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Species swarms and their caterpillar colonisers: phylogeny and polyphenols determine host plant specificity in New Guinean Lepidoptera

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The majority of multi-cellular terrestrial life is found in tropical forests and is either an invertebrate or a plant: for decades ecologists have sought to understand why. As global change erodes the list of extant species on our planet quantifying what species remain, along with their origins and ecology, contributes to our ability to preserve ecosystem functioning and resilience. Here we study three feeding guilds of caterpillars (Lepidoptera) and seek to understand the drivers of their diet breadth across four diverse tropical plant genera in Papua New Guinea. Host specificity is central to biodiversity estimates and the resilience of ecological networks. Specifically, we calculate distance-based host specificity in relation to plant phylogenetic relationships alongside chemical and mechanical traits of leaves. In terms of chemical defenses, we focus on the major polyphenol groups, a compound class shared across many plant species. We refine our data exploration using food webs and ordinations to pick out specific traits of relevance to insect host specificity. Our results showed that the degree of specialization for caterpillars took the following order: phylogenetic>polyphenol>mechanical, such that insect specificity was explained best by host phylogeny and polyphenol chemistry in our study system. Leaf mining insects had higher host specificity than those feeding externally. Of the traits studied hexahydroxydiphenol derivatives, galloyl derivatives, trichome density, quinic acid derivatives, myricetins and successional index explained the most variation in overall insect community structure. Our findings build on earlier studies of New Guinean rainforest communities and add a mechanistic explanation to previous findings that host

genera are functional islands for insect herbivores. Further, we demonstrate that different plant genera combine different defensive traits that appear to drive associated insect diversity. Our approach integrates trait data and phylogeny to explore dimensions of specialization and we welcome metabolomic studies that will provide more detailed explanations for insect-herbivore host use. Finally, chemical diversity is directly linked to organismal diversity and by studying a range of insect herbivore guilds we make a connection between feeding ecology and specialization that will help to predict species interactions and, potentially, the persistence of ecological networks.

KEYWORDS

Ficus, *Macaranga*, *Syzygium*, *Psychotria*, leaf miner, rainforest, phytochemical, biodiversity

1 Introduction

Plants and their associated insect communities represent 75% of terrestrial macro-diversity (Price, 2002). By understanding the ecology and evolution of herbivorous insects we can shed light on the origins and distribution of myriad extant species. A large share of woody-plant diversity in tropical forests is represented by a relatively small number of species-rich plant genera (Gentry, 1982; Foster and Hubbell, 1990). These genera, such as *Ficus*, *Inga*, *Piper*, *Psychotria*, and others have fascinated ecologists for decades (Gentry, 1982; Basset et al., 1997; Sedio et al., 2012). They can form abundant and diverse assemblages composed of multiple congeneric species occurring in sympatry, or ‘species swarms’ (Gentry, 1982). Due to their abundance and diversity, species-rich woody-plant genera serve as particularly important hosts of tropical insects (Basset et al., 1997; Novotny et al., 2010). For example, a single *Ficus* tree can harbor dozens of insect herbivore species from various taxonomic groups and feeding guilds (Novotny et al., 2010). Understanding what factors allow for the coexistence of so many closely related plant species facing diverse herbivore communities can reveal important rules of plant community assembly. In turn this can contribute toward understanding why tropical forests are so diverse (Sedio and Ostling, 2013).

Most insect herbivores show some level of specialization. Typically, insect herbivores are specialized at the level of host-plant genus, with both monophagous and extremely polyphagous species being rather rare (Forister et al., 2015). This is because the functional and defensive traits that govern insect host-choice show high variation among tropical plants (Cardenas et al., 2014; Sedio et al., 2017; Sedio et al., 2018). This variation in traits can be attributed to different evolutionary history and habitat preferences among the species from different genera. Plant genera frequently occur at distinct successional stages (as gap specialists, understory shrubs or canopy trees, for example) and share adaptations to these habitats. In some cases, diversity and identity of neighboring trees

may even explain insect specialization better than leaf traits (Jia et al., 2023). Similar processes also act among congeneric plants (Sedio et al., 2017). In addition, there seems to be a trend toward divergence in chemical traits among related sympatric species of tropical plants that further helps them to avoid sharing their insect herbivores (Kursar et al., 2009; Endara et al., 2017; Salazar et al., 2018; Endara et al., 2023). Indeed, chemical variation seems to increase toward the wet tropics where specialized herbivores are more prevalent (Sedio et al., 2018; Sedio et al., 2021). In turn, variation among congeneric species can be of a similar extent or even larger than between species from different genera (Sedio et al., 2017).

Various herbivores show differential responses to host-plant traits (Volf et al., 2018; Leong et al., 2022). These responses depend on the feeding guild and dietary specialization of the given herbivore species (Ali and Agrawal, 2012; Leong et al., 2022). Our study considers the larvae of Lepidoptera (caterpillars) that show different levels of specialization and belong to various feeding guilds. Generalist free-living caterpillars can be deterred by the toxic chemistry of their hosts. In contrast, specialized caterpillars can prefer to feed on toxic plants and may even benefit from their specialized metabolites (Volf et al., 2018). For example, *Asota* (Erebidae: Aganainae) caterpillars preferentially feed on alkaloidal *Ficus* species, sequestering host alkaloids and likely using them for anti-predator defense (Volf et al., 2018; Fontanilla et al., 2022). Other specialized herbivores are found among concealed caterpillars, such as those mining leaves. By feeding on leaf parenchymal tissue, leaf miners can avoid some leaf defenses associated with the leaf cuticle (Connor and Taverner, 1997). Additionally, many mining and non-mining insects are able to manipulate leaf quality and chemical composition of their hosts, which further contributes to the intimacy of their interactions (Giron et al., 2016).

In this study, we analyze host-specialization in free-living caterpillars, semi-concealed caterpillars (leaf-rollers and leaf-tiers),

and leaf miners associated with speciose tree genera in the tropical forest in Papua New Guinea. Papua New Guinea is a diversity hotspot and a center of endemism for both plants and insects (Novotny and Toko, 2015). Despite covering only ca 5% of the dry land surface of our planet, it is home to at least 13,634 plant species (Höft, 1992; Novotny and Toko, 2015; Cámara-Leret et al., 2020), which represents 3.5% of the global total (Antonelli et al., 2023). Unlike many other tropical islands, it is still partially covered by vast areas of primary forest mixed with secondary forests. These habitats contain diverse plant communities, including some particularly species-rich woody plant genera (Novotny and Toko, 2015). We focus on four of them – *Ficus*, *Macaranga*, *Syzygium* and *Psychotria* (Whitfeld et al., 2012a): together these genera include 6% of all New Guinean plant species (Conn, 2008). While *Syzygium* and *Psychotria* are typically climax species *Macaranga* is generally a pioneer (Whitmore, 1989). Successionary status varies among species within *Ficus*. We combine plant defensive traits and phylogenetic data with insect community data to explore what factors drive host specialization of caterpillars associated with *Ficus*, *Macaranga*, *Syzygium* and *Psychotria*. In terms of chemical defenses, we focus particularly on polyphenols, including various types of tannins and flavonoids. Polyphenols are broadly distributed among the plant kingdom and represent a group of defensive metabolites found in all four genera we studied. Polyphenols affect insect herbivores through various mechanisms, such as causing oxidative stress or precipitating proteins in the insect gut (Salminen and Karonen, 2011; Salminen, 2014). Due to their universal presence in plants, they have been shown to be important predictors herbivore host-choice in tropical forests of Papua New Guinea when considering host-plants from various lineages (Segar et al., 2017). It is important to acknowledge that differences across methods and habitats can determine the role of polyphenols in deterring insect herbivores, here we consider all major groups of polyphenols (as well as the well-studied condensed tannins) and include measures of biological activity. While some studies in tropical habitats have demonstrated mixed findings (Coley, 1983; Kurokawa and Nakashizuka, 2008; Cardenas et al., 2014; López-Carretero et al., 2016), a consideration of individual groups, compounds or biological activities has demonstrated a pronounced effect of polyphenols on insect herbivore damage (Moctezuma et al., 2014) or host use (Segar et al., 2017). We note that colorimetric assays for condensed tannins and quantification of total polyphenol content can provide meaningful results in the right context, but that high resolution data (quantification of major groups or even individual compounds) are more suitable for studying insect chemical ecology, especially when combined with measures of biological activity.

We first hypothesize that the four tree genera will harbor insect assemblages dissimilar from each other, while congeneric hosts will share most of their herbivores. This expectation is based on phylogenetic conservatism in plant defensive traits and existing evidence that insect herbivores are generally specific at the level of host genus (Novotny et al., 2010). Second, we expect that insect specialization will reflect host phylogeny and habitat preferences rather than polyphenols and physical traits (because phylogenetically conserved host traits determine insect

preference). And third, we expect that when partitioned into feeding guilds, the level of specialization will decrease from miners, through semi-concealed caterpillars, to free-living caterpillars. We aim to explore how sympatric species-rich genera of tropical trees share caterpillars from different lepidopteran guilds and identify the factors that govern the patterns in host-use through combining defensive trait and phylogenetic data.

2 Methods

2.1 Study system

Ficus (Moraceae) is a pantropical genus including ca 800 species (Cruaud et al., 2012). It is considered to be one of the key-stone tree genera in tropical forests as it supports a high diversity of herbivores, frugivores, pollinators, and their natural enemies (Berg and Corner, 2005; Cruaud et al., 2012; Volf et al., 2020). The genus occurs in both primary and secondary forests and it possesses diverse chemical defenses that show high variability among species (Berg and Corner, 2005; Volf et al., 2018). *Macaranga* (Euphorbiaceae) is distributed primarily in the secondary forests of the Old-World tropics. Some of its ca 300 species are myrmecophilous and rely on defense by ants (Davies, 2001; Davies et al., 2001). Such strict myrmecophily is rare among the New Guinean species. Additionally, *Macaranga* species produce chemical defenses including various phenolics (Eck et al., 2001). *Syzygium* (Myrtaceae) includes economically important species. The genus is known for being taxonomically challenging. Currently it is estimated at ca 1,200 species (Govaerts et al., 2008; Low et al., 2022). In Papua New Guinea, it occurs mainly in primary forests (Novotny et al., 2010). Being a member of the Myrtaceae family, the genus is known for potent chemical defenses (Dixon et al., 2005; Mohamed et al., 2013). Finally, the genus *Psychotria* (Rubiaceae) contains over 1,500 species and represents one of the World's largest flowering plant genera; it is an important component of tropical forests (Sedio et al., 2017). While *Psychotria* is primarily neotropical there are almost 150 species in New Guinea, 95% of them being endemic. This understory genus is well known for its secondary metabolites, including, among others, the entheogen dimethyltryptamine (Callaway et al., 2005; Cámara-Leret et al., 2020).

2.2 Insect community and phylogenetic data

The insect data were taken from Novotny et al. (2010), these authors sampled all caterpillars from a locally representative selection of 88 host plants in a 10 x 20 km area matrix of primary and secondary lowland rainforest in Madang province, Papua New Guinea. Specifically, the original dataset includes trees and shrubs from plant lineages that comprise a majority of forest woody species in the area. It comprises multiple representatives of locally occurring large genera including *Ficus*, *Macaranga*, *Psychotria*, and *Syzygium*. In terms of species selection, the dataset focuses

on species common in the area that allowed to sample insect herbivores from 1500m². The insect data include only reared individuals, with host associations confirmed by feeding trials. We focused on Lepidoptera as a large insect order with multiple and well represented feeding guilds. Guilds were defined as follows: leaf miners, semi-concealed caterpillars and free-living caterpillars. Leaf miners feed on the leaf mesophyll, semi-concealed caterpillars include leaf rollers and tiers while free-living caterpillars are exposed (Hrcek et al., 2013). We included recent taxonomic revisions for Choreutidae (Volf et al., 2018) and mining caterpillars (O. Kaman, unpublished data). Singleton and doubleton species were removed from all statistical analyzes because it is impossible to assess host-specificity in cases where one individual is found only on one plant and challenging for doubletons (Novotný and Basset, 2000).

We generated multigene molecular phylogenies for hosts using sequence data generated in previous studies (Novotný et al., 2010; Whitfield et al., 2012b). We used rbcL sequences in combination with additional sequence data for the following groups: Euphorbiaceae (ndhF), *Ficus* (ITS) and Rubiaceae (RpS16). We used the resultant topologies as constraints in a larger rbcL only analysis for all 88 plant species from 31 families. We also used the ordinal and family level relationships as derived from the APGIII (2009) tree for constraining the higher-level angiosperm relationships Szefer et al. (2017). We estimated ultrametric phylogenies using BEAST v2.3.1 (Bouckaert et al., 2014) using substitution models derived from jMODEL Test 2 (Darriba et al., 2012) and a relaxed log-normal molecular clock (set to a rate of 1.0). We partitioned our five gene data set, grouping codons one and two (modeling codon three separately) and defined a separate substitution model for each partition. We ran two separate MCMC chains for 20,000,000 generations and assessed the effective sample sizes (ESS) of the combined chains using Tracer v1.6 (Rambaut et al., 2014) to ensure adequate sampling and convergence of the analysis (ESS values over 200 for all parameters sampled). We used the CIPRES portal v3.3 for all phylogenetic analyzes (Miller et al., 2010) and pruned this larger tree to include only our focal species.

2.3 Trait data

Our methods followed Volf et al. (2018), but we give an overview here for clarity. Plant tissue was collected in the field over 251 days between 2013 and 2014, and we sampled leaf discs of 2.4 cm in diameter from 10 young but fully expanded leaves per individual tree for between three to six individuals per species. We avoided sampling from plants heavily damaged by herbivores or pathogens to avoid measuring individuals with strongly induced levels of their defenses. We also avoided sampling from saplings and young trees as we expect that their chemical traits can differ from mature trees. Half of the leaf discs were stored in HPLC grade acetone in order to prevent enzymatic degradation and oxidation of the studied metabolites in the field. They were transferred to a dark -20°C freezer on return to the New Guinea Binatang Research

Centre. These discs were later used for secondary metabolite analysis. The other half of leaf discs were weighed fresh and dry in order to estimate both the percentage of water per leaf disc, and the dry weight contained in each tube of acetone. Leaf discs collected for polyphenol analysis (ca 0.6 g of dry leaf tissue in total for each individual: 0.55 g ± 0.22 SD) were stored in 40 ml of HPLC grade acetone. Concentrations were calculated in mg/g dry weight using individual values for each sample because field collected tissue mass varied.

In the laboratory, this first acetone extract was transferred into a 50 ml falcon tube. Five ml of ultrapure water was added, and the solution was concentrated to water phase under a flow of nitrogen at room temperature. The leaf disks were cut into smaller blades and transferred into grinding tubes (DT-50, IKA-Werke GmbH & Co. KG, Germany) containing 35 ml acetone/water (80:20, v/v). The polyphenols were extracted from the leaves by grinding them for 30 min using tube dispensers at room temperature (Ultra-Turrax Tube Drive, IKA-Werke GmbH & Co. KG, Germany). The leaf material was removed and the extract was combined with the water phase obtained from the first acetone extraction above. The combined extract was diluted with acetone to a uniform volume of 50 ml. Polyphenols were extracted from the samples using 50 ml of acetone/water (80:20, v/v). This volume of extract was split, with 10 ml being taken for polyphenol analysis.

To quantify polyphenols, we ran two separate sets of assays. Firstly, we quantified total concentrations of eight main polyphenol sub-groups (in mg/g dry weight) by UPLC-QqQ-MS/MS with the methods of Engström et al. (Engström et al., 2014; Engström et al., 2015) as described in e.g. Malisch et al. (2016). The measured polyphenol sub-groups included hydrolysable tannins that were divided into (1) galloyl derivatives and (2) hexahydroxydiphenoyl derivatives (HDDP, ellagitannins), proanthocyanidins that were divided into (3) procyanidin and (4) prodelphinidin subunits, flavonol glycosides that were divided into (5) kaempferol, (6) quercetin and (7) myricetin derivatives, and (8) quinic acid derivatives. In addition, we ran two activity assays to quantify two major functions of polyphenols in anti-herbivore protection – oxidative activity and protein precipitation capacity. Polyphenol oxidative activity was measured following Salminen and Karonen (2011) using gallic acid as the standard. Protein precipitation capacity was measured following Hagerman's radial diffusion assay (Hagerman and Butler, 1978) using pentagalloylglucose as the standard. Both assays provided activities in mg/g dry weight.

The total number of trichomes per 10 mm² and their average length was measured on five leaf discs per individual tree (from five leaves per tree), avoiding the central vein. We used a DinoLite USB microscope to draw on a circle of the appropriate diameter and to quantify trichome density and length. Values for dorsal and ventral sides of the discs were averaged. Specific Leaf Area (SLA) was measured as the area per mass using ten dried leaf discs (twenty for *Ficus*, due to tissue being collected for other studies) from ten leaves per individual tree, which were cut avoiding the central vein. Total carbon and nitrogen content were determined by dry combustion using ca 0.6 g of homogenised dry leaf material across the same leaves sampled for polyphenols.

2.4 Statistical analysis

We calculated the rescaled distance-based specialization index (DSI*) (Jorge et al., 2014; Jorge et al., 2017) for each herbivore guild using host phylogenetic distance, polyphenol distance (Euclidean distance derived from a matrix quantifying the concentration of eight polyphenol sub-groups and the two types of activities) and mechanical distance (Euclidean distance derived from C:N, SLA, mean trichomes per disc and mean trichome length). DSI weights the degree of specialization by the phylogenetic and trait similarity of hosts and their availability, rather than using counts of host species, or higher taxonomic categories. In this sense, DSI measures distance-based specialization accounting for differences in the pool of available hosts, such variation in their polyphenol chemistry or phylogenetic relatedness. The rescaled DSI index (DSI*), accounts for differences in abundances and sampling intensities. Rescaled DSI has upper bounds of 1 (monophagy) and the lower bounds -1 (maximum generalization).

We used generalized linear mixed models with a beta error term and logit link function to test for differences in phylogenetic, polyphenol and mechanical specialization among guilds. The response variable in each model was DSI* scaled to be within 0.00001 and 0.99999 (to satisfy the requirements of beta-regression) five models were compared using AIC, each model included species as a random effect. The simplest model had an intercept of one while the next model included only guild as an explanatory variable, the third model included only the DSI metric (phylogenetic, polyphenol or mechanical) and the fourth included both previous explanatory variables together. The fifth model included the interaction term between these two variables. All models were fitted in the R package 'glmmTMB' (Magnusson et al., 2017) and models were compared using an AIC table generated using the R package 'bbmle' (Bolker and Bolker, 2016). Testing of model fit was conducted using the R package 'DHARMA' (Hartig and Hartig, 2017) (specifically: dispersion, homogeneity of variance in the residuals, correct distribution test and distribution of outliers). The model with the lowest AIC was examined further with type II ANOVA: Wald Chi-square tests were used to summarize overall significance of the variables and pair wise tests using a Tukey adjustment were used to compare significance across both categorical variables simultaneously (implemented using the R package 'emmeans' (Lenth et al., 2020)).

Further, we calculated the mean insect specialization per host plant (e.g., the sum of DSI* for each insect herbivore divided by the number of species on that host). Overlap of insect communities across host genera was visualized by plotting the mean of shared species among congeners and among *Ficus*, *Macaranga*, *Psychotria* and *Syzygium*. Here, 0 means that not a single species overlaps with another species or genus, whereas 1.0 means that all members of a guild are shared with other hosts. We constructed food-webs for each guild using the R package 'bipartite' (Dormann et al., 2008). We also calculated connectedness and modularity for each food web.

We used Principal Components Analysis (PCA) to summarize all 14 defensive traits measured across the 34 host plant species

(Supplementary Table 1) as implemented in the R package 'vegan' (Oksanen et al., 2019). We included an additional variable, successional index, to account for host habitat. Successional index ranges from 0 to 100 and represented the % of basal area in primary forest plots (data derived from Lepš et al., 2001). Community data from all three guilds were combined for this analysis. Species were scaled to unit variance. Further, we performed Canonical Correspondence Analysis (CCA) to test for correlations between insect community composition across host species and the defensive variables. Model simplification proceeded in both directions, used 999 permutations and specified R^2 scope as true and was implemented using the 'ordistep' function in the R package 'vegan'.

3 Results

The mixed model with the lowest AIC included both guild and specialization metric (phylogenetic, polyphenol or mechanical) as explanatory variables describing the deviance in DSI*.

No interaction term was included. Both guild ($\chi^2 = 45.343_{2,454}$, $p < 0.001$) and specialization metric ($\chi^2 = 80.203_{2,454}$, $p < 0.0001$) explained significant amounts of deviance in DSI*. Miners were significantly more specialized than either semi-concealed (z-ratio = 6.651, $p < 0.0001$) or free-living chewers (z-ratio = -5.347, $p < 0.0001$) but semi-concealed and free-living chewers did not differ significantly in their specialization (z-ratio = 0.791, $p = 0.7083$). Specialization metric differed significantly in each pairwise comparison (polyphenol-mechanical: z-ratio = 4.317, $p = 0.0001$, polyphenol-phylogenetic: z-ratio = -5.049, $p < 0.0001$ and mechanical-phylogenetic: z-ratio = -8.955, $p < 0.0001$). Miners were more specialized than the other guilds for all metrics and specialization metric takes the following order: phylogenetic > polyphenol > mechanical (Figure 1). The best model was diagnosed as having goodness of fit, indices indicated no significant difference between observed and measured dispersion, had uniform variance and there were no outliers with extreme influence. The quantile-quantile plot demonstrated visual normality of residuals, although there was significant deviation in the correct distribution test. The same model with a Gaussian error term had extreme deviation in variance and normality of residuals as well as outliers with extreme influence.

Plant mean specialization varied across guild and metric. *Macaranga* herbivores were notable for their low specialization toward polyphenols and mechanical defenses. In contrast, *Ficus* herbivores showed the highest mean phylogenetic and polyphenol specialization of all plant genera studied. *Syzygium* insect herbivores were most specialized toward mechanical defenses (Figure 2).

Host specificity varied across guilds when considering the overlap of insect species (Figure 3). For example, there was not a single miner species shared between host species from different genera. In contrast, the congeneric host species on average shared ca 75% of their miner species and more than 90% of their free-living and semi-concealed caterpillars. Food webs were generally modular, with miners being restricted to one host genus (modularity of 0.88,

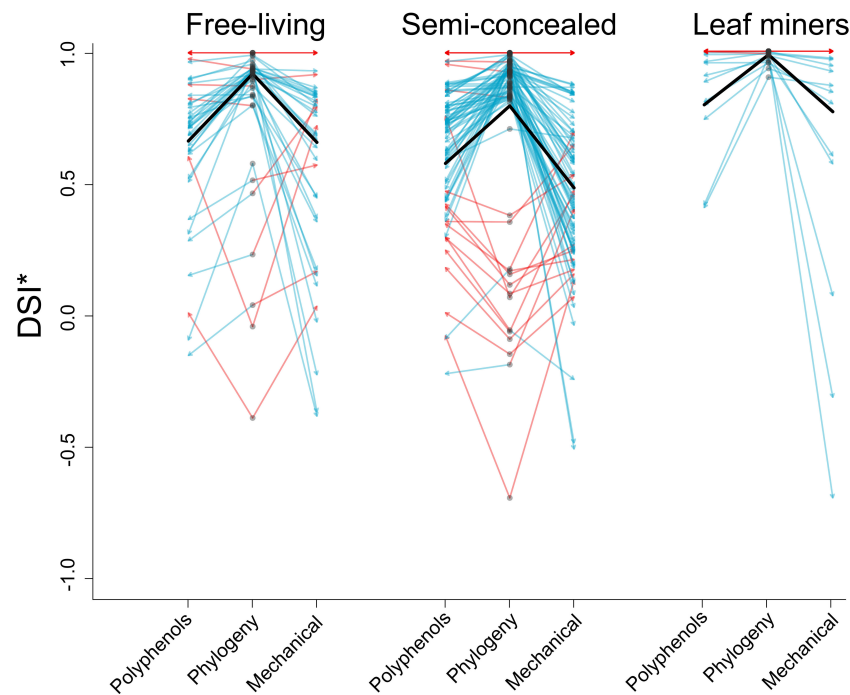


FIGURE 1

Plot showing rescaled specialization DSI* of leaf mining, free-living and semi-concealed Lepidopteran larval leaf-chewing guilds. Grey circles represent a species' phylogenetic specialization, arrows to the left and right indicate specialization toward polyphenols and specialization toward mechanical defense. Blue arrows represent cases where either polyphenol or mechanical specialization are lower than phylogenetic specialization and red arrows represent the inverse scenario. Black bars represent means.

connectedness of 0.07) while the food webs for free-living (modularity of 0.58, connectedness of 0.11) and semi-concealed (modularity of 0.66, connectedness of 0.16) caterpillars were more connected (Figure 4).

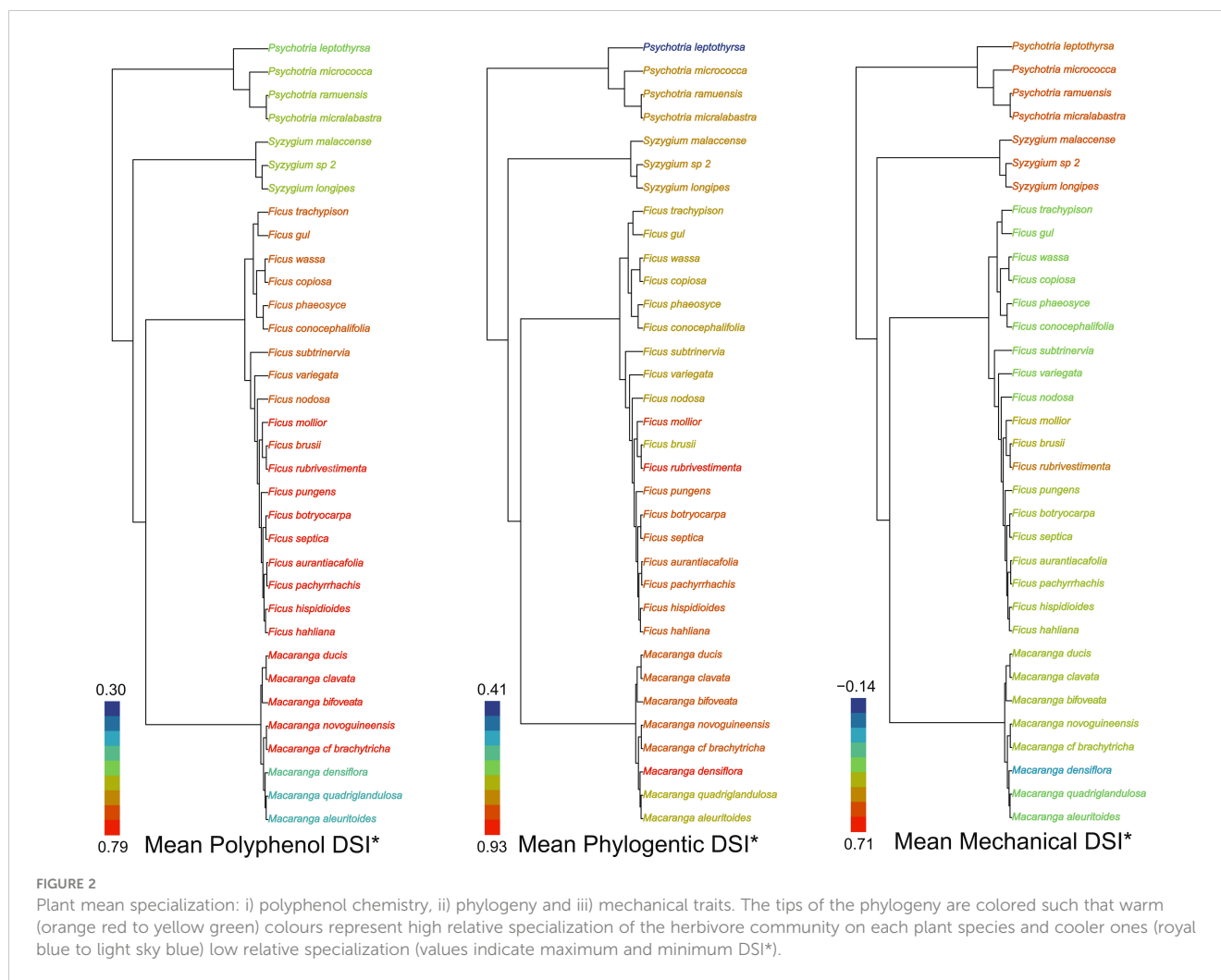
The first two principal components in the PCA analysis explained 48.7% of the total inertia in the studied traits across the host species (Figure 5A). The best CCA model explaining the inertia in herbivore communities consisted of the variables HHDPs ($F=1.3825$, $p=0.062$), galloyl derivatives ($F=1.5191$, $p=0.023$), trichomes per disc ($F=1.5763$, $p=0.010$), quinic acid derivatives ($F=1.4402$, $p=0.019$), CN ($F=1.4509$, $p=0.025$), myricetins ($F=2.3237$, $p=0.001$) and successional index ($F=1.3319$, $p=0.074$). These variables together explained 34.8% of the total inertia in the model ($\chi^2 = 4.3562_{6,27}$, $F=1.9783$, $p<0.001$). The first two CCA axes explained 14.8% of the total inertia (Figure 5B).

4 Discussion

Species-rich plant genera represent an important part of woody plant diversity in tropical forests (Gentry, 1982; Foster and Hubbell, 1990). These 'species swarms' provide resources for a non-trivial component of terrestrial diversity: herbivorous insects. Fundamental to estimating insect species richness is their host-specificity, understanding their diversification requires placing this into a phylogenetic context. In this study we explored phylogenetic and functional patterns of specialization in insect herbivores associated with such hosts, and analyzed what host traits govern

these patterns. Overall, our study shows that *Ficus*, *Macaranga*, *Psychotria*, and *Syzygium* harbor largely distinct herbivore assemblages, with the four genera serving as distinct host islands that support insect diversity in the forest (Novotny et al., 2004). Novotny and Basset (2005) found that only 27% of insect herbivores appear to be monophagous in tropical forest communities where several congeneric hosts are present. Here the recovered numbers of caterpillar species shared among the congeneric hosts are even higher. This suggests that host-shifts may be particularly frequent in caterpillars using species-rich plant genera, as may be the case for other even more generalist herbivores such as adult Coleoptera and Orthoptera.

Insect specialization was most strongly associated with host phylogeny and polyphenol traits conserved at the level of genus. Mechanical traits we measured played a smaller role, apart from in *Syzygium*. Polyphenols affect insect herbivores through protein precipitation and oxidative stress. In caterpillars that have high pH in their mid-gut, the protein precipitation is likely limited. Polyphenol oxidative activity is thus supposed to play a more important role (Salminen and Karonen, 2011; Salminen, 2014). Indeed, the multivariate analyzes picked hydrolysable tannins (HHDPs) with high oxidative activity as one of the traits affecting assemblages of caterpillars associated with hosts from the four genera we studied. A distinct polyphenol profile also characterized *Syzygium*, which was largely driven by the presence of myricetins. The dominance of myricetins has previously been reported in *Syzygium cumini* (Chagas et al., 2018) and these likely influence insect host use and shape food web modularity. Yet, the

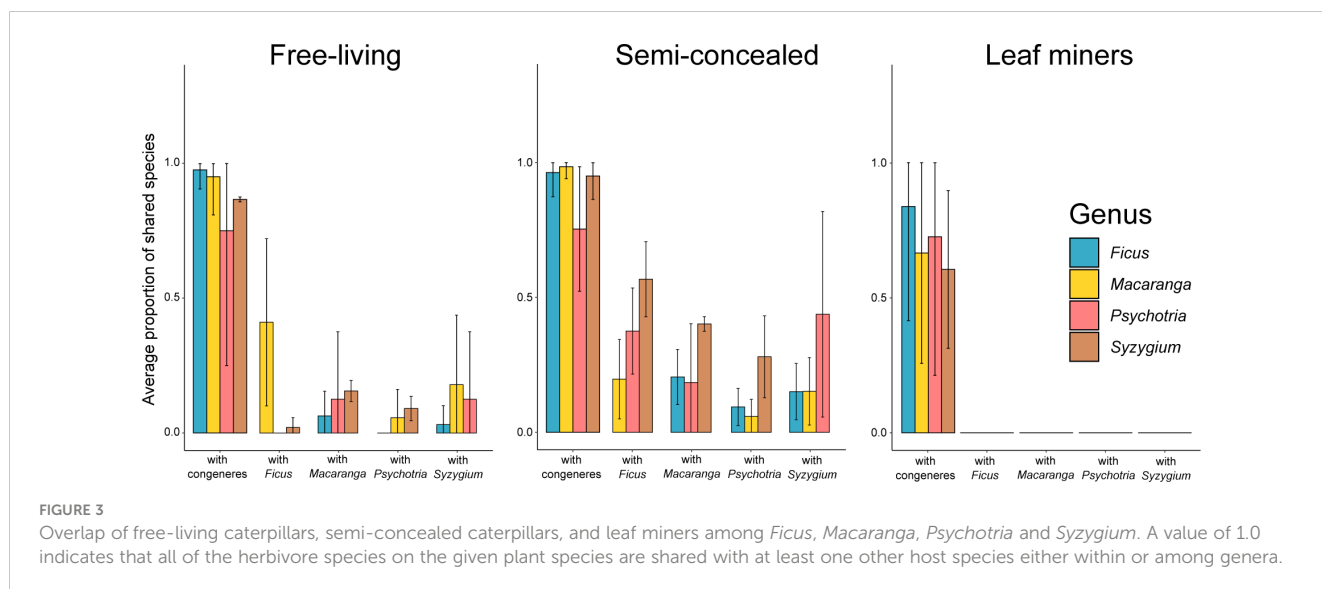


fact that insect specialization was more closely related to host phylogeny than to chemical differences quantified based on polyphenols or physical traits suggests that phylogenetically conserved, but unmeasured, traits are important predictors of host use.

We focused our chemical analyzes on polyphenols that are known to structure insect communities and provide anti-herbivore defense in all four genera we studied (Sagers and Coley, 1995; Segar et al., 2017; Volf et al., 2018). While our targeted approach works well when considering a subset of (well-studied) chemical diversity for which commercial standards are widely available, it fails to capture the higher order complexity of the sample. Our focal plant hosts also possess characteristic specialized metabolites. For example, *Ficus* produces phenanthroindolizidine alkaloids and leaf proteases that affect its interactions with insect herbivores (Volf et al., 2018; Volf et al., 2020). Phenanthroindolizidine alkaloids are even sequestered by some of *Ficus*-specialized herbivores and largely govern their host choice (Fontanilla et al., 2022). Specialized metabolites showing some level of lineage-specificity can be also found in the other studied genera, such as indole alkaloids in *Psychotria* (Gregianini et al., 2004). Unfortunately, we do not have data to analyze the effect of these

metabolites on the differences in insect specialization and assemblage structure between *Ficus*, *Macaranga*, *Psychotria*, and *Syzygium*. However, results from other studies suggest a key role of lineage specific metabolites in promoting turnover of insect species among host-plants (Richards et al., 2015; Volf et al., 2020). Together with the variation that occurs among congeneric species, these trends make chemical variation among tropical plants one of the key factors supporting diversity of insect herbivores in tropical forests (Richards et al., 2015; Endara et al., 2017; Salazar et al., 2018). By quantifying polyphenol groups, we were able to detect correlations among them, this allowed us to explain the distribution of insects across host plants with a higher degree of accuracy than using measures of total content. Different compound groups influence insect feeding in different ways.

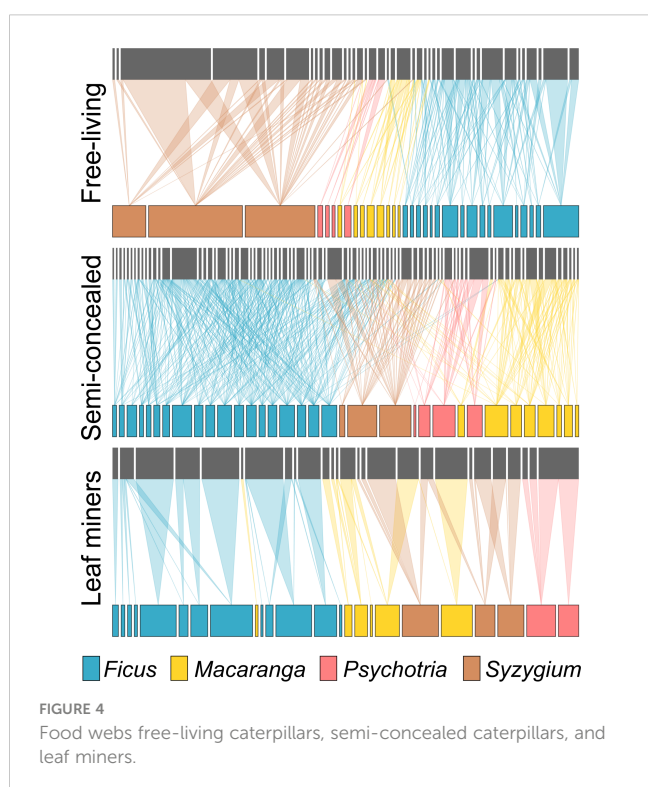
We expected that host-plant habitat preferences could explain significant amount of variation in insect assemblages in addition to the variation explained by the chemical and physical traits of the hosts. The studied tree species differ in their preference for primary and secondary forests where both biotic and abiotic can be profoundly different (Fibich et al., 2016). Habitat preferences are also correlated with variation in leaf chemistry, which could be another factor affecting the herbivores (Sedio et al., 2017). Our



multivariate analyzes did find an effect of host-plant preference for primary vs. secondary forest on the associated insect assemblages, explained largely by the distinct assemblage on *Syzygium*. This relationship, however was not strong. This suggests that, when accounting for host-plant traits, habitat differences between primary and secondary forests *per se* may play a smaller role in structuring caterpillar assemblages than in other insect groups such as ants (Holloway et al., 1992; Klimes et al., 2012). This can be due to the low correlation between host palatability and successional status in tropical forests (Lepš et al., 2001). Here, the observed differences in insect herbivores between hosts preferring either primary or secondary forests were mainly associated with host

species from the genera *Macaranga* and *Syzygium*. Species from *Macaranga* harbored insects with low polyphenol and mechanical specialization, those feeding on *Syzygium* showed specialization toward mechanical defenses. The presence of such host lineages rather than systematic underlying effects can be responsible for a substantial share of differences in specialization and species composition of herbivorous insects between primary and secondary forests (Lepš et al., 2001).

As expected, our results also show important differences in the specialization of various insect guilds associated with the four large genera. The miners were most specialized in all regards, while there was little difference in mean DSI* indices between free-living and semi-concealed caterpillars. These patterns follow the overall guild specialization patterns recovered in tropical forests (Novotny et al., 2010). However, there was also variation within guilds. For example, a sizeable subset of semi-concealed caterpillars was more polyphenol specialized than phylogenetically specialized, illustrating potential convergence of caterpillars onto chemically similar plants. Certain caterpillar lineages seem to be tracking hosts with similar polyphenol profiles. For example, Segar et al. (2017) showed that geometrids predominantly occurred on hosts with high oxidative activity of polyphenols. We observed that caterpillars from the family Tortricidae are frequently associated *Syzygium* while *Dichomeris* spp. (Gelechiidae) are typically associated with *Macaranga* (within the context of this study at least). While chemical turnover among congeneric hosts (chemical β -diversity) is more pronounced in some genera (e.g., *Ficus*) than others (e.g., *Macaranga*), restricted metabolites drive insect herbivore host use. For example, *Macaranga* is distinct among the four genera studied here yet each *Macaranga* species harbours a similar insect community and has similar chemical composition. DSI* measures the deviation of the diet of a given herbivore from the pool of available hosts and it accounts for any imbalance in taxonomic composition of the sampled species. Despite our findings are thus robust, we advocate wider and more balanced sampling of these genera. While inter-generic variation largely exceeds inter-generic variation in our samples, we acknowledge that more balanced



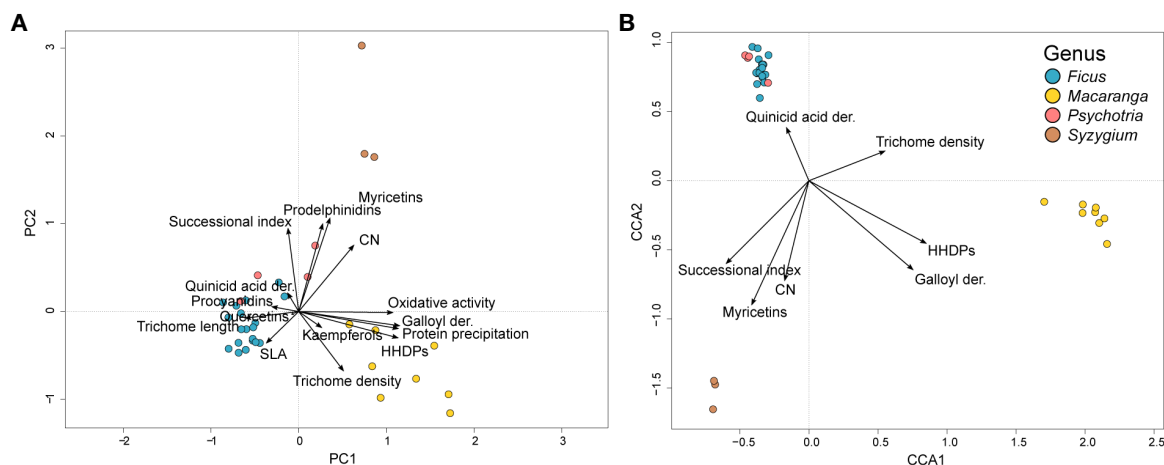


FIGURE 5

Trait variation among the focal tree species as analyzed with PCA (A) and the correlation between traits and insect communities as analyzed with CCA (B). The first two principal components explained 48.7% of the total inertia in trait variation across the tree species when analyzed with PCA. The CCA ordination diagram shows the traits best explaining the variation in herbivore communities. Together, these traits explained 34.8% of the total inertia in the model. Traits are shown as black arrows. Tree species appear as circles colored according to the genus.

sampling would improve the reliability of our multivariate analyses and strengthen conclusions relating to chemical β -diversity.

In conclusion, we show how speciose genera of tropical woody plants harbor largely different caterpillar communities. As proposed by Janzen (1968) such plant lineages can be seen as islands for their herbivore fauna. Host shifts among congeneric hosts from the *Ficus*, *Macaranga*, *Psychotria*, and *Syzygium* seem to be frequent even in highly specialized guilds, such as miners. The overall diversity of insects harbored by individual genera may be particularly affected by their species diversity and variation in functional traits that govern herbivore host specialization (Richards et al., 2015; Volf et al., 2019; Narango et al., 2020). Here, caterpillar specialization seems to be more closely related to phylogenetic relationships between the hosts than to the host traits or successional preference. In particular, while traits such as polyphenols or trichomes that occur across the studied host genera explained some variation in the composition of caterpillar assemblages, we expect that most of the effect of phylogeny we recovered was driven by traits that we did not measure. In miners that are intimately associated with their hosts, such traits can include leaf morphology or host susceptibility to manipulation (Zhang et al., 2016). Indeed, many insect species can manipulate plant defenses (Giron et al., 2016). While our field survey was not designed to detect induced defenses (but rather to avoid manipulated leaves and hosts), we have to expect that some variation can be attributed to up- or downregulated polyphenol defenses (Eyles et al., 2010). We suggest that experiments in more controlled conditions would help to unravel these effects further. Another large share of the variation can be likely attributed to lineage specific specialized metabolites. Rapidly developing fields of metabolomics and bioinformatics offer new possibilities for measuring concentrations of thousands of metabolites in non-model plant species and assessing their structural diversity (Sedio, 2017). Approaches that consider structural or spectral diversity of metabolites are particularly promising in this regard. This is because they allow to quantify

chemical variation among distantly related plants that show only a minimal overlap in the content of individual metabolites (Sedio et al., 2017; Philbin et al., 2022). Combining metabolomic data with information on other types of data, such as other plant traits and habitat preferences that we studied here, can help us to understand the foundational role of speciose plant genera in tropical forest food webs. Greater mechanistic understanding of ecological network structure will ultimately lead to more scientifically informed conservation practices as well as insights into the origins of plant 'species swarms' and their associated insect communities.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

Author contributions

SS: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Software, Supervision, Writing – original draft, Writing – review & editing. LRJ: Conceptualization, Formal Analysis, Investigation, Methodology, Supervision, Writing – original draft, Writing – review & editing. LN: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Writing – original draft. YB: Conceptualization, Funding acquisition, Investigation, Methodology, Writing – review & editing. JR: Data curation, Investigation, Writing – review & editing. OK: Formal Analysis, Investigation, Methodology, Writing – review & editing. MS: Data curation, Investigation, Methodology, Writing – review & editing. BG: Data curation, Investigation, Methodology, Writing – review & editing. CD: Data curation, Investigation, Methodology, Writing –

review & editing. PB: Formal Analysis, Investigation, Methodology, Software, Writing – review & editing. PM: Visualization, Writing – review & editing. SM: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Writing – review & editing. GW: Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Writing – review & editing. J-PS: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Resources, Supervision, Writing – review & editing. VN: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Writing – review & editing. MV: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Supervision, Writing – original draft.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fevo.2023.1308608/full#supplementary-material>

SUPPLEMENTARY TABLE 1

Segar, Simon (2023). Interaction_matrix_large_genera_310723.xlsx. figshare. Dataset. <https://doi.org/10.6084/m9.figshare.24191136.v1>

SUPPLEMENTARY TABLE 2

Segar, Simon (2023). Large_Genera_171123.R. figshare. Software. <https://doi.org/10.6084/m9.figshare.24191184.v1>; Segar, Simon (2023). Means_PNG_results_280618_FORMATTED_MV.xlsx. figshare. Dataset. <https://doi.org/10.6084/m9.figshare.24191133.v1>

SUPPLEMENTARY TABLE 3

Segar, Simon (2023). PNG_Plants_2_SUBST.tre. figshare. Dataset. <https://doi.org/10.6084/m9.figshare.24191187.v1>

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