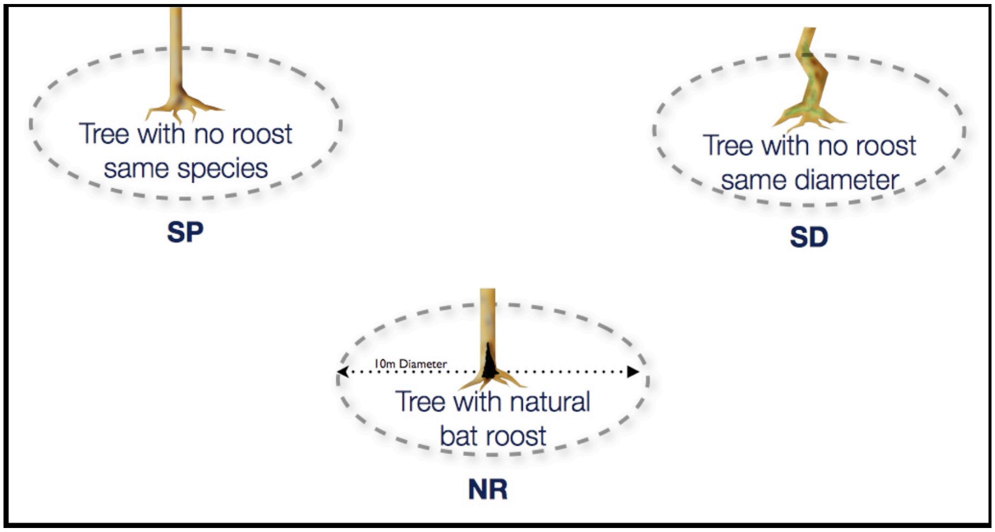

Figure A1: Schematic representation of the "plot triplets" used in the study. NR represents plots made around trees that had natural bat roosts of *Carollia perspicillata*. Same species (SP) plots are made around the nearest tree of the same species as the tree with the bat roost in the no roost (NR) plot. Same diameter (SD) plots are made around the nearest tree with the same DBH as the tree with the bat roost in the natural roost (NR) plot. Trees in the SD do not match the species of tree in the NR plot. Artificial roost (AR) plots spatial structure is independent from the triplet; artificial roosts were made in random locations.

Figure A2: Example of the bat roost studied. (a) Natural roost on *Terminalia oblonga* (Combretaceae). (b) Artificial roost (photogaphies by Salazar, D.).

Appendix B: Supplementary information on the local herbivore community and model selection procedures.

B1: Supplementary information on the herbivore community

Specialist Herbivores:

The genus *Eois* spp. (Geometridae) is by far the most common and well-studied specialized herbivore taxon for Neotropical species of *Piper* species. It has been shown to have a high degree of specialization, in whichone species of *Eois* moth will feed exclusively of one, or rarely, two species of *Piper*. These geometrids can be found along the entire geographical range of *Piper* and are well adapted to the complex secondary chemistry of *Piper* plants (Connahs et al. 2009, Brehm et al. 2011, Strutzenberger and Fiedler 2011, Bodner et al. 2012, Salazar and Marquis 2012). The damage produced by the larvae of *Eois* moths on *Piper* leaves is a very distinctive pattern of leaf skeletonization (Dyer et al. 2010). There are other taxa that are also considered to be *Piper* specialists (e.g., weevils [Curculionidae] (Marquis 1991)). In the current study, these weevils and the damage they cause were not abundant.

Generalist Herbivores:

Generalist herbivores on Neotropical *Piper* are highly diverse, Nevertheless, the majority of the damage at the study site is produced by two taxonomic groups: Lepidoptera (Hesperiidae, Nymphalidae, Limacodidae, Arctiidae, Geometridae, Pyralidae, Notodontidae), and Coleoptera (Curculionidae, and Chrysomelidae). The damage pattern produced by these herbivores varies from simple leaf area removal to elaborated leaf-folds and leaf-rolls (Dyer et al. 2010). Although many of the generalist herbivores that feed on *Piper* also feed on many taxa from other plant families, it is important to note that there is a small but abundant group of generalists that will eat many species of *Piper* but only *Piper* species. Species like *Quadrus cerialis* (Hesperiidae) and some species of *Gonodonta* (Noctuidae) and *Consul* (Nymphalidae) are found feeding almost exclusively on *Piper* plants (Dyer and Gentry 2002, Janzen and Hallwachs 2009).

B2: **Information on generalized linear mixed effects models**

We first built a “beyond optimal” model in which fixed effects included the maximum number of independent explanatory variables based on biological reasonable hypothesis (hereafter: full model). Then we used a “top-down” approach to improve the model by systematically omitting one fixed effect variable at a time. Omitted variables were selected using the ANOVA function (the variable with the least explanatory power based on the data was dropped). The new model was then compared with the previous model (model1 vs. model1 - dropped variable) using AIC with maximum likelihood test; the model that showed smaller AIC’s and $p > 0.05$ was selected. The optimal model was reached when all fixed effects variables achieved $p < 0.05$ on the ANOVA and AICs did not improve with further removal of fixed effects variables. All models used maximum likelihood estimation method. The contribution of the random effect variables on the improvement of the model was also tested comparing the full and the optimal models with and without the random effects variables fallowing the protocol above. Models where analyzed using R 2.15.2 (R Core Team 2012) and the nlme package (Pinheiro et al. 2013) with the exception of the models that have crossed random variables. which were instead analyzed using the lme4 package (Bates and Maechler 2010) and the languageR package (Baayen 2010) to assess significance values using Markov Chain Monte Carlo (MCMC). See table S1 for a complete list of the models and table 2 for results of the Mixed Models.

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Appendix C: Supplementary tables of the results from the generalized linear mixed models and Piper species sampled.

Table C1: Results from the optimal models of the generalized linear mixed models.

A.1 Piper Diversity~Plot Treatment + (1|Block) n=50

		Estimate (SE)	DF	t-value	p-value
(Intercept)		2.313 (0.384)	25	6.02	p<0.001
Treatment SD		-2.342 (0.553)	25	-4.22	p<0.001
Treatment SP		-1.708 (0.543)	25	-3.14	0.004
Treatment AR		-0.148 (0.637)	20	-0.23	0.818

B.1 Specialist Folivory ~*Piper* Diversity + Light + *Piper* Size + (1|fBlock) + (1|*Piper* Species) n=917

		Estimate (SE)	MCMCmean	HPD95% (lower| upper)	p (MCMC)
(Intercept)		-1.303 (0.073)	-1.3022	-1.4173 | -1.1823	p<0.001
Piper Diversity		-0.202 (0.006)	-0.2019	-0.214 | -0.189	p<0.001
Light		0.106 (0.012)	0.1067	0.0825 | 0.1304	p<0.001
Size 2		0.054 (0.020)	0.0557	0.0137 | 0.0967	0.0088
Size 3		-0.027 (0.022)	-0.0254	-0.0679 | 0.0193	0.2558

B.2 Generalist Folivory ~*Piper* Diversity + Light + Non-*Piper* Diversity + (1|fBlock) + (1|*Piper* Species) n=917

		Estimate (SE)	MCMCmean	HPD95% (lower| upper)	p (MCMC)
(Intercept)		-0.789 (0.157)	-0.7824	-1.0598 | -0.4864	p<0.001
Piper Diversity		0.043 (0.006)	0.042	0.0297 | 0.0537	p<0.001
Light		0.207 (0.012)	0.2087	0.1849 | 0.2338	p<0.001
Non-*Piper* Div.		-0.111 (0.014)	-0.1112	-0.1393 | -0.0835	p<0.001

B.3 Total Folivory ~*Piper* Diversity + Light + Non-*Piper* Diversity + *Piper* Size+ (1|fBlock) + (1|*Piper* Species) n=917

		Estimate (SE)	MCMCmean	HPD95% (lower| upper)	p (MCMC)
(Intercept)		-0.031 (0.141)	-0.0319	-0.2925 | 0.228	0.8094
Piper Diversity		-0.121 (0.005)	-0.1213	-0.132 | -0.1102	p<0.001
Light		0.188 (0.010)	0.1895	0.1667 | 0.21	p<0.001
Size 2		0.037 (0.018)	0.0382	0.0024 | 0.0728	0.0308
Size 3		-0.036 (0.019)	-0.0341	-0.0702 | 0.004	0.0738
Non-*Piper* Div.		-0.053 (0.013)	-0.0523	-0.0774 | -0.027	0.0002

C.1 Specialist Folivory ~Plot Treatment + Light + Non-*Piper* Diversity + (1|fBlock) + (1|*Piper* Species) n=917

		Estimate (SE)	MCMCmean	HPD95% (lower| upper)	p (MCMC)
(Intercept)		-1.192 (0.164)	-1.1683	-1.4665 | -0.844	p<0.001
Treatment SD		0.723 (0.028)	0.7229	0.6683 | 0.7799	p<0.001
Treatment SP		0.670 (0.025)	0.6706	0.6166 | 0.7198	p<0.001
an		-0.348 (0.267)	-0.3488	-0.7363 | 0.041	0.0818
Light		0.147 (0.013)	0.1485	0.1219 | 0.1742	p<0.001
Non-*Piper* Div.		-0.067 (0.016)	-0.0695	-0.1019 | -0.0389	p<0.001

C.2 Generalist Folivory ~Plot Treatment + Light + *Piper* Size + Non-*Piper* Diversity + (1|fBlock) + (1|*Piper* Species) n=917

		Estimate (SE)	MCMCmean	HPD95% (lower| upper)	p (MCMC)
(Intercept)		-1.049 (0.148)	-1.0538	-1.3316 | -0.7891	p<0.001

Treatment SD		-0.245 (0.025)	-0.2465	-0.2962 | -0.1981	p<0.001
Treatment SP		-0.324 (0.023)	-0.324	-0.3695 | -0.2795	p<0.001
Treatment AR		-0.477 (0.279)	-0.4793	-0.8453 | -0.1146	0.0114
Light		0.179 (0.018)	0.1791	0.156 | 0.202	p<0.001
Size 2		0.030 (0.019)	0.0313	-0.0048 | 0.0691	0.1002
Size 3		-0.038 (0.019)	-0.0368	-0.0759 | 0.0001	0.0586
Non-*Piper* Div.		-0.062 (0.014)	-0.0621	-0.09 | -0.0352	p<0.001

C.3 Total Folivory ~Plot Treatment + Light + *Piper* Size + Non-*Piper* Diversity + (1|fBlock) + (1|*Piper* Species) n=917

		Estimate (SE)	MCMCmean	HPD95% (lower| upper)	p (MCMC)
(Intercept)		-0.048 (0.158)	-0.039	-0.3405 | 0.2414	0.797
Treatment SD		0.364 (0.026)	0.3631	0.3069 | 0.4141	p<0.001
Treatment SP		0.283 (0.024)	0.2839	0.2353 | 0.3319	p<0.001
Treatment AR		-0.469 (0.290)	-0.469	-0.851 | -0.0877	0.019
Light		0.201 (0.012)	0.2021	0.1783 | 0.2268	p<0.001
Size 2		0.009 (0.020)	0.0104	-0.0308 | 0.0491	0.6052
Size 3		-0.036 (0.021)	-0.0341	-0.0756 | 0.0066	0.104
Non-*Piper* Div.		-0.079 (0.015)	-0.0799	-0.1093 | -0.0495	p<0.001

Notes: All models were selected using the optimization method described in the Appendix. *Piper* diversity and non-*Piper* diversity are calculated using the Gini-Simpson index. Light is measured as the percentage of canopy openness. Folivory, diversity, and light are logit function transformed.

Table C2: Linear mixed effects models used for model selection.

MODEL	D.F.	AIC	P-VALUE	Δ AIC
Simpson~treatment+light+Non-*Piper* Simpson+(triplet)	8	185	-	5
Simpson~treatment+Non-*Piper* Simpson+(triplet)	7	183	0.36	3
Simpson~treatment +(triplet)	6	182	0.41	2
Simpson~treatment+light+Non-*Piper* Simpson	7	182	0.99	2
Simpson~treatment	**5**	**180**	**0.62**	**0**
Specialist Folivory~Simpson+Light+Non-*Piper* Simpson+Size+(triplet)+(*Piper* species)	9	185	-	1
Specialist Folivory~Simpson+Light+Size+(triplet)+(*Piper* species)	**8**	**184**	**0.32**	**0**
Specialist Folivory~Simpson+Light+(triplet)+(*Piper* species)	6	194	<0.001	10
Specialist Folivory~Simpson+Light+Size+(triplet)	7	246	<0.001	62
Specialist Folivory~Simpson+Light+Size+(*Piper* species)	7	785	<0.001	601
Generalist Folivory~Simpson+Light+Non-*Piper* Simpson+Size+(triplet)+(*Piper* species)	9	101	-	1
Generalist Folivory~Simpson+Light+Non-*Piper* Simpson+(triplet)+(*Piper* species)	**7**	**100**	**0.15**	**0**
Generalist Folivory~Simpson+Light+Non-*Piper* Simpson+(triplet)	6	123	<0.001	23
Generalist Folivory~Simpson+Light+Non-*Piper* Simpson+(*Piper* species)	6	776	<0.001	676
Total Folivory~Simpson+Light+Non-*Piper* Simpson+Size+(triplet)+(*Piper* Species)	**9**	**-97**	**-**	**0**
Total Folivory~Simpson+Light+Non-*Piper* Simpson+(triplet)+(*Piper* species)	7	-86	<0.001	11
Total Folivory~Simpson+Light+Non-*Piper* Simpson+Size+(triplet)	6	-58	<0.001	39
Total Folivory~Simpson+Light+Non-*Piper* Simpson+Size+(*Piper* species)	6	719	<0.001	816
Specialist Folivory~treatment+Light+Non-*Piper* Simpson+Size+(triplet)+(*Piper* species)	11	297	-	2
Specialist Folivory~treatment+Light+Non-*Piper* Simpson+(triplet)+(*Piper* species)	**9**	**295**	**0.10**	**0**
Specialist Folivory~treatment+Light+Non-*Piper* Simpson+(triplet)	8	340	<0.001	45
Specialist Folivory~treatment+Light+Non-*Piper* Simpson+(*Piper* species)	9	537	<0.001	240
Generalist Folivory~treatment+Light+Non-*Piper* Simpson+Size+(triplet)+(*Piper* pecies)	**11**	**231**	**-**	**0**
Generalist Folivory~treatment+Light+Non-*Piper* Simpson+(triplet)+(*Piper* species)	9	237	0.01	6
Generalist Folivory~treatment+Light+Non-*Piper* Simpson+(triplet)	10	251	<0.001	20
Generalist Folivory~treatment+Light+Non-*Piper* Simpson+(*Piper* species)	10	481	<0.001	250
Total Folivory~treatment+Light+Non-*Piper* Simpson+Size+(triplet)+(*Piper* species)	**11**	**300**	**-**	**0**
Total Folivory~treatment+Light+Non-*Piper* Simpson+(triplet)+(*Piper* species)	9	303	0.02	3
Total Folivory~treatment+Light+Non-*Piper* Simpson+Size +(triplet)	10	336	<0.001	36
Total Folivory~treatment+Light+Non-*Piper* Simpson+Size+(*Piper* species)	10	588	<0.001	240

Notes: Variables in parenthesis are random variables. Δ AIC is the difference between a model and the "optimal model". Models in **bold** are the "optimal" models.

Table C3: Results from the generalized linear mixed effect model for non-*Piper* diversity.

Non *Piper* diversity ~*Piper* Diversity + Light + Plot Treatment + (1|Block) n=50

	Value	Std.Error	DF	t-value	p-value
(Intercept)	0.8886	0.035	22	24.76	<0.001
Piper Diversity	0.0066	0.006	22	1.05	0.30
Light	0.0136	0.010	22	1.27	0.21
Treatment NB	-0.0206	0.027	22	-0.75	0.45
Treatment SD	0.0384	0.030	22	1.23	0.21
Treatment SP	0.0201	0.028	22	0.70	0.48

Notes: *Piper* diversity and non-*Piper* diversity are calculated using the Gini-Simpson index. Light was measured as the percentage of canopy openness. Folivory, diversity, and light are logit function transformed (values range from -5 to 5).

Table C4: List of *Piper* species found in the four plot types and their total abundances.

***Piper* Species**	**N**
P. asymmetricum DC.	14
P. auritifolium Trel.	34
P. biolleyi C. DC	1
P. biseriatum C. DC.	2
P. cenocladum C. DC.	102
P. colonense C. DC.	45
P. concepcionis Trel.	1
P. darienense C. DC.	1
P. decurrens C. DC.	4
P. euryphyllum C. DC.	10
P. evasum Trel.	28
P. friedrichsthalii C. DC.	1
P. garagaranum C. DC.	44
P. generalense Trel.	124
P. glabrescens (Miq.) C. DC.	42
P. holdridgeanum W. C. Burger	54
P. melanocladum C. DC.	48
P. multiplinervium C. DC.	137
P. nudifolium C. DC.	15
P. paulowniifolium C. DC.	9
P. peracuminatum C. DC.	5
P. perbrevicaule Yunck.	6
P. prismaticum C. DC.	1
P. pseudobumbratum C. DC.	22
p. reticulatum L.	20
P. sancti-felicis Trel.	7
P. silvivagum C. DC.	2
P. tonduzii C. DC.	1
P. urophyllum C. DC.	5
P. urostachyum Hemsl.	102
P. xanthostachyum C. DC.	18

Appendix D: Supplementary information on the variation of non-Piper species diversity and canopy openness across the four plot treatments.

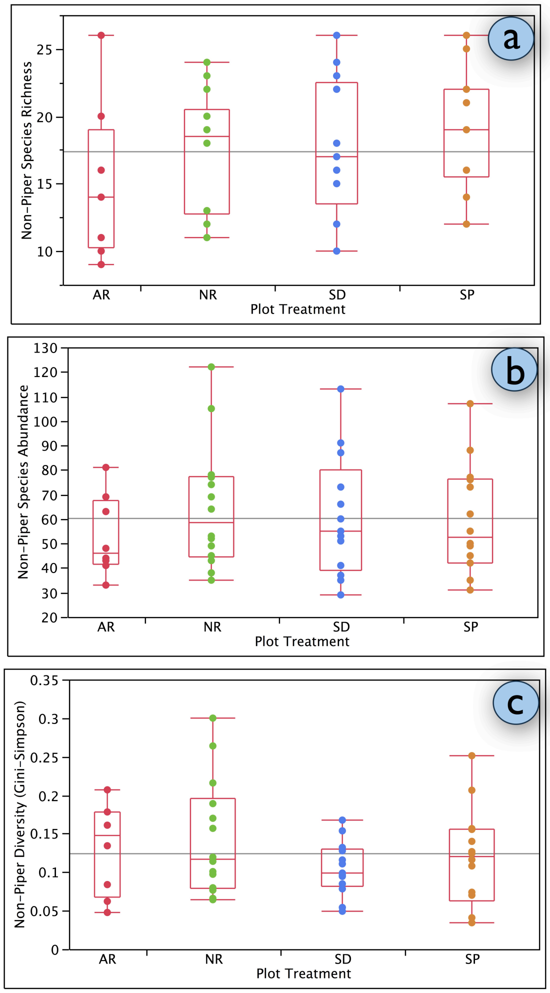

Figure D1: Non-*Piper* species richness (a), species abundance (b), and diversity (c, calculated as Gini-Simpson Index) for the four plot treatments. AR: artificial bat roost plots, NR: natural bat roost plots, SD: same diameter tree plots and, SP: same species tree plots.

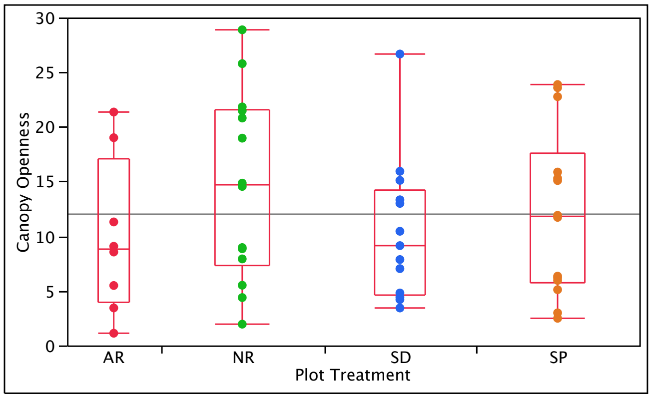

Figure D2: Canopy openness for each of the four different plot treatments. Canopy openness is measured as the percentage of sky area covered by canopy vegetation. AR: artificial bat roost plots, NR: natural bat roost plots, SD: same diameter tree plots, and SP: same species tree plots.

Chapter 3

Exploring the relationship between plant chemical diversity and intraspecific chemical variation in *Piper*

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Plants have a great diversity of secondary metabolites that can have a significant role in the protection against many biological and environmental negative interactions. Nevertheless, this diversity is not evenly distributed across plant taxa and can vary significantly between and within species. Despite the great interest in species diversity and the potential economical value of these secondary metabolites, far less attention has been given to the patterns and the ecological importance of chemical diversity. Here we quantify the relationship between plant intraspecific chemical variation and plant chemical diversity by comparing four species of *Piper* (Piperaceae) that vary in their chemical diversity. We tested the hypothesis that plants with low chemical diversity are likely to have lower amounts of intraspecific

chemical variation than plant species with high chemical diversity due to the high ecological cost associated with these compounds. We found that species with high chemical diversity showed significantly higher amounts of chemical intraspecific variation than species with lower chemical diversity, a pattern that was repeated across two collection sites and apparent despite the multiple potential sources that can cause secondary compound variation in natural systems. We discuss possible mechanisms that could be responsible for this pattern.

Introduction

Plants produce an immense diversity of secondary compounds, often variable among species but predictable in general type along phylogenetic lines (Hadacek 2002). This rich plant chemical diversity is further enhanced by the countless combinations of compounds that can comprise the chemical profile of a given plant species (Jones and Firn 1991). In addition to the variation in chemical composition that exists between plant species, plants of the same species can also exhibit great variation depending of the interaction between the plant genotype, plant ontogeny, and a multitude of biotic and abiotic factors (Boege and Marquis 2005, Nyman and Julkunen-Tiitto 2005, Kleine and Müller 2011).

Nevertheless, not all plant species possess high chemical diversity or high intraspecific chemical variation. Even within a particular plant genus, not all species will have the same chemical diversity. While some plants could have an array of co-dominant secondary metabolites, others can produce as little as one dominant secondary metabolite (Jones and Firn 1991). Given the major ecological role that secondary metabolites can play in plant-herbivore interactions, the variation in chemical diversity among and between plant species could correspond to distinctively different defensive strategies with equally different tradeoffs.

On one end of the chemical diversity spectrum, in plant species that have low chemical diversity, single secondary metabolites are likely to have a very high ecological significance to the plant. This is because these few compounds will be responsible to directly or indirectly reduce the herbivore pressure applied by a diverse array of herbivores species with very different evolutionary and co-evolutionary histories. Similarly, these few compounds will also have to perform their protective function across a diversity of abiotic environments. Given the high ecological importance of single metabolites in plant species with low chemical diversity, we predict that plants containing one or few dominant secondary metabolites for chemical defense will have a low intraspecific variation in their chemical profile.

In contrast, on the other end of the chemical diversity spectrum, distinct secondary metabolites are likely to play a smaller individual role, protecting against individual species or a single guild of herbivores (Lankau 2007, Bidart-Bouzat and Kliebenstein 2011, Kleine and Müller 2011). Because herbivores are not evenly distributed in space and time, it will be reasonable to expect that plants with high secondary metabolite diversity to exhibit higher levels of intraspecific variation in their chemical profile, compared to species protected by one or a few compounds.

The goal of this study was to explore the relationship between plant chemical diversity and intraspecific chemical variation. Our main hypothesis is that plants with low chemical diversity (low secondary metabolite richness) are likely to have lower amounts of intraspecific chemical variation than plant species with high chemical diversity. This negative relationship arises as a result of the high ecological cost associated with these compounds.

We tested this hypothesis using genus *Piper*, a pantropical plant genus with more that 1500 species native to the Neotropics. The natural range of the genus in the New World is from northern Mexico to northern Argentina. *Piper* species are abundant in low- and mid-elevation forests (but rarely reaching 2,500 m) and are often among the top ten most speciose genera in Neotropical forests (Marquis 2004). Most Neotropical species occur in discrete, multi-species patches that can contain up to 22 different species. *Piper* represents an appropriate system to test our hypothesis because the secondary chemistry of this group is very diverse and highly variable from one species to the other (Parmar et al. 1997, Parmar et al. 1998, Dyer et al. 2004, Kato and Furlan 2007, Sun et al. 2007, Fischer et al. 2008, Friedman et al. 2008). Furthermore, the tendency of *Piper* to grow in multispecies patches allows testing this hypothesis in species that are exposed to identical biotic and abiotic conditions.

To test our hypothesis we have compared the intraspecific chemical variation on the dominant leaf secondary metabolites between four sympatric *Piper* species that vary greatly in terms of secondary chemistry and represent a gradient along the *Piper* chemical diversity spectrum. *Piper* species were compared among and between two distinct geographical sites.

Materials and methods

Collection Sites

Samples were collected in two sites along the Atlantic slope of Costa Rica (Central America). The first group of samples (lowland site) was collected in the vicinity of the northern limits of the Gandoca-Manzanillo National Wildlife Refuge, located at 73 km north of Limon City (9°37’45” N, 82°40’06” W; Talamaca, Limon). This site is a lowland tropical rain forest with an average elevation of 25 m. The second group of samples (mountain base site) was collected in the vicinity of the eastern limits of Braulio Carrillo National Park (10°09’51” N, 83°53’45” W; Vasquez de Coronado, San Jose). This site is located in the transition between the lowland rain forest and cloud forest and has an average elevation of 550 m.

Target Species

For this study we worked with four pioneer *Piper* species: *P. umbellatum* (L.), *P. peltatum* (L.), *P. auritum* (Kunth), and *P. aduncum* (L.). Even though most *Piper* species are understory shrubs, adapted to low light conditions, these four species are common and abundant in secondary forests, forest gaps, and forest edges. In combination, they represent the full spectrum of variation in leaf defense profiles in the genus. *Piper auritum* is the species that shows the simplest secondary chemistry of the group in terms of the number of abundant secondary compounds. This species has only a single dominant secondary metabolite, **safrole** (McBurnett et al. 2007, Monzote et al. 2010). *Piper peltatum* is slightly more chemically diverse that *P. auritum* due to the presence of two abundant secondary metabolites. One of these metabolites is **4-nerolidylcatechol**, a prenylated catechol that can be found in *P. peltatum* plants as well as in other members of the Pothomorphe clade (Kijjoa et al. 1980, Rezende and Barros 2004, Pinto et al. 2010, Lopes et al. 2013, Mendanha da Cunha et al. 2013). The other very abundant metabolite in *P. peltatum* is the sesquiterpene **germacrene D** (Parmar et al. 1997, Cicció and Segnini 1998). *Piper umbellatum* (the sister species of *P. peltatum*) is highly diverse in sesquiterpenes. The major component of *P. umbellatum* chemical profiles is **caryophyllene**, a very common compound in other *Piper* species but that is found in high abundance in this taxon (Martins et al. 1998, Núñez et al. 2005, Tabopda et al. 2008, Cruz et al. 2012, Tabopda et al. 2012). In addition to caryophyllene, high abundances of **humulen**, **spathunelol**, and **copaene** are also found (Martins et al. 1998, Núñez et al. 2005, Tabopda et al. 2008, Cruz et al. 2012, Tabopda et al. 2012). Finally, the *Piper* with the highest chemical diversity of the four pioneer species is *P. aduncum.* The most abundant secondary metabolite of this species is **dillapiol**, a propenylphenol that has been found in all plants of *P. aduncum* (Cicció and Ballestero 1997, Vila et al. 2005, De Oliveira et al. 2006, De Almeida et al. 2009, Parise-Filho et al. 2011). Additionally, *P. aduncum* can have an array of abundant secondary metabolites that vary in identity from one population to the other (Cicció and Ballestero 1997, Parmar et al. 1997, Vila et al. 2005, De Oliveira et al. 2006, Bernardo et al. 2008, De Almeida et al. 2009). For our populations in Costa Rica the most abundant metabolites are **β-asarone**, **β-cubebene**,

eudesmol, and an additional unknown compound (mw. 264) (Cicció and Ballestero 1997).

Sample collection

We collected 20 leaf material samples for all four *Piper* species, each from a different individual. Half of the samples were collected in the lowland site and half from the montane site. All leaf material was collected from young, fully extended leafs. Additionally, all samples where selected to have similar herbivore damage (between 5 and 10%, damage was assessed visually). All collected plants where found growing in the edge of secondary forests. Samples where dried with silica gel and transported to the University of Missouri-St Louis for chemical analysis.

Sample analysis

For each sample 0.4 g of material was measured and pulverized under liquid nitrogen. Samples were extracted using 1:1 methanol-chloroform solution and sonicated for 25 min. As an internal standard, 10 mg of piperine were added to all samples. Samples were finally filtered and stored at -80c° until analysis.

Identification of the dominant secondary metabolites was performed using GC-MS (HP 5890 coupled with a quadrapole 6872) with helium as a carrier gas and a HP-5 capillary column (30 m). The mass spectra of the different compounds in the samples were compared with NIST and MassBank Databases as well as primary literature. To assess the intraspecific variation between the samples we used a GC-FID (HP 5890) with an identical capillary column and identical chromatographic conditions than the GCMS. Chromatograms of each sample were integrated and the area of the peaks of the dominant metabolites in each species was calculated. Chromatograms were then aligned using COTW (Correlation Optimized Time Warping).

Statistical analysis

To visualize the difference in chemical variation between and among species we performed a hierarchical cluster analysis (Ward algorithm) with heat maps. To quantify the amount of chemical variation within and among species we used two approaches. First we assessed the amount of variation within the most abundant compound in each species. Chemical variation for each major compound within each sample was calculated as the absolute value of the difference between the peak area of the metabolite for that sample and the average peak area for that metabolite for that species. All chemical variation values were standardized using z-scores to make the variation between species and metabolites statistically comparable. We used ANOVA to compare the chemical variation values.

Our second approach was to calculate intraspecific chemical distances based on the hierarchical cluster analysis. Distances between the samples in a cluster analysis represent the differences in chemical composition across multiple secondary metabolites. Thus, these distances function as a proxy for assessing the chemical variation across multiple secondary metabolites. We used ANOVA to test if there were significant differences in terms of chemical distances among the four *Piper* species. Finally, to assess differences in intraspecific variation within species across the two collection sites we compared chemical distances (calculated as above) with t-tests (JMP 8.01).

Results

The hierarchical clustering analysis grouped all species in well-defined clusters (Fig. 1). Nevertheless, the analysis also showed intraspecific variation within each of the studied *Piper* species. This variation was especially high in *P. umbellatum* and *P. aduncum* where plants vary greatly in the relative abundance of the major secondary metabolites (Fig. 1). Samples within collection sites grouped in discrete clusters for the two *Piper* species with the lowest chemical diversity (*P. auritum* and *P. peltatum*) but did not form single clusters in the two species with high chemical diversity (*P. umbellatum* and *P. aduncum*; Fig. 1).

In our first statistical approach major secondary metabolites for each species across the two sites showed a wide range of mean intraspecific variation (lowland /mountain base): safrole in *P. auritum* 0.34/2.98, 4-neroligylcatechol in P. *peltatum* 0.68/1.14, caryophyllene in *P. umbellatum* 0.84/1.70 and, dillapiol in *P. aduncum* 2.84/6.65. However, after the analysis of variance, only dillapiol in *P. aduncum* showed a significant higher amount of variation at both sites than compounds in the other three sites (lowland: F=11.10, df=3, p<0.0001; montane: F=13.22, df=3, p<0.0001; Fig. 2).

After our second statistical approach, the intraspecific variation in chemical distances showed a similar pattern to the variation in major secondary metabolites. Nevertheless, the variation between the species was more pronounced (species; mean intraspecific chemical distance: lowland/mountain base): *P. auritum*; 0.40/1.46, P.

peltatum; 1.05/1.75, *P. umbellatum*; 2.78/3.51 and, *P. aduncum*; 4.38/3.85 (Fig. 3). Moreover, despite that *P. peltatum* and *P. auritum* did not show a significant difference in the intraspecific variation at either site (measured as chemical distances), *P. umbellatum* and *P. aduncum* showed significantly higher intraspecific variation that the two former species for the lowland and the mountain base sites (F=33.71, df=3, p<0.0001 and F7.58, df=3, p=0.0006 respectively; Fig. 3). Furthermore, *P. aduncum* had significantly higher variation than *P. umbellatum* at the lowland site (Fig. 3). Finally, mirroring the results from our first approach, *P. aduncum* also had significantly higher intraspecific variation than the other three species (F=33.71, df=3, p=<0.0001; Fig 3).

Finally, only *P. auritum* showed a significant difference in level of chemical production between the two collection sites (t=12.3, df=11.02, p<0.0001).

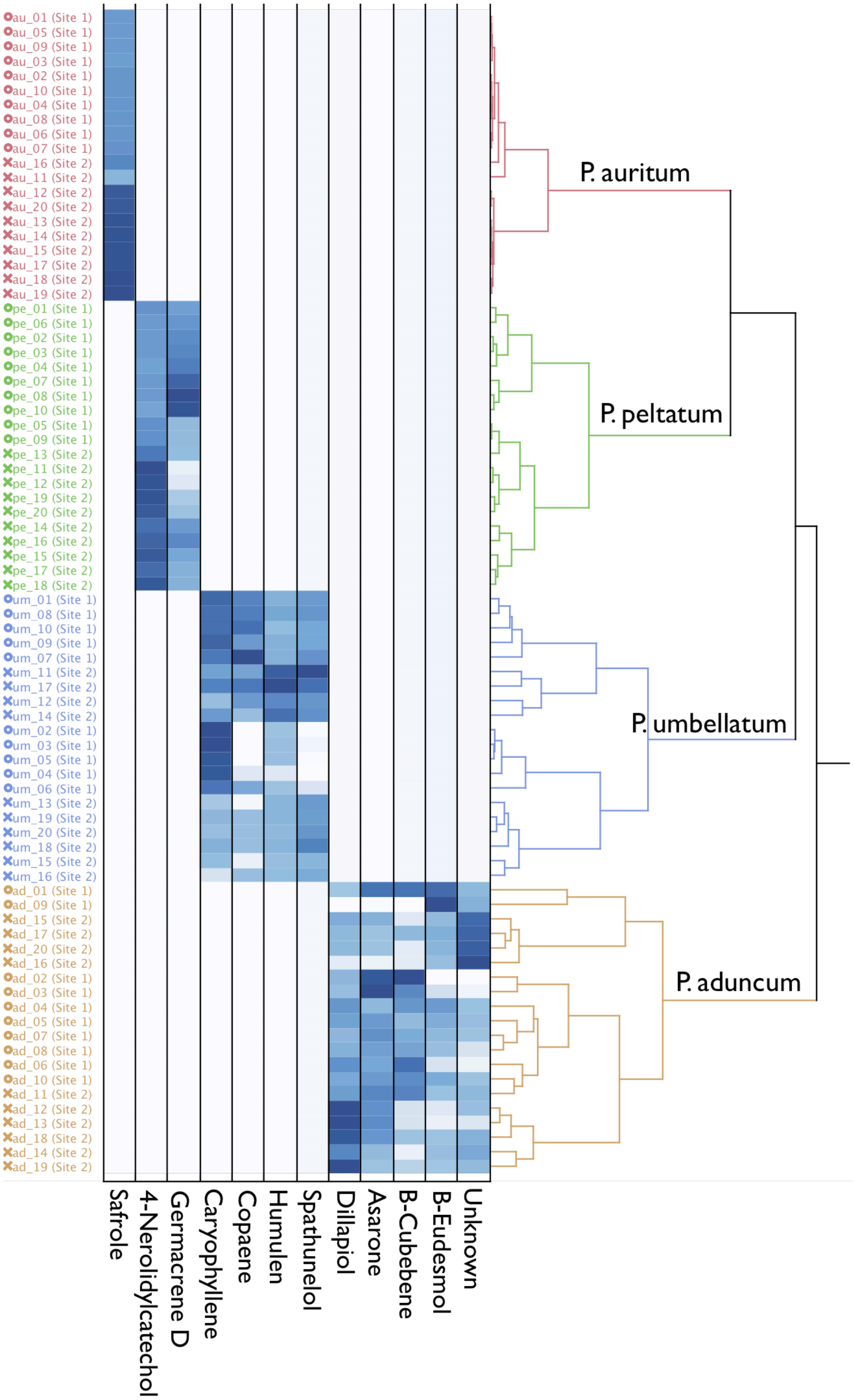

Figure 1. Chemical hierarchical clustering dendrogram base on z-transformed data for abundances of the major secondary metabolites of the four *Piper* species studied. Branch lengths represent chemical distance between the samples. Darker tones represent higher abundances of the compounds.

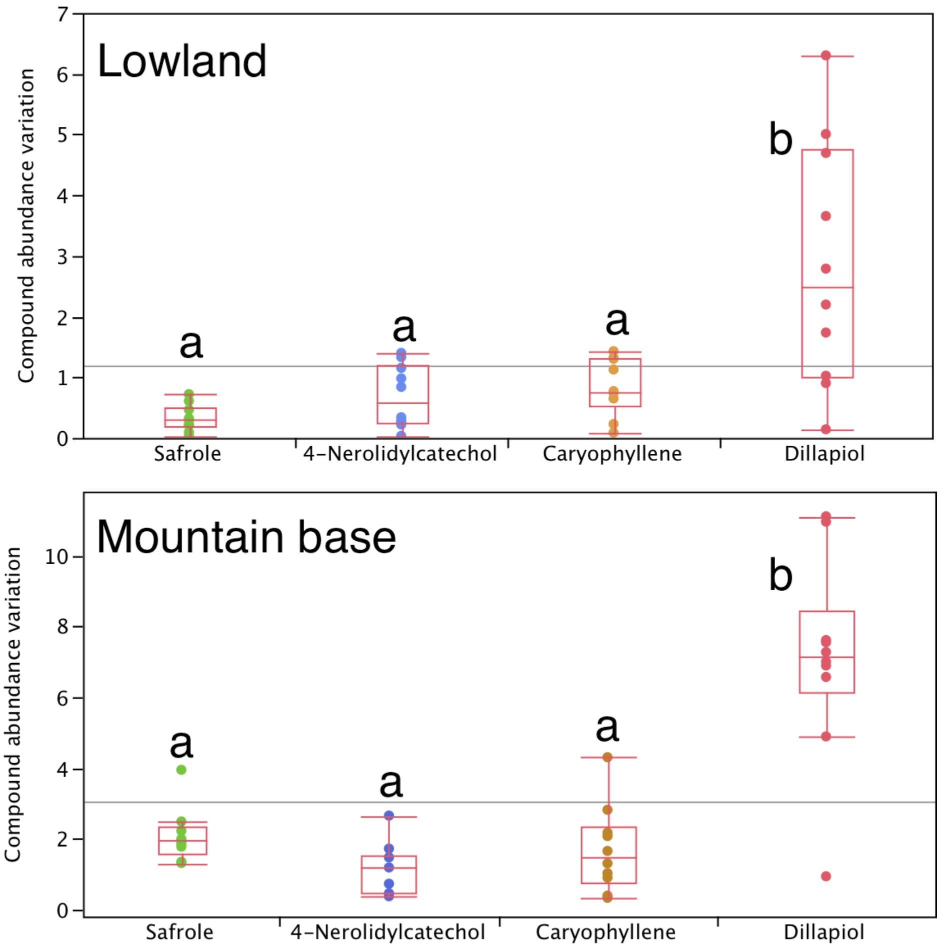

Figure 2: Variation in the abundance of the major compound for all species studied: safrole (*P. auritum*), 4-nerolidylcatechol (*P. peltatum*), caryophyllene (*P. umbellatum*), and dillapiol (*P. aduncum*). Compounds not sharing a letter are significantly different. Horizontal line denote the mean of means.

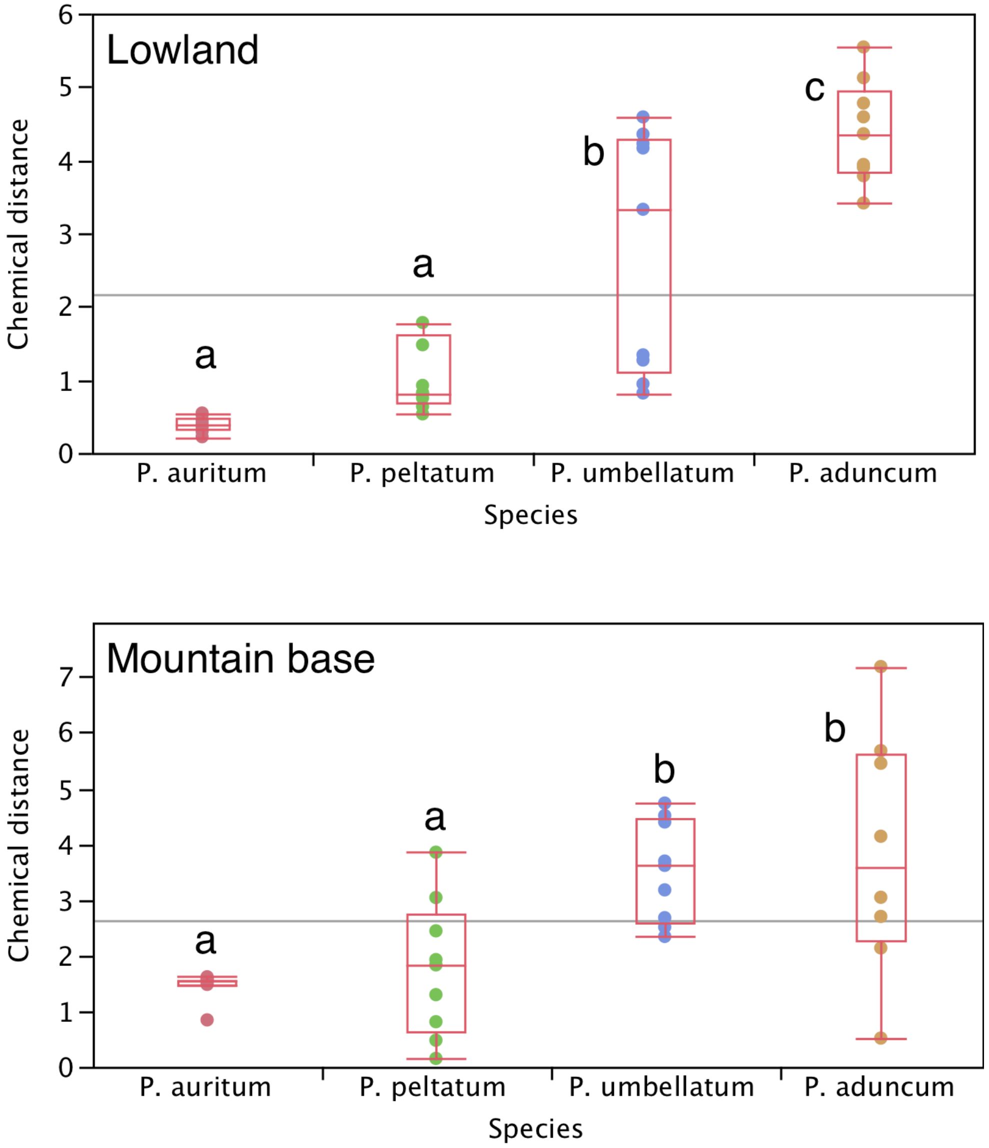

Figure 3: Chemical variation between the four target species measured as the chemical distances calculated by hierarchical clustering. Species not sharing a letter are significantly different. Horizontal line denotes the mean of means.

Discussion

Although there is widespread interest in processes that lead to the creation and maintenance of species diversity, much less consideration has been given to addressing the processes of creation and maintenance of biological chemical diversity. Even though most researchers agree on the importance of plant secondary metabolites for mediating plant species evolution and interactions of plants with other organisms (Hadacek 2002), very few models have been proposed to specifically explain the high variability in chemical diversity seen across plant taxa. Evolutionary models, for example like the "arms race" hypothesis (Ehrlich and Raven 1964), the "chemical defense escalation" hypothesis (Vermeij 1994, Agrawal and Fishbein 2008, Becerra et al. 2009), and the "screening hypothesis" (Jones and Firn 1991) have generated frameworks for evolutionary research and bio-prospection applications. However, they shed limited light on the mechanisms that will allow closely related species to vary greatly in their secondary metabolite diversity. In order to disentangle the mechanisms that account for the great interspecific variation on chemical diversity found in nature, it is crucial to first understand the potential tradeoffs associated with changes in plant chemical diversity.

In this study we explored the relationship between secondary chemical diversity produced by individual plants and variation in that chemical production among plant species. We tested that hypothesis that plants with low chemical diversity are likely to have lower amounts of intraspecific chemical variation than plant species with high chemical diversity due to the potentially high ecological cost associated with these compounds. Overall, the results of this study support this hypothesis. By comparing the intraspecific variation on dominant secondary metabolites across a gradient of plant chemical diversity we have found suggestive evidence of a potential tradeoff between metabolite richness and the relative ecological value of secondary compounds. This tradeoff consisted of an increase in intraspecific variation among individuals of plant species that produced a greater variety of secondary compounds, compared to species that produce few.

This pattern was found in both sampling sites despite the potential differences between the two locations. One important difference between the sites that can affect levels of intraspecific variation is the difference in soil heterogeneity. The high diversity of soil types that can be found at the montane site (Sollins et al. 1994) could also explain the increase in variability at this location via changes in resource availability (Coley et al. 1985).

There are two potential alternative explanations for the patterns found here. First, differences in intraspecific variation could be due to differences in resource availability between the *Piper* species (Coley et al. 1985). However, all *Piper* species used in the study are secondary growth specialists. They all share the same habitat and are exposed to identical light conditions (full sunlight) and similar soil heterogeneity. A second possibility is that the difference in metabolite intraspecific variation between the species is associated with different herbivore communities. It could be that plants with higher chemical diversity (in our case *P. aduncum* and *P. umbellatum*) have a more diverse herbivore fauna and thus, more likely to exhibit higher levels of intraspecific variation. Nevertheless, feeding records based on sampling more than 15 years (Janzen and Hallwachs 2009) show that there is no obvious relationship between herbivore diversity and intraspecific chemical variation on this four *Piper* species. *Piper auritum*, the plant species that showed the lowest secondary metabolite variation has a total of 32 genera of herbivores (97 species), while *P. aduncum* has 24 genera of herbivores (48 species). Only 9 genera of herbivores (16 species) have been found to feed on *P. umbellatum*, which has the second highest intraspecific variation. Furthermore, many of the herbivores species share three or all four *Piper* species (*Dysodia* spp. [Thyrididae]; *Quadrus* spp. [herpesiidae]; *Anacrusis* spp. [Tortricidae]; *Consul* spp. [Nymphalidae]; *Gonodonta* spp. [Noctuidae])(Janzen and Hallwachs 2009).

It is also important to note that the pattern found in this study was apparent despite many factors that can contribute to plant-to-plant chemical variation, most notably intraspecific genetic variation (Mauricio and Rausher 1997, Osier and Lindroth 2001), induction (Agrawal 1998), and changes in resource availability (Coley et al. 1985). Furthermore, the plants with different chemical diversities are likely to interact differently with these factors creating equally distinctive patterns of chemical variation within each species, and that these different interactions can also help explain the pattern found here.

In terms of intraspecific genetic variation, is also likely that plant genetic variability can interact with changes in secondary metabolite diversity to create different patterns of intraspecific variation. Because the different secondary metabolites associated with different chemical defense genotypes can potentially play distinctive defense roles, species with high chemical diversity are more likely to respond differently to different selective pressures. Consequently, changes in the diversity and in the spatial and temporal distribution of herbivores (as well as pathogens and environmental stresses) can change the relative abundance of chemical defense genotypes (Agrawal et al. 2012, Hare 2012, Züst et al. 2012) and therefore generate high amounts of intraspecific variation (Iason et al. 2011). Contrastingly, dominant secondary metabolites in plants with low chemical diversity are likely to play multiple functions (e.g., emodin (Izhaki 2002)) and for that reason, increasing the relative ecological importance of these metabolites for plant fitness. As a result of the multiple functions and high ecological value, multiple selective pressures are likely to select for similar defense genotypes and therefore, generate lower amounts of intraspecific chemical variation.

Similarly, plant induced defenses can also generate different patterns of chemical intraspecific variation depending on the chemical diversity of the plant taxon. Research has shown that different species of herbivores can potentially induce different chemical responses from the plant host (Delphia et al. 2007, Dicke et al. 2009, Clavijo McCormick et al. 2012). These differential responses are due to the specificity that these compounds can have with either the herbivores (direct defenses) or their predators (indirect defenses). Thus, different compounds will have potentially different ecological importance to the plant depending of the presence (or absence) of specific herbivores. For this reason, it is reasonable to expect that plants with high chemical diversity will exhibit a broad array chemical induction profiles that will inevitably increase the intraspecific chemical variation of the species. Conversely, less chemically diverse plants whose secondary compounds are more likely to have less specific direct and indirect effects on herbivores, will possess a more limited combination of chemical responses in the presence of inductive forces, even under intrinsically different herbivore pressures. For this reason it could also be expected that plants with extremely low chemical diversity (one or two dominant compounds) are more likely to rely on constitutive defenses than inducible responses to herbivore pressures.

Finally, with respect to resource availability, plants with high chemical richness are likely to have more variation due to the potentially diverse pathways involved in the production of the secondary compounds (Coley et al. 1985). Given a limited amount of resource, plants with multiple dominant metabolites will also have multiple alternatives on where to invest such resources. For example, the nitrogeneted compounds (e.g., alkaloids) present in a chemically diverse species are more likely to show higher amounts of variation depending on the spatial and temporal distribution of available nitrogen (Massad et al. 2011, Jamieson and Bowers 2012). Contrastingly, species that have a simpler chemical profile are likely to invest resources more evenly in chemical defense when compare to a species with more diverse chemistry given a similar amount of resources available.

We acknowledge that this pattern was found using a small number of species and that further research is needed to test the relationship between chemical diversity and intraspecific chemical variation. Moreover, besides greater sample sizes, experimental approaches manipulating resource availability and inductive pressures while controlling for genetic variability will be needed to further explore this relationship. Nevertheless, we believe that there is a strong need for more studies that address the potential evolutionary and ecological consequences of chemical diversity.

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Chapter 4

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

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We tested the hypothesis that leaf herbivores determine local community composition in the hyper diverse genus *Piper* at one lowland wet forest location in Costa Rica. We first characterized the entire chemical composition of 27 of the most abundant species of *Piper*. We then tested whether species more different in chemical composition were more likely to coexist, as predicted by the resource concentration hypothesis. Finally, we assessed the degree to which secondary composition in any one plant species is related to the phylogeny of the species studied. We found that co-occurring species were more likely to differ in chemical composition than expected by chance. Contrary to what was expected, there was no

phylogenetic signal for secondary chemical composition. Because chemical composition is widely divergent among closely related species, it appears that this composition is evolutionarily very labile. In addition we found that species in local communities were much more closely related than expected by chance, suggesting that functional traits other than those measured also influence local assembly. Selection by herbivores for divergent chemistries between closely related species allows such coexistence of closely related species to occur.

Introduction

A historical 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 is determined by two major ecological processes at the local scale: 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 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.

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, Frodin 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). 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 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 rule of limiting similarity. In many of these genera (e.g., *Piper* and *Peperomia* [Piperaceae], *Miconia* and *Clidemia* [Melastomataceae], *Elaphoglassum* [Dryopteridaceae], *Psychotria* [Rubiaceae], *Bursera* (Burseraceae), and *Inga* [Mimosaceae]), there appears to be insufficient morphological and functional differentiation to allow local coexistence.

Currently, one of the most common strategies to assess species similarity is to describe their functional traits (McGill 2006). Using this approach one measures species characteristics that can determine the quantitative and qualitative use of a specific set of available resources (Swenson 2013). Species with similar values of functional traits are expected to undergo stronger competitive interactions than species that differ more in those 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 strongly associated with a set of critical resources for plants: enemy free space (e.g., direct and indirect defense against herbivores, pathogen resistance), the benefits of mutualistic interactions (pollinator and seed disperser attractiveness), and protection against abiotic factors. Given that secondary chemistry can play a critical role in the use of locally available resources by plant species, it is reasonable to expect that chemical similarity between sympatric taxa can also play an important role in determining species coexistence.

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). A good example of this kind of interdisciplinary approach for studies of 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. The results suggest the presence of divergent selection (and resulting rapid evolution) on antiherbivore 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 other species-rich plant genera.

Specifically we used a metabolomic and phylogenetic approach to assess interspecific chemical similarity for 27 sympatric Neotropical *Piper* species with the goal of determining the role that plant secondary chemistry and plant phylogeny play for species coexistence of *Piper* in a low land tropical forest. We applied both a species-pair and a community-based approach. The use of a metabolomic technique allowed us to assess chemical similarity across a wide 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.

Material and methods

Site and System

The study was conducted May-August 2007 at the La Selva Biological Station in Costa Rica of the Organization for Tropical Studies (OTS). Located in the Atlantic lowlands of Puerto Viejo de Sarapiquí (Heredia province), the station possesses more than 1600 ha of tropical wet forest and receives approximately 4000 mm of rainfall annually. Currently, 1850 species of vascular plants have been found in La Selva; 50 of these species are in the genus *Piper*.

Piper is a pantropical genus with more that 1000 species in the Neotropics (Frodin 2004, Jaramillo 2006). The natural range of the genus in the New World is from northern Mexico to northern Argentina. *Piper* species are abundant in low- and mid-elevation forests (but rarely reaching 2,500 m) and are often among the top ten most speciose genera in Neotropical forests(Gentry 1990, Marquis 2004). At La Selva, most species occur in discrete, multi-species patches that can contain up to 21 different species (Salazar et al. 2013). *Piper* secondary chemistry has been extensively studied and there is an important body of published methods for compound isolation, chemical profiling, and synthesis. Additionally, the effects of their secondary chemistry on herbivores, pathogens, and seed dispersal are well-documented (Parmar et al. 1997, Dyer et al. 2001, Mikich et al. 2003, Kato and Furlan 2007, Fincher et al. 2008, Marques et al. 2010).

Species coexistence

To assess *Piper* species coexistence we sampled 81 multi-species patches of *Piper* throughout the primary forest of La Selva. Patches were located by performing transects parallel to the station trails with transects between 50 and 100 m from the trail. Patches selected were a minimum 250 m from each other. Each patch was sampled using a 10 m diameter plot, which was large enough to include all *Piper* individuals within each of the patches. All *Piper* species within the plot were identified and their relative abundances determined.

We used two different indices of species coexistence. First, we calculated the species-pair matrix of the species co-occurrence c-score index based on presence/absence data from each patch (Stone and Roberts 1990).

Second, to take into account the effect of species abundance, we calculated a species-pair matrix of niche overlap. Here, plot was considered as the species niche and we used plot occupancy as a measure of the niche use. Occupancy was calculated for all Piper species as the relative abundance of each species in every sampled plot. Niche overlap was calculated using Pianka's Index (Pianka 1973) based on 1000 iterations. Both measures of species coexistence were calculated using EcoSim 7.1 (Gotelli and Entsminger 2012) and yielded two full, 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 where 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 dried with silica gel and transported to the University of Missouri-St Louis for chemical analysis. 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 and sonicated for 25 min. As an internal standard, 10 mg of piperine was added to all samples. Samples were finally filtered and stored at -80°C until analysis.

Qualitative chemical analysis of the extracts was performed using GC-MS (HP 5890 coupled with a quadrapole 6872) with helium as the carrier gas and using a HP-5 capillary column (30 m). Because the abundance of the secondary compounds can vary between individuals of the same species due to factors like induction, genetic variability, and resource availability, we only used data on the presence and absence of chromatographic features.

We assessed chemical similarity between all sample species in two different ways. Our first approach consisted in building a mass spectra library containing all chromatographic features for each species. The libraries of all species were then crossed referenced using AMDIS (Automated Mass Spectral Deconvolution and Identification System) to identify features common to all sampled *Piper*s (Stein 1999, Stein et al.

2005). Our second approach used mass spectra and retention time chromatogram alignment. This approach uses mass spectra and expected retention times to find common chromatographic features across samples. Chromatograms where aligned using MZmine and the RANSAC (random sample consensus) algorithm (Pluskal et al. 2010). Given that the two approaches yielded similar results, we only used the results from the former approach for the statistical analysis.

Using the chemical similarity data from the above methodology, we performed a hierarchical clustering analysis (Ward's algorithim, R package pvclust (Suzuki and Shimodaira 2011, 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 components analysis (JMP 10.0, (SAS 2007)) to the presence/absence data on chromatographic features to generate continuous values of chemical diversity for the plant species in order to calculate the phylogenetic signal of chemical diversity across our *Piper* species pool (see below).

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. All 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 a) control for phylogenetic non-independence of the chemical similarity between the sampled species, and to b) assess the effect of phylogenetic relatedness on species coexistence, we used the Picante package (R-software, (Kembel et al. 2010, R Core Team 2012))to generate a species-pair matrix of phylogenetic distances for all sampled species based on the species 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 accumulative 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 controlling for phylogenetic non-independence (1000 permutations each). Each analysis was done 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 (1000 permutations; PASSaGE 2.0,(Rosemberg and Anderson 2011)).

Community based approach: We quantified the community phylogenetic and trait 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 independent of deeper node clustering patterns (Webb et al. 2002, Webb and Donoghue 2005, Kembel and Hubbell 2006). Positive values of –

NTI and -NRI indicate that similar species (phylogenetically or in this case chemically) co-occur more than expected by chance; negative values indicate that similar species are not likely to co-occur. The randomization to generate null communities was done by shuffling phylogeny tip labels in order to calculate the standardized effect sizes for -NRI and -NTI (abundance weighted model, n=100 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 27 focal species (Picante package).

Results

We sampled a total of 2035 individuals and 27 species of *Piper* across the 81 sampled plots (Table S1). The mean number of individuals present per plot was 25.2 (SE =1.1; Max-Min = 4-51), and the mean number of *Piper* species per plot was 5.2 (1.4; 3-11).

The GCMS analysis yielded more than 1100 chromatographic features. Approximately 40% of all features were present in all *Piper* species (e.g., phytol, stigmasterol, sitorterol, 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, benzenoids, and alkaloids (table S2). The hierarchical clustering showed six discrete chemical phenotypes (Fig. 1, S1), while the first principal component from the PCA encompassed 16% of the total chemical variation among species.

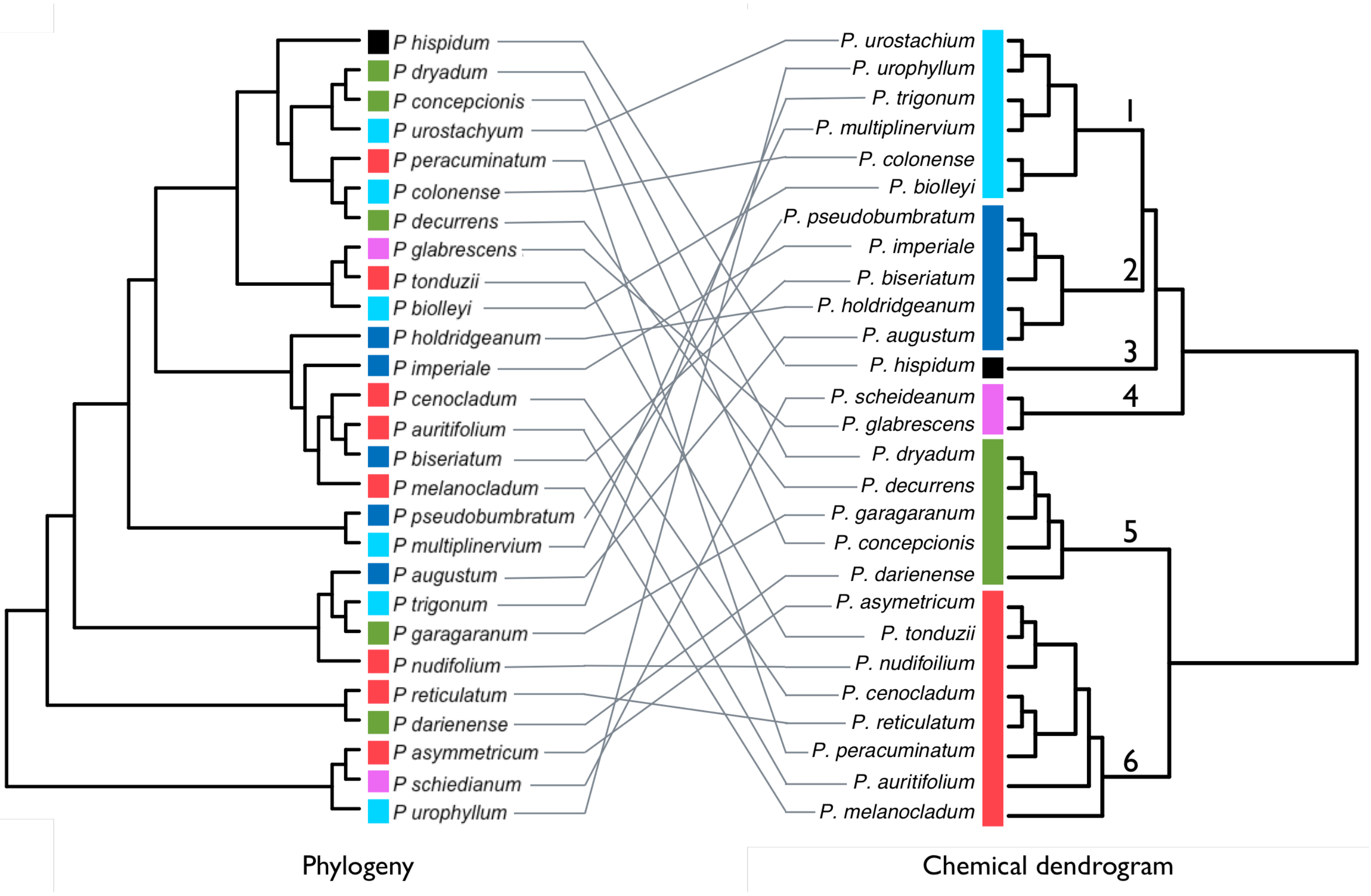

Figure 1. Comparison of the phylogenetic tree (left) and the chemical dendrogram (right) of the 27 *Piper* species sampled across the 81 natural patches. A description of the chemical characteristics of the six *Piper* chemical clusters is included in the appendix.

We did not find a strong phylogenetic signal for secondary chemical composition in our 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 species co-occurrence (Gotelli's C score) (Mantel test: r=0.20, p=0.0001), on the one hand, and species chemical distance and plot niche overlap (Pianka's index) (Mantel test: r=0.17, p=0.0014), on the other. Similar results were obtained when controlling for phylogenetic non-independence: species co-occurrence (partial Mantel test: r=0.20, p=0.002) and plot niche overlap (partial Mantel test: r=0.18, p=0.0015). Both results suggest that the more species are chemically distinct from each other, the more likely they will co-occur. In contrast, we found a significant negative relationship between species phylogenetic distance and species 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 plot niche overlap (r=0.03, p=0.54).

In our community-based approach we found that, for our 81 plots, *Piper* species were on average more overdispersed (positives values of –NRI and –NTI) with respect to their secondary chemical composition than expected by chance. The -NRI was significantly different from zero (t=1.83, df=80, p=0.03). 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).

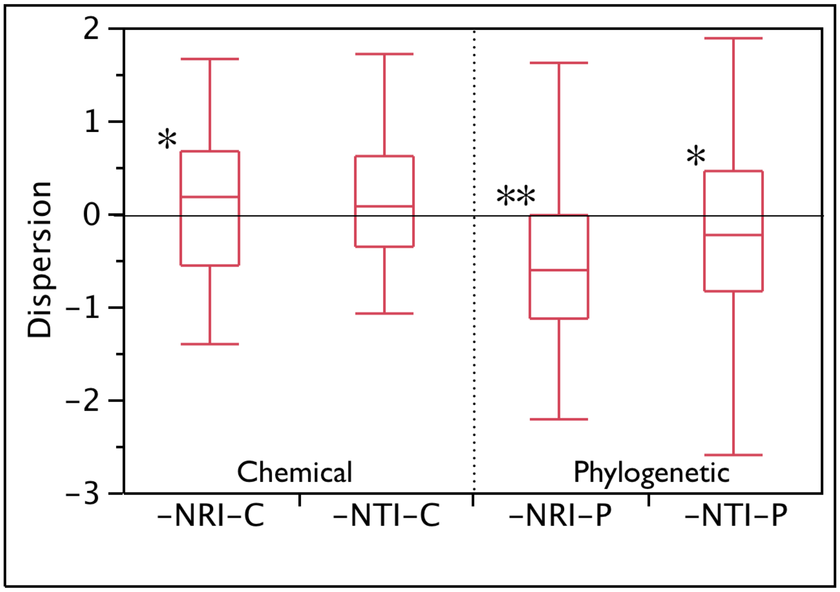

Figure 2. Standardized chemical and phylogenetic community dispersion measured as Inverse Nearest Relative Index (-NRI) and Inverse Nearest Taxon Index (-NTI). Values above 0 indicate over dispersion and values bellow 0 indicate under dispersion. Asterisks indicate values significantly different than 0 under a null model (communities assembled at random; * < 0.05, ** < 0.005).

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 if communities were assembled at random. In contrast, species that were more closely related phylogenetically were more likely to coexist. Counter to one of our original predisctions, we found that the 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 (see below), are strongly conserved across the phylogenetic history of our target *Piper* species. Furthermore, given that phylogenetic similarity was only significant for our presence/absence coexistence measure, it seems probable that these unknown traits have a weak effect on ecological interactions that are density dependent. 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).

Our results at the community level agree with those of the species-pair approach. Patches showed a significant underdispersion in terms of *Piper* chemical composition (positive values of -NRI and –NTI, Fig. 2) implying that species within a patch are less chemically similar than expected by chance. Furthermore, these results suggest that the role of 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 metabolic groups (e.g., flavonoids, amides, phenylpropanoids; see Fig. S1), it is likely that the effect of chemical composition on community assembly could be largely driven by differences with respect to these major metabolic groups.

Finally, the higher "chemical repulsion" between species from the major chemical sub-clusters, compared to the species within sub-clusters, is likely to create strong evolutionary pressures for the divergence of major secondary chemical groups in sympatric *Piper* species, rather than increase the diversity of compounds of the particular chemical groups.

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 concurs with the species-pair approach. Nonetheless, phylogenetic underdispersion was significant for both –NRI and –NTI, which 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 encountered secondary metabolites as anti-herbivore defenses, we believe that plant herbivore interactions are responsible for a great proportion of the effect that chemical composition has on community assembly. Most of the chemicals found by our analysis are well known to confer direct and indirect anti-herbivore defenses to plants (Fig. S1). One possible mechanism to explain an interaction between chemical composition, plant herbivore interactions, and community assembly is the resource concentration hypothesis (Root 1973). The hypothesis states that in diverse plant assemblages, a particular resource will have a lower relative abundance than in a more simple community. It is expected that the relative diversity of host plants in a community will affect the encounter rate between a specific herbivore and its plant host, and as a result, the amount of herbivory (Brown and Ewel 1987, Andow 1991, Otway et al. 2005, Scherber et al. 2006). Within this particular context, increments in local chemical diversity, rather than species richness alone, could reduce the encounter rate between herbivores and plant hosts due to (1) chemical disorientation or (2) chemical cues masking via higher chemical complexity (*see* (Randlkofer et al. 2010)). Thus, *Piper* species that are more different chemically than those species already present would be more likely to invade and persist in a patch. We acknowledge that the potential role of 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.

It is important to note that, for our study system, there seem to be two distinctive set of traits that are acting in opposite directions; while communities tend to be chemically overdispersed, the same communities are also phylogenetically underdispersed. This is not only an example of species partitioning available niche space, but it also has potentially interesting evolutionary outcomes. It will be 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 patterns that agrees with that of Kursar *et al.* 2009), we believe that this is not a reason to expect that there will be not a strong phylogenetic signal for chemical composition across the *Piper* phylogeny. 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" (*Piper* s.s., *sensu* Jaramillo et al. 2008). A similar pattern can be found for other compounds like piperlonguminine, methysticin and yangonin (Napralert 2011 (Loub et al. 1985)). Similarly, compounds like 4-nerolidylcatechol can be found in multiple Neotropical *Piper* species (especially, but not exclusively, in the Pothomorphe clade, Napralert 2011 (Loub et al. 1985)) but not in species of the Asian *Piper* clades. Thus, we argue that the lack of phylogenetic signal found in our local species pool could be the result of insufficient species sampling and local and regional filtering processes.

Piper, like other species-rich plant groups, can have very high local species richness and yet it appears to have very low ecological diversity. 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, Fleming 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, challenges 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 a large number of species. 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 communities, as well as the evolutionary processes that has driven tropical plant radiation.

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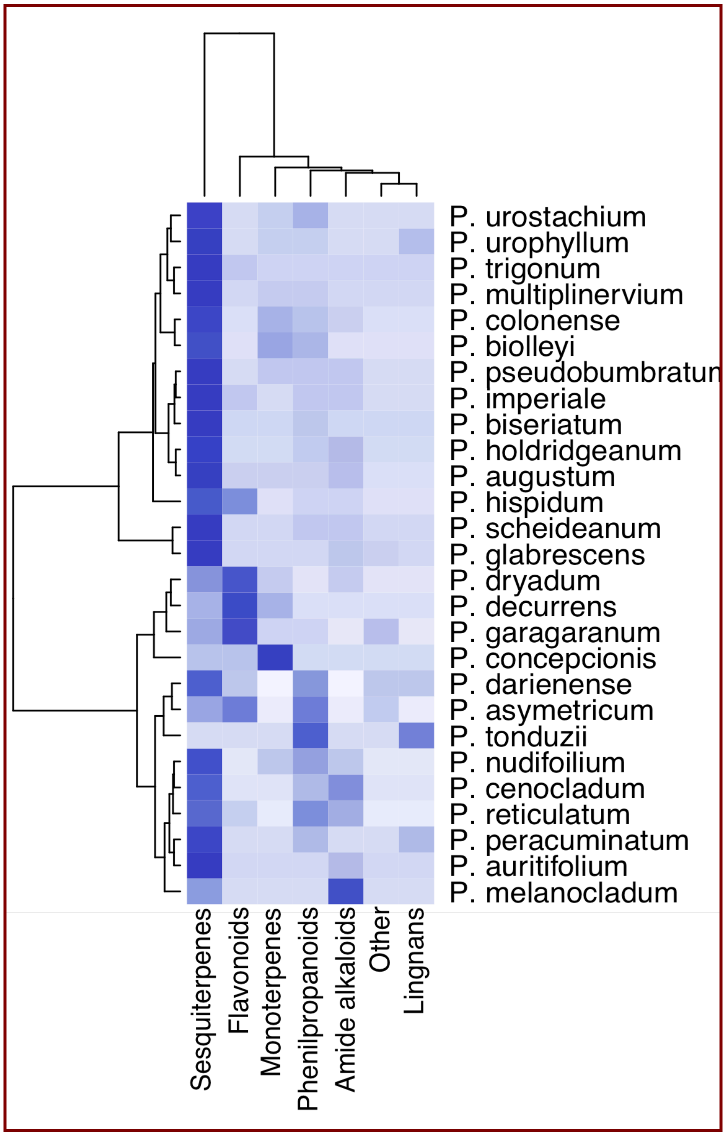

Figure S1: Total chemical similarity dendrogram for the 27 species of *Piper* found within the 81 plots. A non-exhaustive heat map has been added to help visualize the differences in chemical composition between the species. Colors show the secondary compound richness (number of compounds found) for different chemical groups. Darker colors signify higher number of compounds. Note that the dendrogram was calculated using the complete chemical data from the chemical analysis, and not from the values of the heat map. Heat map is added as a visualization tool.

Table S1: List of *Piper* species found in the study and their total abundances.

Piper species	N / 0.2 Ha
P. asymmetricum	28
P. augustum	3
P. auritifolium	77
P. biolleyi	1
P. biseriatum	4
P. cenocladum	227
P. colonense	93
P. concepcionis	1
P. darienense	1
P. decurrens	11
P. dolichotrichum	13
P. dryadum	36
P. euryphyllum	20
P. friedrichsthalii	1
P. garagaranum	91
P. glabrescens	87
P. hispidum	14
P. holdridgeanum	108
P. imperiale	56
P. melanocladum	100
P. multiplinervium	294
P. nudifolium	33
P. peracuminatum	10
P. pseudobumbratum	49
P. reticulatum	44
P. sancti-felicis	14
P. schiedeanum	21
P. silvivagum	4
P. tonduzii	1
P. trigonum	346
P. urophyllum	10
P. urostachyum	240

Table S2: Non-exhaustive list of some of the compounds found in the chemical extractions of the *Piper* species in this study.

Compound	Compound Class
Eucalyptol	Terpene
Isoledene	Terpene
Linalol	Terpene
(-)-Spathulenol	Terpene
(E)-β-Farnesene	Terpene
1,3-Cyclohexadiene, 1-methyl-4-(1-methylethyl)-	Terpene
1,3-Dimethyl-5-(propen-1-yl)adamantane (Guaiene)	Terpene
1,5-Cyclodecadiene, 1,5-dimethyl-8-(1-methylethenyl	Terpene
1,6-Cyclodecadiene, 1-methyl-5-methylene-8-(1-methylethyl)	Terpene
12-Oxabicyclo[9.1.0]dodeca-3,7-diene, 1,5,5,8-tetramethyl	Terpene
1H-3a,7-Methanoazulene, 2,3,6,7,8,8a-hexahydro-1,4,9,9-tetramethyl	Terpene
1H-Cyclopenta[1,3]cyclopropa[1,2]benzene, octahydro-7-methyl-3-methylene-4-	Terpene
3-Carene, 4-isopropenyl-	Terpene
9-Methyltetracyclo[7.3.1.0(2.7).1(7.11)]tetradecane	Terpene
Andrographolide	Terpene
Aromadendrene	Terpene
Bicyclo[2.2.1]heptane, 2-cyclopropylidene-1,7,7-trimethyl-	Terpene
Cadinol	Terpene
Calacorene	Terpene
Carotene, 5,6-dihydro-5,6-dihydroxy-	Terpene
Carotol	Terpene
Caryophyllene	Terpene
Caryophyllene oxide	Terpene
Copaene	Terpene
Copaene-8-ol	Terpene
Cubebene	Terpene
Cycloisolongifolene	Terpene
Eicosane	Terpene
Epi-bicyclosesquiphellandrene	Terpene
Eucalyptol	Terpene
Farnesene	Terpene
Isoledene	Terpene
Linalool	Terpene
Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)	Terpene
Phellandrene	Terpene
Phytol	Terpene
Pyrimido[1,6-a]indole, 1,2,3,4-tetrahydro-2,5-dimethyl-	Terpene
Sabinene	Terpene
Selinene	Terpene
Spathulenol	Terpene
Spiro[4.4]non-1-ene (Thujene)	Terpene
Squalene	Terpene
Thymol	Terpene
trans-‡-Bergamotene	Terpene
Veridiflorol	Terpene
α-Humulene	Terpene
α-Phellandrene	Terpene
α-Pinene	Terpene
α-Thujene	Terpene
Zingiberene	Terpene
Solavetivone	Terpene
1,3-Benzodioxole, 5-(1-propenyl)-	Phenolic
1,3,5-Benzenetriol, dihydrate	Phenolic
Apiole	Phenolic
Benzene, 1,2-dimethoxy-4-(2-propenyl)-	Phenolic
Cyclopentanol, 1,2-dimethyl-3-(1-methylethenyl)	Phenolic
Desaspidinol	Phenolic
Dillapiole	Phenolic
Isoasarone	Phenolic
Maltol	Phenolic
Myristicine	Phenolic

Safrole	Phenolic
trans-Cinnamic acid	Phenolic
Vanillin	Phenolic
Phloroglucinol	Phenolic
Myristicin	Phenolic
Isosafrole	Phenolic
Isovanillin	Phenolic
Isohomogenol	Phenolic
Eugenol	Phenolic
Desaspidinol	Phenolic
Cinnamic acid	Phenolic
Cerulignol	Phenolic
Benzenecarboxylic acid	Others
3,5-Dihydroxy-4',7-dimethoxyflavone	Others
3,7,11,15-Tetramethyl-2-hexadecen-1-ol	Others
4-nerolidylcatechol	Others
Conanine	Others
Pyrrolidine, 1-[5-(1,3-benzodioxol-5-yl)-1-oxo-2,4-pentadienyl]	Others
Sitosterol	Others
Sitosterol acetate	Others
Stigmasterol	Others
Tocopherol	Others
Vitamin E	Others
Vitamin E	Others
Aspidinol	Ketone
Borneol	Ketone
Flemi chapparin	Flavonoid
Galangin	Flavonoid
Dimethoxyflavanone	Flavonoid
Chrysin	Flavonoid
Pinocembrin	Flavonoid
Naringenin	Flavonoid
Chrysin	Flavonoid
Cinnamamide, N-(p-hydroxyphenethyl)-	amide
Cenocladamide	amide
Piplartine	amide

Piper chemical clusters

After using the chemical similarity data to perform a hierarchical clustering analysis (Ward's algorithim, R package pvclust (Suzuki and Shimodaira 2011, R Core Team 2012)) the 27 sampled *Piper* species grouped in to six discrete chemical clusters (see figure one of the main text). These six species groups are not characterized by the presence or absence of specific secondary compounds or metabolite chemical groups; they are characterized by sharing similar chemical composition in terms of the chemical compound richness. Table S3 shows a synthesis of the chemical characteristics of the *Piper* chemical clusters. In the table the categories "high", "medium", and "low" depict the relative chemical compound richness of each metabolite group for the six clusters. The thresholds for each category where defined by partitioning the compound richness distribution for a particular metabolite group between species that where below 33rd percentile (low), species within 34th and 66th percentile (medium), and species above the 67th.

Table S3: Chemical compound richness of the six *Piper* chemical clusters yielded by the chemical similarity clustering analysis.

	Cluster 1	**Cluster 2**	**Cluster 3**	**Cluster 4**	**Cluster 5**	**Cluster 6**
Sesquiterpenes	high	high	high	high	medium	high
Flavonoids	low	medium	high	low	high	low
Monoterpenes	medium	low	low	low	medium	low
Phenilpropanoids	medium	low	low	low	low	high
Amide alkaloids	low	medium	low	medium	low	high
Lignans	low	low	low	low	low	low

The terms high, medium, and low indicate the chemical compound richness for a particular compound.

Chapter 5

Exploring the role of plant secondary chemistry on plant herbivore interactions at the community level: a metabolomic approach.

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Understanding the role of diversity on ecosystem processes and species interactions is a central goal of ecology; however, the underlying mechanisms that link one to the other are still unclear. For plant-herbivore interactions, it has been observed that when plant species diversity is reduced, loss of primary production to herbivores increases. Nevertheless, little is known on the effect of plant chemical diversity on plant-herbivore interactions at the community level. Here we use a metabolomic and phylogenetic approach to assess the effect that secondary chemical diversity has on patterns of leaf damage caused by generalist and specialist herbivores on 81 natural multi-species plots of the neotropical genus *Piper*. Total chemical diversity had a significant negative effect on total and specialist herbivore

damage and this pattern was independent of taxonomic and phylogenetic diversity. Furthermore, we found strong differences between the effects that volatile and non-volatile chemical diversity had on herbivore damage. Volatile chemical diversity showed a negative effect on specialist herbivory and non-volatile chemical diversity had a negative effect on generalist herbivory. Our data suggest that, although changes in the complexity of the plant community have a significant effect on plant-herbivore interactions, this effect is likely to be mediated by the changes in the diversity of anti-herbivore plant traits and the strength of the relationship between herbivores and their plant hosts. This study contributes to our understanding of how plant secondary chemical diversity can affect local trophic interactions as well as the ecological and evolutionary processes that forge plant and herbivore communities.

Introduction

Community ecologists have struggled to find rules that govern both the assembly of communities they study and the processes that occur within those communities (Lawton 1999, Simberloff 2004, McGill et al. 2006, Ricklefs 2008). The search for these rules has been especially challenging in tropical species-rich communities where multiple, closely related species, from highly diverse taxa, can coexist despite of an apartment lack of ecological and functional differentiation. Thus, the mechanisms that allow the close coexistence of multiple species from taxa like *Inga* (Mimosaceae), *Piper*, *Peperomia* (Piperaceae), *Psychotria* (Rubiaceae), *Miconia* and *Clidemia* (Melastomataceae), *Bursera* (Burseraceae), and *Solanum* (Solanaceae), among others, are yet to be fully understood. Given that about one fifth of the world,s seed plant biodiversity occurs in such clades (Frodin 2004), disentangling the mechanisms that forge and maintain the high biodiversity of these genera is crucial for understanding the general processes of species diversification and species maintenance.

Two recent studies have found suggestive evidence of the role of secondary chemistry for structuring communities of species-rich genera (Becerra 2007, Kursar et al. 2009, Salazar et al. 2012b). These studies have shown that plants with similar secondary metabolite composition are less likely to coexist at the local scale. Furthermore, both Kursar et al. (2009) and Salazar et al. (in review) found that local communities of two species-rich genera (*Inga* and *Piper* respectively) had higher chemical diversity than expected by chance. Given the evidence for the role of plant secondary chemistry as defenses against herbivores (Hartmann 2007), it is reasonable to expect that plant-herbivore interactions are likely to be influenced by and are driving these patterns.

One possible mechanism to explain the relationship between chemical composition, plant- herbivore interactions, and community assembly is the resource concentration hypothesis (Root 1973b, Randlkofer et al. 2010). This hypothesis states that in diverse plant assemblages, a particular resource will have a lower relative abundance than in a less diverse community. It is expected that the relative diversity of host plants in a community will affect the encounter rate between a specific herbivore and its plant host, and as a result, the amount of herbivory (Brown and Ewel 1987, Andow 1991, Otway et al. 2005, Scherber et al. 2006, Jactel and Brockerhoff 2007). Increased diversity would reduce the frequency of any particular species, thus making it more difficult to find. In addition, for herbivores searching for host plants via volatile compounds, increases in local chemical diversity, rather than species diversity per se, could reduce the encounter rate between herbivores and plant hosts due to chemical disorientation via higher chemical complexity (Randlkofer et al. 2010). Consequently, plant communities with higher chemical diversity could suffer less herbivore pressure and therefore, be more likely to persist.

The strength of the above relationships will depend not only on the specific composition of the plant community, but also on the diet breadth of the herbivores (Andow 1991, Coll and Bottrell 1994, Specht et al. 2008, Sobek et al. 2009, Schuldt et al. 2010). Herbivore diet breadth is a commonly overlooked component when assessing the relationship between plant diversity and plant herbivore interactions. Because the relationship between plant diversity and herbivore damage is partly based on the reduction of the encounter rate between herbivore and plant host, the strength of this relationship is expected to correlate with the diet breadth of the herbivore (Andow 1991). For specialist herbivores that feed on one or a small number of plant species, an increment in the diversity of the plant community is expected to have a strong reduction in their encounter rate with their specific hosts. In contrast, generalist herbivores with a greater diet breadth will have a smaller reduction in their plant host encounter rate after an equal increment in diversity (Andow 1991, Barbosa et al. 2009). Within the context of chemical diversity, herbivores that are adapted to particular plant secondary metabolites will suffer a reduction on their encounter rate with potential host plants as the chemical diversity increases of a community increases.

Finally, it is important to note that not all secondary plant metabolites are likely to act similarly on all insect herbivores (Lankau 2007, Orians and Ward 2010). Volatile secondary compounds, like low molecular weight terpenoids and phenylpropanoids, are likely to affect search for host plants by herbivores at medium to long distances (Bruce et al. 2005, De Bruyne and Baker 2008, Bruce and Pickett 2011). Contrastingly, non-volatiles compounds, like alkaloids, cyanides, and cardenolides, are more likely to affect close range recognition of host plants on specialist herbivores (Barker et al. 2002, Macel and Vrieling 2003, Macel et al. 2005). These latter types of plant metabolites are more likely to have a deterrent effect on generalist herbivores (Macel et al. 2005, Lankau 2007).

In this study we use phylogenetic and metabolomic techniques to assess the role of plant secondary chemistry on plant herbivore interactions (taking into account herbivore diet breadth) at the community level for the neotropical species-rich genus *Piper*. Here we measure the effect of chemical diversity on total, specialist, and generalist herbivore damage on a set of naturally occurring *Piper* communities in a low land neotropical forest. Additionally, we assess how the chemical diversity of different secondary compound groups (volatile vs. non-volatile) affects the levels of specialist and generalist herbivory.

Materials and methods

Site and System

The study was conducted at the La Selva Biological Station in Costa Rica (Organization for Tropical Studies; OTS) between May and August 2007. The station is located in the Atlantic lowlands of Puerto Viejo de Sarapiquí (Heredia) and contains more than 1600 ha of tropical wet forest and receives approximately 4000 mm of rainfall annually. To date, approximately 1850 species of plants have been found in La Selva and around 50 of these species are *Piper*.

Piper is a pantropical genus with around 1000 species in the Neotropics (Jaramillo 2006). The natural range of the genus in the New World extends from northern Mexico to northern Argentina. *Piper* species are 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 (Gentry 1982, Marquis 2004). Most species occur in discrete, multi-species patches that can contain up to 21 different species (Salazar et al. 2012a). *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. Furthermore, the effects of their secondary chemistry on herbivores, pathogens, and seed dispersal are well documented (Parmar et al. 1997, Dyer et al. 2001, Mikich et al. 2003, Dyer et al. 2004b, Kato and Furlan 2007, Fincher et al. 2008, Marques et al. 2010).

Piper communities and herbivore damage

In order to assess the effect of *Piper* chemical diversity on herbivore damage we sampled 81 naturally occurring multi-species patches of *Piper* throughout the primary forest of La Selva. Patches were selected by performing parallel transects to the station trails (transects were between 50 and 100 m from the trail). All patches were selected to be at least 250m from each other. 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.

For each *Piper* we visually assessed the percentage of specialist and generalist herbivory, measured as the percentage of leaf area removed. Every plant was given a value between 0% and 100%, in increments of 5%, for each herbivory type. Specialist and generalist herbivory in all *Piper* plants were easily distinguishable due to the characteristic skeletonization of leaves by *Piper* specialist herbivores from the genus *Eois* (Geometridae (Connahs et al. 2009b, Dyer et al. 2010)). The neotropical species of this genus of caterpillars are highly specialized and have been found to feed on one or rarely on two species of *Piper* (Connahs et al. 2009). All other damage was assigned to generalist herbivores (Dyer et al. 2010). In addition, plant size data for each *Piper* individual were also collected to account for the possible effect of plant ontogeny on herbivore damage (Boege et al. 2007). Plants were classified into one of three size categories: size 1 (smaller than 20 cm), size 2 (between 20 and 60 cm), and size 3 (taller than 60 cm).

To assess the effect of chemical diversity independently from taxonomic species diversity we calculated each plot's taxonomic diversity (hereafter, *Piper* diversity) using the Gini-Simpson's index (also known as Hurlbert's probability of interspecific encounter: PIE, (Hurlbert 1971)). Furthermore, to account for the effect that the surrounding plant diversity could have on *Piper* herbivore damage, we also counted and identified all non-*Piper* plants present within all plots (hereafter, non-*Piper* diversity). Non-*Piper* diversity was calculated for each plot also using the Gini-Simpson's index (Hurlbert 1971). Diversity indices were calculated using EcoSim 7.1 (Gotelli and

Entsminger 2012). Finally, given that light availability can influence leaf quality and leaf palatability, and in so doing, potentially affecting levels of leaf herbivory (Angulo-Sandoval and Aide 2000, Blundell and Peart 2001, Takafumi et al. 2010, Salgado-Luarte and Gianoli 2011), we measured canopy openness (hereafter: Light) for each plot using hemispherical photography (Frazer et al. 1999).

Chemical analysis

For all *Piper* species we collected leaf material samples from young, fully extended leaves with similar herbivore damage (between 5 and 10%). Samples were dried with silica gel and transported to the University of Missouri-St Louis for chemical analysis. Plant chemical extraction was performed following the protocol by Salazar et al. (in review). Qualitative chemical analysis of the extracts was performed using GC-MS (HP 5890 coupled with a quadrapole 6872) with helium as a carrier gas and a HP-5 capillary column (30 m). Because the abundance of the secondary compounds can vary between individuals due to factors such as induction, genetic variability, and resource availability, we only use data on the presence and absence of chromatographic features. To assess chemical dissimilarity between all sample species we used mass spectra and retention time chromatogram alignment. This approach uses mass spectra and expected retention times to find common chromatographic features across samples. Chromatograms where aligned using MZmine and the RANSAC algorithm (random sample consensus; (Pluskal et al. 2010)). The mass spectra of the different compounds in the samples were compared with NIST/EPA/NIH and MassBank (Horai et al. 2010) databases as well as primary literature. Metabolites that did not have a match from the available sources and databases were classified as unknown.

Plot chemical diversity

To assess the chemical diversity of the 81 sampled plots we first used the pvclust R package (Suzuki and Shimodaira 2011, R Core Team 2012) to generate chemical dendrograms (hierarchical clustering analyses; Ward's algorithm) based on the chemical data of the aligned chromatograms. We generated three different dendrograms: (1) a species total chemical dendrogram, (2) a species volatile chemical dendrogram (including only low molecular weight terpenes and phenylpropanoids (mw<230)), and (3) a species non-volatile chemical dendrogram (including high molecular weight secondary compounds like amides, flavonoids, lignans, catechols, and cinnamic acids (mw>250)). Subsequently, we extracted the species-pair matrices of chemical distances from the dendrograms (pvclust package). Finally we used these three matrices to calculate the total, volatile, and non-volatile chemical diversity for all sampled plots. All chemical diversities were assessed using Rao's quadratic entropy index in the Picante package for R (Rao 2010, Ricotta and Moretti 2011, Kembel et al. 2012). Using Rao's index allowed us to take into account the different relative abundances of *Piper* species that contain specific secondary metabolites within each plot.

Phylogenetic diversity

To control for the potential effect of phylogeny on *Piper* herbivory and chemical similarity, we constructed a phylogenetic tree of all sampled *Piper* species. 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 we used the resulting phylogenetic reconstruction to calculate the Rao's phylogenetic diversity index of each plot using the Picante package (Kembel et al. 2010). Similar to the chemical indices, the Rao's phylogenetic diversity also takes into account the species relative abundances.

Statistical analysis

We analyzed the data using generalized linear mixed effects models (GLMM). To test the role of chemical diversity on *Piper* herbivory we assessed the effect of total chemical diversity, *Piper* phylogenetic diversity, and non-*Piper* diversity (as fixed effects) on *Piper* total, generalist, and specialist herbivory (models A.1, A.2, and A.3 respectively; see table S1). In all these models we controlled for light, *Piper* size, *Piper* diversity, and *Piper* species (random effects). We also assessed the effect of volatile diversity, non-volatile diversity, *Piper* phylogenetic diversity, and non-*Piper* diversity (as fixed effects) on *Piper* generalist and specialist herbivory (models B.1, and B.3 respectively). In these latter models we also controlled for light, *Piper* size, *Piper* diversity, and *Piper* species (random effects). In all models the experimental unit was the *Piper* individual within each plot. Model fit was evaluated via AIC's and maximum likelihood tests (see Appendix for details on model construction and model selection). All models used maximum likelihood estimation method. Models where analyzed using R 2.15.2 (R Core Team 2012) and the nlme package (Pinheiro et al. 2013).

Results

A total of 2035 individuals from 27 species of *Piper* were found across the 81 plots sampled for this study (see Table S2). The mean number of individuals present in a plot was 25.2 (SE; max-min=1.1; 51-4), and the mean number of *Piper* species was 5.2 (SE; max-min =1.4; 3-11). Levels of herbivory found within the plots were relatively high (mean total: 20.13%; mean generalist: 9.97%, mean specialist: 11.15%) and were highly variable among *Piper* species (Figure S1). Non-*Piper* diversity within the plots was variable with an average of 0.88, a minimum of 0.86, and a maximum of 0.97 (values of the Gini-Simpson index range between 0 for low diversity, to 1 for high diversity).

The GC-MS analysis yielded more than 1100 chromatographic features; however, approximately 40% of these were present in all *Piper* species (e.g., phytol, stigmasterol, sitorterol, tocopherol). Because these shared features were non-informative in the context of a chemical similarity analysis, and most likely related to plant primary chemistry, they were not used for the analysis. Among the remaining features we found a great diversity of terpenes, phenylpropamoids, some lignans, flavonoids, benzenoids, and alkaloids (Table S3). The total, volatile, and non-volatile chemistry hierarchical clustering showed strong variation in chemical composition among species (Fig. 1 and 2). We found no congruence between the patterns of volatile and non-volatile chemical similarity among the sampled species (Fig. 2).

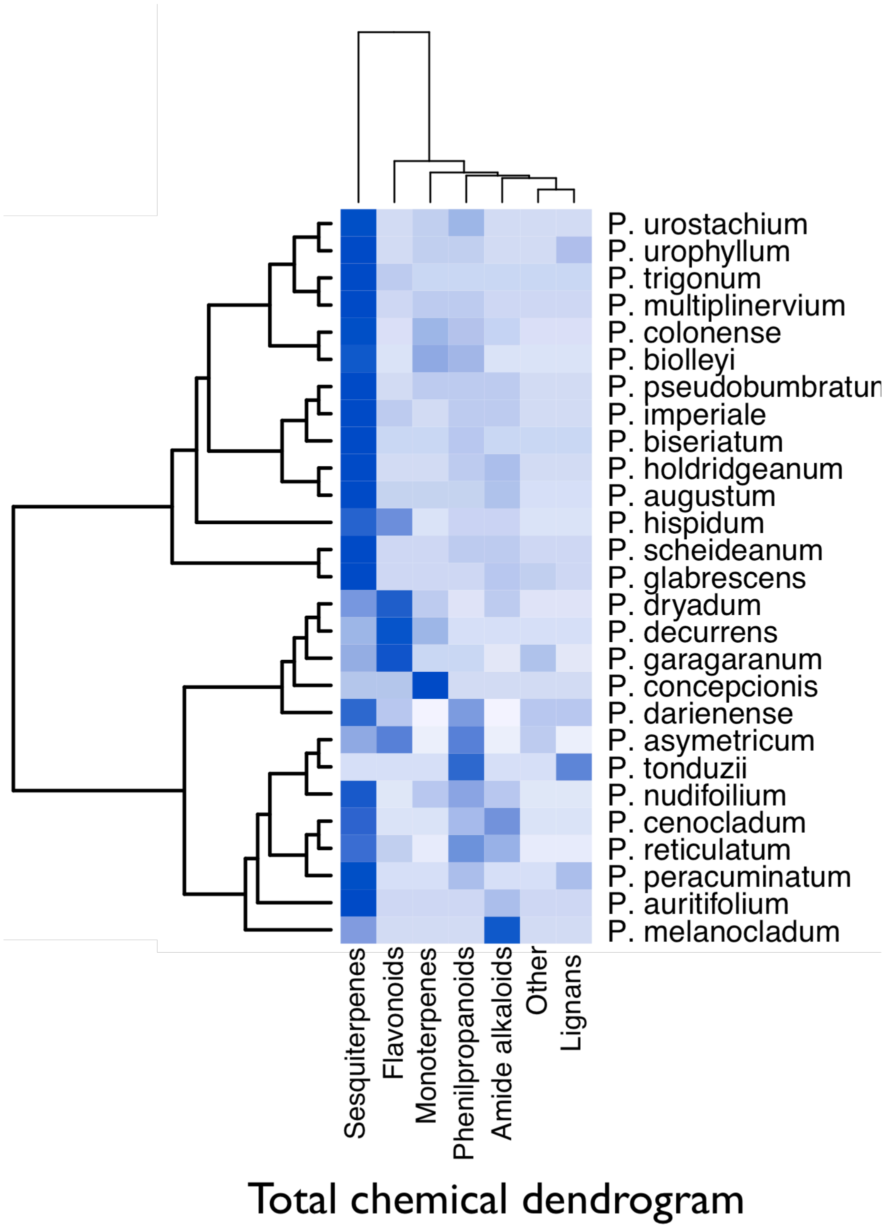

Figure 1: Total chemical similarity dendrogram for the 27 species of *Piper* found within the 81 plots. A non-exhaustive heat map has been added to help visualize the differences in chemical composition between the species. Colors show the secondary compound richness (number of compounds found) for different chemical groups. Darker colors signify higher number of compounds. Note that the dendrogram was calculated using the complete chemical data from the chemical analysis, and not from the values of the heat map. The heat map is added as a visualization tool.

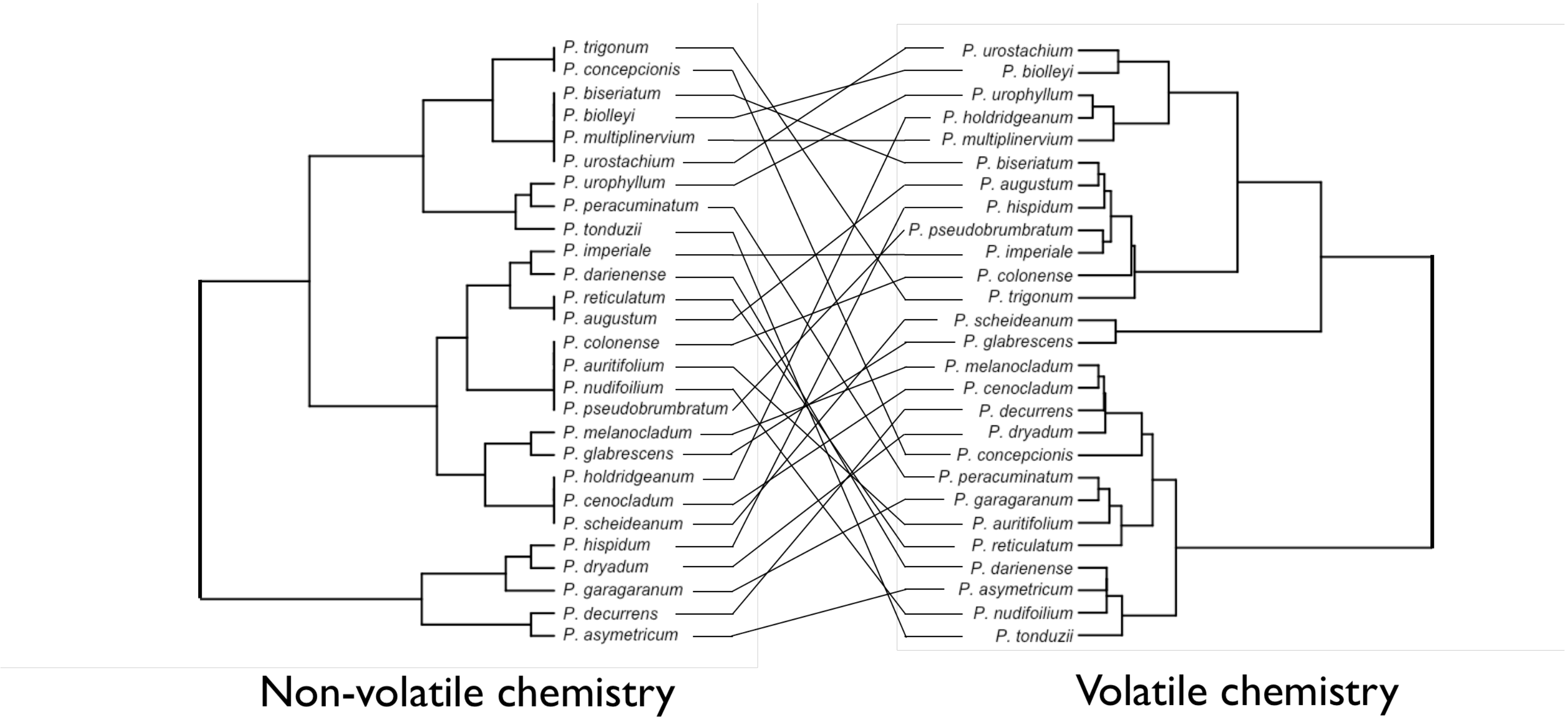

Figure 2: Dendrograms for the volatile and non-volatile chemical similarity for the 27 *Piper* species found within the plots. Lines were added to link the same species in each dendrogram. The complete figure shows the low congruence between the similarity patterns of the two chemical groups.

Chemical diversity and herbivore damage

After controlling for light, plant size, and *Piper* species, the final optimal generalized mixed linear model showed that total chemical diversity had a significant negative effect on total herbivore damage; higher levels of chemical diversity within a plot were associated with lower levels of herbivore damage (model A.1; see Table 1, S1). An identical pattern was found for the effect of total chemical diversity on specialist herbivory (model A.3). We did not find a significant effect of total chemical diversity on generalist herbivory (model A.2; see Table 1, S1), nor did we find a significant effect of non-*Piper* diversity on the amount of leaf area removed by herbivores from *Piper* plants (Table 1).

Table 1: Results from the generalized linear mixed model for the optimal models. Complete models and details of model selection procedure can be found in the supplementary information. Variables in parenthesis are random variables.

Model A.1	Estimat	**df**	**F**	**p-value**
Total herbivory = total chemical diversity + non-*Piper* diversity + (light) + (size) + (Piper diversity) + (species)				
Total chemical diversity*	-0.11	1	14.8	0.0002
Non-Piper diversity	-0.98	1	1.62	0.2
Model A.2				
Generalist herbivory = non-*Piper* diversity + (light) + (size) + (*Piper* diversity) + (species)				
Non-Piper diversity	-0.9	1	2.44	0.12
Model A.3				
Specialist herbivory = total chemical diversity + (light) + (size) + (*Piper* diversity) + (species)				
Total chemical diversity*	-0.09	1	17.28	<0.0001

Model B.1				
Specialist herbivory = volatile chemical diversity + (light) + (size) + (*Piper* diversity) + (species)				
Volatile chemical diversity*	-1.8	1	6.81	0.01
Model B.2				
Generalist herbivory = Non-volatile chemical diversity + non-*Piper* diversity + (light) + (size) + (*Piper* diversity) + (species)				
Non-Volatile chemical diversity*	-2.7	1	9.86	0.002
Non-Piper diversity	-0.8	1	2.9	0.07

Volatile chemical diversity showed a significant negative effect on specialist herbivory (model B.1; Table 1, S1). In contrast, the effect of non-volatile chemical diversity was opposite that of the effect of volatile diversity: higher levels of non-volatile diversity had a significant negative effect on generalist herbivory (model B.2; Table 1, S1). Finally, the model selection procedure showed that volatile chemical diversity did not improved the model explaining generalist herbivory, and that non-volatile chemical diversity did not improve the model explaining specialist herbivore damage (Table 1, S1).

Adding phylogenetic *Piper* diversity did not improve any of the models explaining total, generalist, or specialist herbivory (Table S1).

Discussion

The overall results from this study suggest that community-wide levels of chemical diversity can influence plant-herbivore interactions. For our 81 natural-occurring multi-species *Piper* plots we found that plots with higher levels of total *Piper* chemical diversity were associated with lower levels of total and specialist insect herbivory. Furthermore, this pattern held true after controlling for neighborhood effects (non-*Piper* diversity), *Piper* taxonomic diversity, plant size, light availability, species-to-species variation in average herbivore damage, and *Piper* phylogenetic relationships. Additionally, we also found that different kinds of chemical diversity can differentially affect specific guilds of insect herbivores (in this case specialist and generalist herbivores). Plots with higher levels of volatile chemical diversity had significantly lower specialist insect herbivory, while plots with higher non-volatile chemical diversity showed lower levels of damage caused by generalist insect herbivores. Thus, in addition to its species identity, the composition of *Piper* plants growing around an individual *Piper* plant at the study site determines how much damage that plant receives from its herbivores. This neighborhood effect is apparently influenced mainly by the presence of conspecifics and congeners, not plants from other families. We show here that the effects that chemical diversity has on the amount of herbivore damage appear to be independent of taxonomic and phylogenetic plant diversity.

Neotropical *Pipe*r, like other hyper-diverse tropical genera, challenge our understanding of species coexistence, the maintenance of biodiversity, and the processes that govern the assembly of natural communities. High local species richness, similar pollination and dispersal syndromes, and an apparent overlap in resource usage are common characteristics of these hyper-diverse genera (Kursar et al. 2009, Sedio et al. 2012). Recent work by Becerra (2007) and Kursar et al. (2009) suggests that plant herbivore interactions and plant chemistry could play an important role in the structuring of local assemblies of these taxa. Our results here further support this hypothesis, showing the effect of plant chemical diversity at the community level on the amount of herbivore damage. Because folivory influences growth and seed production in two species of *Piper* at the study site (Marquis 1984, Dyer et al. 2004a), and for plant species in general (Marquis and Lill 2010), leaf chemistry likely then influences whether a plant species occurs in a given local assembly. Ours is the first study to link leaf chemistry, plant diversity, and damage by folivorous insects.

Two mechanisms that could explain the effects of chemical diversity on the local plant herbivore damage found in this study are: (1) a resource concentration effect (Root 1973a), and (2) the semiochemical-diversity hypothesis (Zhang and Schlyter 2003, Randlkofer et al. 2010). Root's resource concentration hypothesis (1973) states that herbivore damage experienced by a given plant will depend on the encounter rate between the plant and its herbivores. In more diverse plant assemblages, a particular plant host will have a lower relative abundance than in a simpler, less diverse community. Consequently, this dilution effect will translate into a similar reduction in the encounter rate between the plant host and its potential herbivores. Insect herbivores are likely to be constrained to feed upon tissue containing a limited set of plant secondary compounds due to evolutionary (Becerra 1997, Becerra and Venable 1999), metabolic (Freeland and Janzen 1974, Scriber 2002, Marsh et al. 2006), and behavioral constraints (Bruce et al. 2005, Fernandez and Hilker 2007, Schröder and Hilker 2008, Cheng et al. 2013, Zakir et al. 2013). Therefore, it is also likely that an increase in local plant chemical diversity will reduce the encounter rate between insect herbivore and chemically compatible plant hosts, resulting in a comparable reduction in the plant herbivore damage.

Another prediction that can be made from Roots' hypothesis is that changes in chemical diversity will have a stronger effect on herbivores that feed on a small set of plant secondary metabolites (specialists). Thus, the significant effect of total plant chemical diversity on specialist herbivores found in this study further supports this hypothesis.

Alternatively, herbivore chemical disorientation due to local plant chemical complexity can influence plant herbivore interactions. The semiochemical-diversity hypothesis (Zhang and Schlyter 2003) states that complex plant communities will have greater secondary compound diversity than simpler communities, and that these higher levels of chemical diversity are likely to pose a strong challenge to herbivores orientation by negatively affecting their ability to find, recognize, or accept a particular plant host (for similar ideas see also: Hambäck et al. 2000, De Bruyne and Baker 2008, Schröder and Hilker 2008, Dicke and Baldwin 2010, Bruce and Pickett 2011, Jactel et al. 2011, Party et al. 2013). For example, combinations of specific plant odors have shown to mask host chemical cues or even repel insect herbivores (Party et al. 2013). By interfering with the herbivore's ability to track critical plant-host chemical cues, higher levels of plant chemical diversity could have a strong effect on herbivore-host encounter rate thus, reducing the local levels of plant damage caused by herbivores. Another prediction that can be drawn from this hypothesis is that the chemical disorientation effect of higher chemical diversity is likely to have a stronger effect on herbivores that rely heavily on volatile secondary metabolites for finding suitable hosts. In the case of *Piper* herbivores, we do not know which species use volatiles to find their host plants, but our results suggest that some subset of or all specialist insect species do so.

This chemical disorientation effect could also alter the ability of the natural enemies of herbivores (i.e., parasitoids) to find their prey (e.g.,Mumm and Hilker 2005). *Eois* caterpillars are frequently parasitized, resulting in a significant proportion of mortality (Connahs et al. 2009a, Richards et al. 2010). Thus, in order to determine the net effect of chemical disorientation on plant herbivore damage, directly on herbivore attack and indirectly on parasitism, specific experiments (in laboratory as well as in the field) need to be performed. It is also important to note that these two mechanisms (resource dilution and chemical disorientation) are not mutually exclusive and that both could act simultaneously in natural systems.

After partitioning the plot's chemical diversity into volatile and non-volatile chemical diversity, it was possible to assess the effect of specific groups of secondary metabolites on plant herbivore damage. These two groups of secondary compounds showed markedly contrasting effects on generalist and specialist herbivore damage. Specialist plant damage was negatively affected by volatile chemical diversity but not by non-volatile diversity. Given that specialist herbivores are generally better adapted to circumvent or overcome specialized non-volatile secondary plant metabolites (e.g.: alkaloids and glucosinolates; Orians 2000, Lankau 2007), it is reasonable to expect that these herbivores will not be especially susceptible to changes in the diversity of non-volatiles. Furthermore, specialist herbivores are expected to feed on a subset of *Piper* species within our plots (Connahs et al. 2009b, Dyer et al. 2010). Thus, it is unlikely that the presence of different non-volatile compounds in the surrounding non-host plants could affect the amount of herbivore damage they cause (Macel et al. 2002, Macel and Vrieling 2003, Cheng et al. 2013). In contrast, despite the fact that specialist herbivores are also expected to have highly developed host search mechanisms, it is likely that higher levels of community wide volatile chemical diversity can affect their ability to track the specific chemical cues from their hosts due to odor masking and semiochemical redundancy (Zhang and Schlyter 2003, Schröder and Hilker 2008, Randlkofer et al. 2010, Togni et al. 2010, Bruce and Pickett 2011, Party et al. 2013, Zakir et al. 2013). This former result further supports the role of the semiochemical diversity hypothesis on the herbivory patterns in this study.

In contrast, generalist herbivore damage was not affected by volatile diversity. Given that generalist herbivores feed on a wide arrange of plants, it is less likely that they track specific volatile chemical cues from a particular host plant in order to find a suitable host (e.g.: Vargas et al. 2005). Thus, changes in the community's volatile diversity are less likely to affect their ability to find a suitable plant to feed upon.

Instead, non-volatile diversity was found to negatively affect generalist herbivore damage. Generalist herbivores have been shown to be less adapted to cope with specialized qualitative defenses of a particular plant host, which is also true for some *Piper* generalist herbivores (Van Dam et al. 1995, Richards et al. 2010, Macel 2011, Ali and Agrawal 2012, Lampert 2012). Given that most of the non-volatile secondary compounds found in this study included alkaloids (including amides and imides), flavonoids, and a few catechols, it is reasonable to expect that higher levels of non-volatile chemical diversity will limit the amount of compatible hosts available to particular generalist herbivores.

After separating chemical diversity into volatile and non-volatile diversity, the mechanisms linking chemical diversity to either generalist or specialist herbivory appear to differ. On the one hand, data suggest that the effect of chemical diversity on the reduction of specialist herbivore damage is the result of semiochemical saturation, redundancy, or odor masking (semiochemical-diversity hypothesis). On the other hand, generalist herbivores appear to be affected by non-volatile diversity. Given that non-volatile compounds are less likely to be used as cues for host finding by generalist herbivores, this pattern suggests that the effect of high non-volatile diversity on generalist herbivory is the result of a "resource dilution" effect (resource concentration hypothesis).

Finally, it is important to underline the fact that including *Piper* phylogenetic diversity did not improve any of the models used for this study. This result is likely due to the fact that *Piper* chemical diversity does not show a significant phylogenetic signal for the 27 focus *Piper* species found within our plots (Salazar et al. in review). It is generally assumed that insect herbivores are more likely to feed on closely related plant because these plants are more likely to share similar traits than distantly related species. When this assumption does not hold true, community phylogenetic complexity will not necessarily have a significant effect on the encounter rate between herbivore and compatible plant hosts.

Given our results, we believe that the interaction between *Piper* and its natural enemies at the community level is significantly affected by local secondary metabolite diversity. Furthermore, this result supports the idea that chemical diversity is likely to play a major role in the community assembly and local coexistence of a large number of species. Additionally, our results also suggest that patterns of plant chemical diversity will have differential effects on different herbivores guilds and that these differences are mediated by two different mechanisms: resource concentration and semiochemical-diversity, for generalist and specialist herbivores, respectively.

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Supplementary material for chapter 5

Table S1: Generalized linear mixed effects models used for model selection.

Model A.1	**d.f.**	**AIC**	**p-value**	**Δ AIC**
Total herbivory = chemical diversity + non-*Piper* diversity + phylogenetic diversity + (light) + (size) + (*Piper* Diversity) + (species)	8	16489	0.56	5
Total herbivory = chemical diversity + non-*Piper* diversity + (light) + (size) + (*Piper* Diversity) + (species)	**7**	**16484**	**-**	**0**
Total herbivory = chemical diversity + (light) + (size) + (*Piper* Diversity) + (species)	6	16485	0.2	1
Model A.2				
Generalist herbivory = chemical diversity + non-*Piper* diversity + phylogenetic diversity + (light) + (size) + (*Piper* Diversity) + (species)	8	15342	0.94	11
Generalist herbivory = non-*Piper* diversity + phylogenetic diversity + (light) + (size) + (*Piper* Diversity) + (species)	7	15334	0.11	3
Generalist herbivory = non-*Piper* diversity + (light) + (size) + (*Piper* Diversity) + (species)	**6**	**15331**	**-**	**0**
Generalist herbivory = phylogenetic diversity + (light) + (size) + (Piper Diversity) + (species)	6	15336	0.001	5
Model A.3				
Specialist herbivory = chemical diversity + non-*Piper* diversity + phylogenetic diversity + (light) + (size) + (*Piper* Diversity) + (species)	8	15671	0.98	7
Specialist herbivory = chemical diversity + phylogenetic diversity + (light) + (size) + (*Piper* Diversity) + (species)	7	15670	0.79	6
Specialist herbivory = chemical diversity + (light) + (size) + (*Piper* Diversity) + (species)	**6**	**15664**	**-**	**0**
Model B.1				
Specialist herbivory = Volatile diversity + non-volatile diversity + non-Piper diversity + phylogenetic diversity + (light) + (size) + (*Piper* Diversity) + (species)	9	15670	0.83	4
Specialist herbivory = Volatile diversity + non-Piper diversity + phylogenetic diversity + (light) + (size) + (*Piper* Diversity) + (species)	8	15668	0.72	2
Specialist herbivory = Volatile diversity + phylogenetic diversity + (light) + (size) + (*Piper* Diversity) + (species)	7	15668	0.05	2
Specialist herbivory = Volatile diversity + (light) + (size) + (*Piper* Diversity) + (species)	**6**	**15666**	**-**	**0**
Model B.2				
Generalist herbivory = Volatile diversity + non-volatile diversity + non-*Piper* diversity + phylogenetic diversity + (light) + (size) + (*Piper* Diversity) + (species)	9	15328	0.71	6
Generalist herbivory = non-Piper diversity + non-Piper diversity + phylogenetic diversity + (light) + (size) + (Piper Diversity) + (species)	8	15327	0.37	5
Generalist herbivory = non-volatile diversity + non-*Piper* diversity + (light) + (size) + (*Piper* Diversity) + (species)	**7**	**15322**	**-**	**0**
Generalist herbivory = non-volatile diversity + (light) + (size) + (*Piper* Diversity) + (species)	6	15322	0.16	0

All models were selected using the optimization method described in the Appendix (see below). *Piper* diversity and non-*Piper* diversity are calculated using the Gini-Simpson index. Light was measured as the percentage of canopy openness. Herbivory, diversity, and light were logit function transformed to achieve normality. Δ AIC is the difference between a model and the "optimal model". Models in bold are the "optimal" models.

Model Selection

We first built a “beyond optimal” model in which fixed effects included the maximum number of independent explanatory variables based on biological reasonable hypothesis (hereafter: full model). Then we used a “top-down” approach to improve the model by systematically omitting one fixed effect variable at a time. Omitted variables were selected using the ANOVA function (the variable with the least explanatory power based on the data was dropped). The new model was then compared with the previous model (model1 vs. model1 - dropped variable) using AIC with maximum likelihood test; the model that showed smaller AIC’s and $p > 0.05$ was selected. The optimal model was reached when all fixed effects variables achieved $p < 0.05$ on the ANOVA and AICs did not improve with further removal of fixed effects variables. All models used maximum likelihood estimation method. Models where analyzed using R 2.15.2 (R Core Team 2012) and the nlme package (Pinheiro et al. 2013) See table S1 for a complete list of the models and table 2 for results of the Mixed Models.

Table S2: List of *Piper* species found in the study and their total abundances.

Piper species	N / 0.2 Ha
P. asymmetricum	28
P. augustum	3
P. auritifolium	77
P. biolleyi	1
P. biseriatum	4
P. cenocladum	227
P. colonense	93
P. concepcionis	1
P. darienense	1
P. decurrens	11
P. dolichotrichum	13
P. dryadum	36
P. euryphyllum	20
P. friedrichsthalii	1
P. garagaranum	91
P. glabrescens	87
P. hispidum	14
P. holdridgeanum	108
P. imperiale	56
P. melanocladum	100
P. multiplinervium	294
P. nudifolium	33
P. peracuminatum	10
P. pseudobumbratum	49
P. reticulatum	44
P. sancti-felicis	14
P. schiedeanum	21
P. silvivagum	4
P. tonduzii	1
P. trigonum	346
P. urophyllum	10
P. urostachyum	240

Table S3: Non-exhaustive list of some of the compounds found in the chemical extractions of the *Piper* species in this study.

Compound	Compound Class
Eucalyptol	Terpene
Isoledene	Terpene
Linalol	Terpene
(-)-Spathulenol	Terpene
(E)-β-Farnesene	Terpene
1,3-Cyclohexadiene, 1-methyl-4-(1-methylethyl)-	Terpene
1,3-Dimethyl-5-(propen-1-yl)adamantane (Guaiene)	Terpene
1,5-Cyclodecadiene, 1,5-dimethyl-8-(1-methylethenyl	Terpene
1,6-Cyclodecadiene, 1-methyl-5-methylene-8-(1-methylethyl)	Terpene
12-Oxabicyclo[9.1.0]dodeca-3,7-diene, 1,5,5,8-tetramethyl	Terpene
1H-3a,7-Methanoazulene, 2,3,6,7,8,8a-hexahydro-1,4,9,9-tetramethyl	Terpene
1H-Cyclopenta[1,3]cyclopropa[1,2]benzene, octahydro-7-methyl-3-methylene-4-	Terpene
3-Carene, 4-isopropenyl-	Terpene
9-Methyltetracyclo[7.3.1.0(2.7).1(7.11)]tetradecane	Terpene
Andrographolide	Terpene
Aromadendrene	Terpene
Bicyclo[2.2.1]heptane, 2-cyclopropylidene-1,7,7-trimethyl-	Terpene
Cadinol	Terpene
Calacorene	Terpene
Carotene, 5,6-dihydro-5,6-dihydroxy-	Terpene
Carotol	Terpene
Caryophyllene	Terpene
Caryophyllene oxide	Terpene
Copaene	Terpene
Copaene-8-ol	Terpene
Cubebene	Terpene
Cycloisolongifolene	Terpene
Eicosane	Terpene
Epi-bicyclosesquiphellandrene	Terpene
Eucalyptol	Terpene
Farnesene	Terpene
Isoledene	Terpene
Linalool	Terpene
Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)	Terpene
Phellandrene	Terpene
Phytol	Terpene
Pyrimido[1,6-a]indole, 1,2,3,4-tetrahydro-2,5-dimethyl-	Terpene
Sabinene	Terpene
Selinene	Terpene
Spathulenol	Terpene
Spiro[4.4]non-1-ene (Thujene)	Terpene
Squalene	Terpene
Thymol	Terpene
trans-‡-Bergamotene	Terpene
Veridiflorol	Terpene
α-Humulene	Terpene
α-Phellandrene	Terpene
α-Pinene	Terpene
α-Thujene	Terpene
Zingiberene	Terpene
Solavetivone	Terpene
1,3-Benzodioxole, 5-(1-propenyl)-	Phenolic
1,3,5-Benzenetriol, dihydrate	Phenolic
Apiole	Phenolic
Benzene, 1,2-dimethoxy-4-(2-propenyl)-	Phenolic
Cyclopentanol, 1,2-dimethyl-3-(1-methylethenyl)	Phenolic
Desaspidinol	Phenolic
Dillapiole	Phenolic
Isoasarone	Phenolic
Maltol	Phenolic
Myristicine	Phenolic
Nitrous acid, 3-phenylpropyl ester	Phenolic
Safrole	Phenolic
trans-Cinnamic acid	Phenolic

Phloroglucinol	Phenolic
Myristicin	Phenolic
Isosafrole	Phenolic
Isovanillin	Phenolic
Isohomogenol	Phenolic
Eugenol	Phenolic
Desaspidinol	Phenolic
Cinnamic acid	Phenolic
Cerulignol	Phenolic
Benzenecarboxylic acid	Others
3,5-Dihydroxy-4',7-dimethoxyflavone	Others
3,7,11,15-Tetramethyl-2-hexadecen-1-ol	Others
4-nerolidylcatechol	Others
Conanine	Others
Pyrrolidine, 1-[5-(1,3-benzodioxol-5-yl)-1-oxo-2,4-pentadienyl]	Others
Sitosterol	Others
Sitosterol acetate	Others
Stigmasterol	Others
Tocopherol	Others
Vitamin E	Others
Vitamin E	Others
Aspidinol	Ketone
Borneol	Ketone
Flemi chapparin	Flavonoid
Galangin	Flavonoid
Dimethoxyflavanone	Flavonoid
Chrysin	Flavonoid
Pinocembrin	Flavonoid
Naringenin	Flavonoid
Chrysin	Flavonoid
Cinnamamide, N-(p-hydroxyphenethyl)-	amide
Cenocladamide	amide
Piplartine	amide

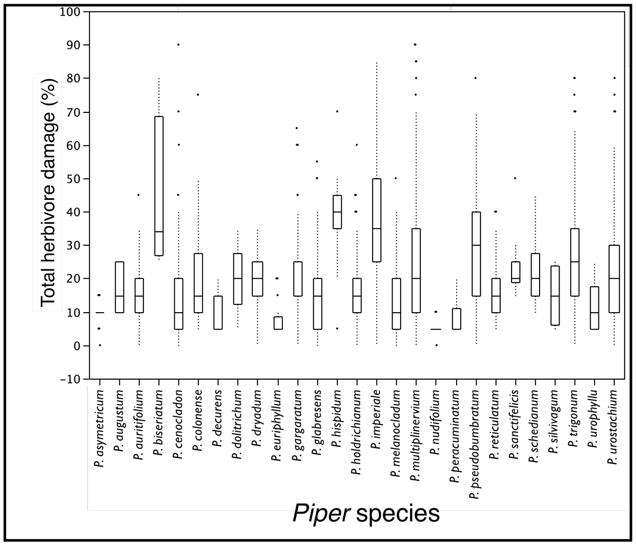

Figure S1: Boxplot of the variation in total herbivore damage among the 27 *Piper* species found within the plots.