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Macroevolution of defense syndromes in *Ficus* (Moraceae)

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Abstract. Recursive adaptations and counter-adaptations of plant-feeding insects are thought to have driven chemical and physical diversity in plant defenses. Among existing theories on defensive diversity, the syndromes hypothesis predicts that plants may evolve suites of covarying defense traits along evolutionary arms races with herbivores. We use the large, phenotypically diverse angiosperm genus *Ficus* (Moraceae) to test the major predictions of this hypothesis: (1) plant species will form distinctive combinations of defensive traits; (2) these traits will be correlated within each syndrome. Trait combinations need not map well onto phylogenies because plant species can converge onto similar trait values, but strong phylogenetic signal driven by selection (as opposed to drift) suggests roles for escalation and coevolution. Finally, *Ficus* species with complementary combinations of defenses will be less susceptible to insect damage and harbor distinct insect communities. We quantified susceptibility to insect herbivory and nine leaf traits related to resource acquisition and defense in 36 *Ficus* species growing in a common-garden setting in dry and wet seasons over 2 yr. We recovered a set of three syndromes defined by relatively small sets of trait combinations. Broadly speaking, these syndromes grouped fig species with different life forms. For example, epiphytic figs had nutrient-poor, tough, tannin-rich leaves, while free-standing trees tended to have leaves covered in trichomes and full of alkaloid-rich latex. When season and species identity were accounted for, the combination of two traits, higher C:N and higher latex tannin content, provided significantly stronger defense than did either trait taken singly. Several individual traits (C:N, latex tannin, and trichome density) were significantly negatively correlated with herbivore damage, while alkaloid content was positively correlated (perhaps as a result of feeding by adapted herbivores). Several defensive traits influenced insect herbivore community structure. Finally, traits followed different evolutionary trajectories. While latex tannin, C:N, and leaf tannin fit a Brownian-motion model of evolution, the first two escalating across *Ficus* phylogeny, others appeared to have more limited phylogenetic signal or tended to de-escalate. Overall, the patterns we detected support the concept of coordinated defense syndromes, demonstrating that evolutionary arms races can drive combinations of traits in this genus.

Key words: *defense syndrome;* Ficus*; herbivory; life form; macroevolution; phylogenetic generalized linear mixed model; phylogeny; trade-off.*

INTRODUCTION

Insects on plants are of great economic and ecological interest; they dominate terrestrial ecosystems in terms of species richness and abundance (Price 2002). While a large proportion of plants rely on insects for pollination, many are also under attack by insect herbivores (Ehrlich and Raven 1964). Multiple traits help to defend plants against leaf-chewing insects, including, but not limited to (1) limitations to nutritional quality (e.g., proteins and antiproteins; Green and Ryan 1972, Ryan 1990), (2) physical defenses (e.g., spines, trichomes, and leaf toughness; Wagner 1991, Lucas et al. 2000), (3) toxicity to insects (e.g., cyanogens and alkaloids; Bennett and Wallsgrove 1994), (4) adaptive phenology (Feeny 1970, Takahashi and Yamauchi 2010), (5) high regrowth capacity (i.e., tolerance; Fornoni 2011), and (6) indirect defenses (e.g., volatile compounds that attract enemies of phytophagous insects; Aljbory and Chen 2016). Which trait, or suites of traits, contribute(s) to effective defense, and whether these traits are constrained by host phylogeny or ecological factors, are fundamental questions in plant defense (Agrawal and Fishbein 2006, Agrawal 2007). Although a range of different theories exist as to the origin and diversification of plant defenses, our understanding of the relative contribution

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of local ecological conditions vs. historical or physiological constraints in shaping the observed interspecific variation in defenses is still limited (Agrawal 2007, 2011, Nuñez-Farfan et al. 2007, Zust and Agrawal 2017).

Plants have evolved different combinations of traits (Wright et al. 2004, Díaz et al. 2015), and typically employ a broad arsenal of defensive traits against herbivores (Duffey and Stout 1996, Romeo et al. 1996). Several hypotheses envisaging syndromes of defensive traits have been developed. Silvertown and Dodd (1996) showed that herbaceous and woody plants have distinct types of chemical defenses (alkaloids and tannins, respectively). Because growth and defense are interlinked (Kursar and Coley 2003, Zust and Agrawal 2017), defense syndromes also include growth-related traits. The repeated appearance of a particular defense syndrome might be driven by convergent adaptation of multiple traits in unrelated plant species growing under similar ecological conditions and herbivore pressures (Coley et al. 1985, Fine et al. 2006). Certain combinations of traits have been repeatedly selected across unrelated species for defense against herbivores in tropical forest. Kursar and Coley (2003) argue that in their production of young leaves, trees fall along an escape–defense continuum: young leaves of extreme "escape" species are predicted to have few chemical defenses, but rapid synchronous leaf expansion and low leaf nutritional quality during expansion; young leaves of extreme "defense" species have high chemical defense, low nutritional quality, and asynchronous leaf expansion. Ecologists also hypothesize that shared defensive traits or strategies could reflect a common ancestry; for example, Pearse and Hipp (2009) found that interactions between introduced oaks and their herbivores are driven independently by traits that track plant phylogeny.

When pairs of traits are considered separately from others, trade-offs between traits can be found (Steward and Keeler 1988, Herms and Mattson 1992). If defensive traits are costly, or to some extent redundant, then trade-offs will occur, especially when plants are nutrient-limited. Trade-offs can involve not only different types of chemical-defense compounds but also physical and chemical defenses (Steward and Keeler 1988, Eichenberg et al. 2015) and the ways in which defenses are deployed, e.g., between constitutive (always active) and inducible defenses (activated through a previous consumer and conferring some degree of resistance to subsequent attacks; Morris et al. 2006, Kempel et al. 2011). However, some studies have found limited evidence of defense syndromes and trade-offs. Mason et al. (2016) did not observe any defense syndrome based on trade-offs. Instead, they found leaf defenses to be strongly related to a plant's position on the leaf economic spectrum, with higher defenses being found in *Helianthus* species with more resource-conservative leaf economic traits supporting a slower-growth persistence strategy. However, there was little support for the evolution of higher defenses in low-resource habitats (but see Fine et al. 2006). The contrasting results of previous studies might suggest that coadaptation among defensive traits could be lineage-dependent (Agrawal 2011, Johnson et al. 2014). More empirical studies of additional lineages are necessary to address whether and how defensive traits of a plant are co-adapted, while data on the specialization and identity of the insect herbivores provides much-needed context (Volf et al. 2019).

Ficus is a large, pantropical genus with about 800 species (Berg and Corner 2005), representing an important component of tropical floras. The genus is particularly species rich in Southeast Asia (Berg 1989, Stevens 2001). Figs are also ecologically diverse, with different life forms, including epiphytes, hemi-epiphytes (epiphytes that become free-standing trees as they grow), and free-standing trees. Fig plants are attacked by a wide range of different herbivores (Novotny and Basset 2005). Furthermore, figs are latex-bearing and latex is well known for its defensive role against herbivores (Agrawal and Konno 2009, Bauer and Speck 2012). Several important studies have explored the functional traits of *Ficus* in relation to defense against herbivores and their evolutionary implications (Volf et al. 2018, Villard et al. 2019). For example, in a recent study, Volf et al. (2018) showed an evolutionary escalation of alkaloid diversity and of oxidative activity in fig species, which may have resulted from adaptation to generalist herbivorous insects. In contrast, they found significant divergence in the density of leaf trichomes between closely related fig species, which may have been driven by specialist herbivores. Other studies, conducted at our field site, have demonstrated that there is a trade-off between leaf water flux capacity and leaf drought tolerance-related traits such as LMA (leaf mass per area) across hemi-epiphytic and free-standing *Ficus* species (Hao et al. 2010). As the inverse of LMA, SLA (leaf area per mass) is considered to be related not only to plant palatability but also to plant growth (Schädler et al. 2003, Wright et al. 2004), implying that there is no strict boundary between traits evolved in response to biotic or to abiotic pressures. Some traits that affect leaf water exchange can also affect plants' resistance to herbivores or their ability to regrow after herbivory.

In this study, we combine comparative phylogenetics, detailed plant trait data, insect herbivore data, and field observations to investigate the macroevolution of leaf defenses and resistance to herbivory in *Ficus* (Moraceae) in a common-garden setting. The use of common-garden experiments can minimize the contribution of plastic phenotypic adjustments to local site conditions, allowing the detection of genetically based species differences (Garland and Adolph 1991). In this study, the individuals of the different *Ficus* spp. we studied are distributed in a relatively small area (about 1.3 ha), meaning that they are all exposed to a relatively uniform local community of herbivores.

Specifically, we addressed the following four questions: (1) Can defensive phenotypes of *Ficus* spp. be classified into defense syndromes? (2) Are there correlations among defensive traits within each syndrome? (3) Are phylogenetic relationships among *Ficus* species congruent with patterns of defense trait similarity? (4) Which traits best predict observed herbivore damage and insect herbivore community structure, and do combinations of traits better predict resistance to attack than single traits?

METHODS

Study system and study site

The study was conducted in Xishuangbanna Tropical Botanical Garden (XTBG; 21°56′ N, 101°15′ E, elevation 580 m), Chinese Academy of Sciences, Yunnan Province, southwestern China. The climate is dominated by the southwest monsoon, with a well-defined alternation of wet and dry seasons. Mean annual precipitation is about 1,500 mm with 80% occurring during the May–October wet season. Mean annual air temperature is 22.7°C, with monthly means ranging from 14.8°C in January to 25.5°C in June. The garden is surrounded by a river and retains an ~255-ha patch of relatively undisturbed tropical rain forest. The garden also adopts an organic management strategy, prohibiting the use of pesticides and chemical fertilizers, thus allowing many members of the natural herbivore community to colonize plants growing within the garden. Furthermore, the individual fig plants studied are distributed haphazardly in this common garden and are attacked by a wide range of different herbivores, including leaf beetles and larvae of butterflies and moths (Fig. 1). All fig plants studied were planted as seeds or seedlings introduced from the field at different years (Appendix S1: Table S1) and were planted without a specific arrangement in mind. Thirty-six species of *Ficus* belonging to five subgenera, including four epiphytes, 15 hemi-epiphytes, and 17 free-standing tree species, were selected to address our questions. All of the *Ficus* species in our study are native to southwestern China. Our study focused on leaf defenses and susceptibility of figs to naturally occurring herbivores. All plants sampled were mature and growing under similar conditions at XTBG, providing a common environment for the analysis of evolutionary differences (Garland and Adolph 1991). All measurements were performed during 10–25 March (dry season) and 10–25 August (wet season) in both 2014 and 2015.

Measurement of leaf defenses

Six traits of leaves and three traits of latex present in leaves were included in this study: specific leaf area (SLA, cm^2/g dry mass), leaf water (%), C:N ratio, leaf tannin content (%), toughness (g), trichome density (no./mm^2), latex dry content (g/cm^2), latex tannin content (g/L), and latex alkaloid content (g/L). SLA, leaf water, C:N, leaf tannin, and toughness are traits commonly correlated (positively or negatively) with leaf palatability and digestibility. Higher C:N ratio, toughness, and tannin content are often also correlated with reduced herbivory and slower herbivore growth rates (Choong et al. 1992, Hanley et al. 2007, Clissold et al. 2009, Kitajima et al. 2010, Kitajima et al. 2012, Prado et al. 2014). SLA and C:N are also considered as indices of resource capture ability and leaf quality, respectively. The latter might affect herbivore damage (Pérez-Harguindeguy et al. 2003), whereas the former affects the plant's ability to tolerate herbivory (e.g., by regrowth) because of its connection with carbon storage and photosynthetic capacity (Wright et al. 2004). Trichomes block or ensnare small herbivores such as insects or gastropods, interfering with feeding or oviposition, and may keep pathogen-containing water droplets away from the leaf surface (Hanley et al. 2007). Glandular trichomes are also known to secrete secondary compounds that may deter small herbivores and inhibit colonization and growth of pathogens (Hanley et al. 2007). Tannins are polyphenolic compounds classically recognized for their protein-precipitating capacity and more recently for their strong oxidative activity against herbivores (Salminen and Karonen 2011). Tannins are widespread in plants, and have been demonstrated to reduce herbivory by reducing leaf protein digestibility, damaging the digestive system, and generally interfering with metabolism and growth (Moles et al. 2011). Latex has been strongly implicated in defense against herbivorous insects not only because its sticky nature provides mechanical defense, but also owing to the bioactive defense-related compounds it contains, including (depending on the plant) tannins, alkaloids, cardiac glycosides, and digestive cysteine proteases (Agrawal et al. 2008, Agrawal and Konno 2009, Konno 2011). Together, the nine traits we studied reflect a wide variety of different classes and mechanisms of putative leaf defenses present in plants.

Three individuals per species were selected; six to ten leaves from each individual were collected for measuring leaf traits. Because latex immediately began flowing from the detached leaves, the first step was to collect the latex. We measured latex exudation on all plants by detaching the youngest fully expanded undamaged leaf from the plant and then cutting its tip off and collecting the latex in a pre-weighed microcentrifuge tube. We collected latex exuded from the apex of the leaf; the tip was repeatedly cut to keep the latex flowing until no more latex exuded. These tubes were weighed by electronic balance in the laboratory nearby. The tube containing latex was oven-dried at 45°C for 72 h to measure its dry mass. The latex dry content was calculated as mass per leaf area. Furthermore, 10 μL of latex (from the cut petiole when the leaf was detached) was collected from 1–10 additional leaves and dissolved with 90 μL purified water, and then diluted 10 times. The mixture was filtered through a 0.45-μm membrane. The extract was fully blended with 50 mL acetone for 40 minutes and

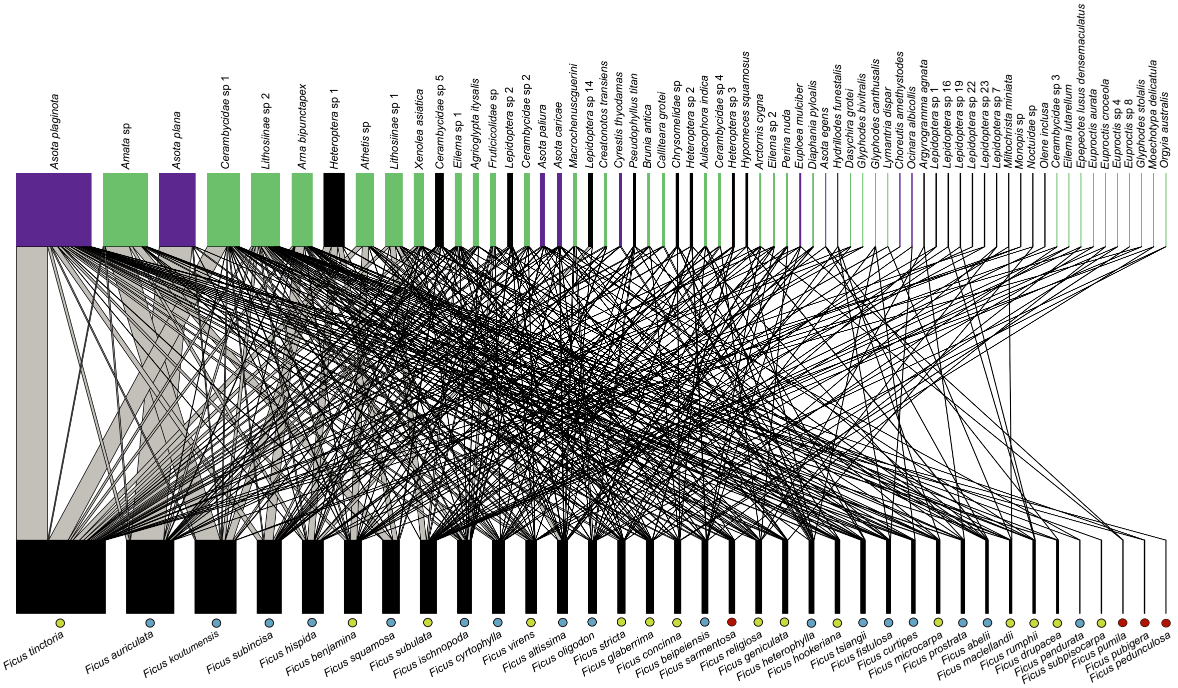

FIG. 1. Interaction networks between *Ficus* and herbivorous insects, constructed from observations in the wet season of 2019. The bars above represent insect morphospecies (purple bars represent herbivore species that are specialists on *Ficus*, green bars represent generalists), the black bars below represent *Ficus* species. Bar width corresponds to the proportion of interactions contributed by each species to the network. Links between species are indicated by lines that are proportional to interaction strength. Red circles represent epiphytic, yellow circles represent hemi-epiphytic, and blue circles represent free-standing *Ficus* species, respectively.

the mixture was filtered through a medium-speed quantitative filter. Then 1-mL filtrate was mixed with 2.5 mL of sodium tungstate-phosphorus molybdenum acid and 5 mL sodium carbonate. The bromothymol blue colorimetric method (418 nm) was used to determine the latex alkaloid content (Shamsa et al. 2008).

Tannin content was measured by a colorimetric method (760 nm) using the Folin-Denis reagent (Bajaj and Devsharma 1977). Many different methods have been used to quantify tannin content, particularly content of condensed tannin (Schofield et al. 2001). Despite limitations of the method using Folin-Denis reagent (Schofield et al. 2001), measures of total phenolics by this simple colorimetric method, like those obtained by other methods, are negatively correlated with growth rates of phytophagous insects (overview in Appel 1993).

Following latex extraction, we used the same leaves to measure other traits. Leaf area was measured via a flatbed scanned image or digital photograph using ImageJ (Abràmoff et al. 2004). Leaves were stored in plastic bags on ice in the field, then brought into the lab and weighed. Leaf toughness was measured using a leaf punch. We built a device consisting of a flat-ended cylindrical steel rod (punch, 2 mm diameter) mounted onto the moving head of the testing machine and a stationary base with a sharp-edged hole with a 0–1 mm clearance (following Sanson et al. 2001). The punch was set to go through the hole without any friction. The punch moved downward at a constant speed of 10 mm/s. The leaves were positioned to avoid primary and secondary veins where possible. Trichome density was measured under a light stereoscope by counting the trichomes in a circle 5 mm in diameter on the abaxial side of the leaf. We then oven-dried leaves for 48 h (70°C) in the laboratory. Leaf water was estimated as the difference between the wet and dry leaf mass. Leaf area and dry mass were then used to calculate SLA. Additional leaves were collected for analysis (following exudation of all latex) of total C and N and leaf tannin, which required more material than the other analyses. Our measures of leaf dry mass, leaf water content, SLA, and total C and N thus excluded latex from the calculations. Because latex accounted for only very small proportions (0.3–3.5%) of the total dry mass of leaves, its exclusion in these measures had little effect on our results. Owing to the fact that our measures of leaf tannin were conducted on leaves from which latex had been extracted, leaf tannin and latex tannin contents are two independent measures. Dry samples were then sent to the Biogeochemical Laboratory of the Kunming Division of Xishuangbanna Tropical Botanical Garden for chemical analyses. Total C and N were determined using a wet digestion procedure (Kalra and Maynard 1991).

Susceptibility to naturally occurring herbivores

The susceptibility of different *Ficus* spp. to herbivores was assessed in both dry and wet seasons for 2 yr by measuring the proportion of leaf surface lost to chewing herbivores. Similar-aged leaves were sampled in wet and dry seasons and in both years. We sampled at least 100 of the youngest fully expanded leaves, from four to five branches extending in different cardinal directions. These leaves were photographed, and the initial area of the intact leaf was reconstructed, with the percentage of lost leaf surface being calculated using ImageJ.

For comparative purposes we also assessed the degree of damage to two of our focal species (*F. subulata* and *F. auriculata*) in the relatively undisturbed rain forest adjacent to XTBG in the wet season of 2017. This allowed us to assess how our results from the common-garden experiment aligned with those from wild populations. Ten individuals of each species standing at least 50 m apart were selected and the above methods followed.

Phylogenetic reconstruction

Phylogenetic relationships among the 36 *Ficus* species were inferred from genotypes at three loci: ITS, ETS, and G3pdh sequences (Appendix S1: Table S2). The phylogeny of *Ficus* was reconstructed using Bayesian inference as implemented in MrBayes. *Antiaropsis decipiens* and *Castilla elastica* were used as outgroups. The following substitution models were used for individual loci: ITS: GTR + I + G, ETS: HKY + G, G3pdh: GTR + G. Models were selected according to Bayesian information criteria (BIC) using jModelTest 2.1.10 (Darriba et al. 2012). Sampling was carried out every 1,000 generations for 10^7 generations, the first 20% of all generations were discarded as “burn in” and the results were summarized with a maximum clade credibility tree. The phylogenetic tree of *Ficus* published by Cruaud et al. (2012) was used to confirm the accuracy of the placement of the 36 *Ficus* species in our study. For the species represented in both studies, topology of the tree we generated was congruent with that presented by Cruaud et al. (2012).

Evolution of traits

We treated each life form (epiphyte, hemi-epiphyte, and free-standing) as a binary variable (zero or one) to analyze the evolutionary model of life form (*D* statistic in the caper package, Fritz and Purvis 2010). We estimated phylogenetic signal in traits and herbivore damage using the *K* statistic in the picante package (Kembel et al. 2010) for the R programming language (R Core Team 2012). The *K* statistic provides a Brownian-motion-based estimate of phylogenetic signal. $K > 1$ indicates a greater degree of trait similarity among related taxa than expected under Brownian motion, while $K < 1$ indicates trait convergence that exceeds the expectation under Brownian-motion models. Estimated variance is obtained by permuting trait values across the tips of the tree.

The effects of herbivore pressure on evolution of defense traits can vary, including divergence (predicted when pressure is exerted by specialists), escalation (predicted when generalists exert pressure) and even decline combined with increase in tolerance to herbivory (when defenses become ineffective) (Agrawal and Fishbein 2008, Agrawal et al. 2009*c*, Volf et al. 2018). Three evolutionary models were fitted for each trait in this study: Brownian motion (the correlation structure among trait values is proportional to the extent of shared ancestry between species); white noise, a non-phylogenetic null model; and Pagel’s lambda, allowing a more complex model of evolution with strong ($\lambda = 1$) to weak ($\lambda = 0$) phylogenetic covariation. The fitContinuous function in the R package Geiger was used for fitting these models (Harmon et al. 2008). $\mathrm{AIC_c}$ weights of each model were compared to evaluate the best-fitting one. We also used the values of trait disparity through time (DTT) from the root to tips using the function dtt in the R package Geiger to further examine the evolution of individual traits through time (Harmon et al. 2008). Furthermore, we used the average square distance metric to calculate trait disparity, and created a null distribution of DTT with 95% confidence intervals using 999 simulations under Brownian motion. We also used Permutational Multivariate Analysis of Variance (PERMANOVA) as implemented in the function adonis in the R package vegan (Oksanen et al. 2017) to test the correlations between leaf traits and phylogenetic distance among figs. To test the general directional changes in trait values from the root of the tree, the correlation between Abouheif’s distance (distance from the root) and trait values was determined as calculated in the R package adephylo (Jombart et al. 2010). Because evolutionary trends may not be linear over time (e.g., in late-diverging vs. early-diverging lineages), we also examined whether polynomial regressions gave better fits than linear correlations.

We also analyzed the effects of life form, season, year, and interactions between all of these factors on damage inflicted by herbivores, using three-way ANOVA.

Testing correlation between traits by the convex hull method

We used convex hull methods to explore combinations of traits. Convex hull methods, a construct from computational geometry, provide an *n*-dimensional measure of the volume of trait space occupied by species, and reflect shared ecological tolerances (Cornwell et al. 2006). Convex hull methods have been applied successfully to a wide range of data sets, including those used to examine the global spectrum of plant form and function (Díaz et al. 2015). In our study, we computed a nine-dimensional convex hull volume (H_{ob}) on the basis of the observed values of latex dry, latex tannin, and latex alkaloid content, leaf water content, SLA, C:N, toughness, trichome density and leaf tannin content, and compared it to four null model volumes (H_n: H_1–H_4) constructed under four different sets of assumptions (Díaz et al. 2015). Observed data were log- or square-root-transformed and standardized to zero mean and unit variance (z transformation).

Null model 1 (H_1) supposes that species traits vary independently and each of them comes from a uniform distribution, and the shape of the hypervolume under this null model (H_1) is a hypercube. The uniform and independent distribution of traits under H_1 corresponds to the assumption that any combination of trait values can arise from mutation and escape from the natural selection process with equal probability. Null model 2 (H_2) assumes that species traits vary independently and each of them comes from a normal distribution; the hypervolume of H_2 is a hypersphere. This null model assumes that all the traits evolve independently, as in H_1. However, extreme trait values are selected against during evolution. Simulated data were obtained by randomly and independently selecting from univariate normal distributions with standard deviation determined by the transformed observed data. Null model 3 (H_3) also assumes that species traits vary independently but there is no assumption about the distribution of trait variation; each trait varies according to the observed univariate distributions. Under this null hypothesis (H_3) the hypervolume can take many potential shapes. Null model 4 (H_4) supposes that species traits are normally distributed and follow the estimated correlation structure of the observed data set. H_4 assumes that there are fewer than nine independent axes of specialization because of the correlation among these traits and that extreme values are selected against. The hypervolume of H_4 is a hyperellipsoid.

We compared the observed hypervolume based on the observed values of nine traits to each null model (the average of 999 hypervolumes generated from the assumptions, Monte-Carlo permutations). The reduction in size was indicated by percentages as VD (volume difference = $(H_{ob} - H_n)/H_n \times 100$). In this equation, H_n is the null model (H_1–H_4).

Covariation among traits

Fig trait data were log- or square-root-transformed to fit a normal distribution, and we examined covariation among traits using two methods. First, we estimated pairwise correlation coefficients (Pearson's r) among all traits while accounting for species' mean values across four sampling times ($n = 36$). When the residual errors of the correlation among all traits displayed phylogenetic signal, we also tested the effect of shared evolutionary history in a maximum likelihood phylogenetic generalized least squares (PGLS) framework using Pagel's Continuous, implemented in BayesTraits (Pagel 1999), also using mean values for each species across four sampling times ($n = 36$). When the raw and the PGLS analyses gave the same result, only the latter is presented; otherwise, both analyses are presented. We further analyzed correlations among all traits, using mean values for each fig species in each of the four sampling times ($n = 144$). Secondly, because many traits were correlated, we simplified this covariation using principal components analysis (PCA) using mean values for each *Ficus* species across four sampling times ($n = 36$). PCA was appropriate because our traits varied continuously and any correlation among variables was generally linear, which we assessed visually by examining all pairwise biplots.

Ficus traits correlated with herbivore damage

Four methods were used to analyze correlations between herbivore damage and fig traits. First, data on herbivore damage were square-root-transformed to fit a normal distribution; we then examined the correlation between herbivore damage and individual traits and two PC axes of PCA by Linear Models (LM; $n = 36$). Furthermore, we also conducted an additional Phylogenetic Generalized Least Squares (PGLS) analysis to test the phylogenetic regression of herbivore damage and plant defensive traits and PC axes of PCA for data averaged from four sampling times ($n = 36$).

In addition, we used LM to examine correlations between herbivore damage (response variable) and defensive traits (fixed explanatory variables) for each sampling time considered separately ($n = 144$). Generalized Linear Mixed-Effects Models (GLMM) were constructed in the R package phyr to test the influence of fixed effects (*Ficus* traits) and random effects (species, year, and season) on herbivore damage. Models were fitted using restricted maximum likelihood. We used the mean value for herbivore damage of each sampling time from three individuals of each fig species. These analyses included a correlation matrix derived from a fully unresolved species level phylogenetic tree for *Ficus* as a random effect. Using a star phylogeny effectively excluded phylogeny from the analysis, but provided a modeling framework directly in line with subsequent phylogenetic generalized linear mixed models. Following non-phylogenetic analyses, we conducted a second set of models controlling for phylogenetic relationships. Phylogenetic Generalized Linear Mixed Models (PGLMMs) were used to analyze the response of herbivores (response variable) to *Ficus* traits (fixed explanatory variables), while including a correlation matrix derived from *Ficus* inter-species phylogenetic relationships as a random effect alongside year and season. The only difference between GLMM and PGLMM was that PGLMM included phylogenetic relationships of *Ficus* species. We also tested for phylogenetic signal in the residuals of each analysis. Year and season were also included in the PGLMMs as random effects in order to assess the power of both phylogeny and its covariance with traits to explain the residual variance not captured by our traits

($n = 144$). By including a phylogenetic term, we were able to assess (1) the need to control for phylogeny and (2) the power of phylogenetic relationships in predicting herbivore damage. All the figures were visualized by using the package ggplot2 in R 3.5.1.

Ficus *species and herbivores interaction network*

We explored the identity of key herbivores and the overlap in community structure between figs by collecting herbivorous insects between July and October 2019. Three to 10 individuals of each *Ficus* species were sampled (we checked each sampled plant three times per week), and the total area of foliage sampled over multiple sampling times ranged from 50 to 150 m^2 per species. For externally feeding adult insects such as Orthoptera, we hand collected insects from foliage and provided them with fresh leaves of the plant on which they were found until they fed or died. Only those insects that fed on the leaves of their host fig were included in our analysis (Novotny et al. 2002).

All externally chewing larvae were collected by hand from the foliage, and taken to the laboratory where they were fed with their host plant until pupation and emergence. Leaf miners and piercing-sucking insects (leaf feeders only) were also hand sampled from foliage. Relatively few sucking species were collected. All insects were assigned to morphospecies using external morphological characteristics and identified to the lowest possible taxonomic level with identification keys. We classified insects as specialists or generalists; a third category “other” was used to place species for which no published evidence was available. Specialist insects included those whose diet was largely composed of fig species, while generalists consumed leaves of figs alongside those of a wide range of plants. Classifications were built with the assistance of R. G. S. Tharanga Aluthwattha at XTBG and with reference to the published literature. Insect–plant associations were analyzed using three separate analyses: one univariate analysis considering abundance and two multivariate analyses considering distance measures. First, a chi-square test was used to compare the abundance of specialists and generalists across *Ficus* life forms. Second, we created quantitative adjacency matrices using the leaf-chewer data, and quantified interactions using the number of insect individuals recorded (standardized to leaf area sampled and with singleton species removed). We then used this interaction matrix as the biological matrix in canonical correlation analysis (CCA) to understand how herbivore insect occurrence and abundance (community structure) relates to the defensive traits of figs. Ordination of our interaction matrix was constrained by the set of nine defensive (environmental) trait variables measured. Finally, we used nonmetric multidimensional scaling (NMDS) to group *Ficus* species based on the similarity of their insect herbivore communities. Significance of differences in insect herbivore community between *Ficus* life forms was tested using PERMANOVA using 999 random permutations and Euclidean distance. The response variable was the distance matrix derived from the *Ficus*–herbivore interaction matrix, and the explanatory variable was the categorical variable life form.

Comparing the defensive traits of damaged vs. intact leaves

Because our leaf trait measurements were taken from intact leaves, not herbivore-damaged ones, it was important to explore whether the defensive traits we measured can be induced by herbivory. We carried out measurements of leaf defenses using both intact and artificially damaged leaves of *Ficus* species in August 2019. Three individuals per species were selected; six to ten leaves from each individual were collected for measuring leaf traits. During a period of field observations, we designed a collection regime that allowed us to standardize leaf age sampled. Immediately following bud appearance, we marked the developing leaves with labels. During leaf expansion, we left one-half of the leaves of each species intact and injured the others using a hole punch (5 mm diameter) once every 4 h for three times within one day and ensured that the size of the removed area matched with the mean value of natural herbivory observed over wet and dry seasons for each fig species. Following complete expansion, we collected leaves of the intact and damaged groups for measurement of defensive traits using the methods described earlier. We examined the correlation between defenses of intact and damaged leaves using Linear Models (LM) ($n = 36$). Our approach allowed us to establish (1) if relative levels of defense traits in different species are similar whether leaves are damaged or not and (2), for each species, if damaged leaves have levels of defense similar to those measured in intact leaves.

RESULTS

Susceptibility to herbivores

Season and life form significantly affected herbivory (Appendix S1: Fig. S1, Table S3). Feeding intensity on epiphytic figs was significantly lower than on hemi-epiphytic and free-standing figs, while there was no significant difference between the latter two groups. Leaf area lost was greater on leaves collected in the dry season than on those collected in the wet season. However, results of PGLMM (Table 1) showed that sampling time did not contribute significantly to explaining relationships between herbivory and leaf traits. As such, seasonal variation will not be discussed further. We also measured leaf area loss in natural populations in the relatively undisturbed rain forest near XTBG in the wet season. For *F. subulata*, the leaf area lost in natural populations was 0.5–27.3% (mean 8.1%), while this figure was 0.2–15.5% (mean 5.8%) for *F. auriculata*. In the wet

TABLE 1. Contributions of leaf traits and other variables to explaining variation among species in herbivore damage to leaves, using PGLMM analyses (n = 144).

Variables	R^2	P
Leaf traits	0.38	<0.001***
Variation among species	0.16	<0.001***
Sampling times	0.03	0.14
Phylogeny	0.005	0.69

***P < 0.001.

season in our common garden, *F. subulata* suffered leaf area losses of 18.1–21.1% (mean 19.6%), while *F. auriculata* lost 13.4–32.8% (mean 23.1%) of its leaf area. Levels of herbivory of natural populations were lower than plants on the common-garden setting. Furthermore, the percentage of leaf area lost was larger than has been reported from other studies of wild *Ficus* populations in the Australasian region, e.g., Sam et al. (2020) report a maximum value of 6.1%.

Evolution of traits in Ficus

In analyses of each trait, strong phylogenetic signal of individual traits was recovered (Table 2). Latex tannin, SLA, C:N, and leaf tannin all displayed strong phylogenetic signals. Life form also showed strong phylogenetic signal (epiphyte, D = −0.99, P = 0.01 when compared to phylogenetic randomness, P = 0.84 when compared to Brownian threshold model; hemi-epiphyte, D = −0.57, P = 0.00 when compared to phylogenetic randomness, P = 0.90 when compared to Brownian threshold model; free-standing, D = −0.22, P = 0.00 when compared to phylogenetic randomness, P = 0.68 when compared to Brownian threshold model). Strong phylogenetic signal was also detected for PC1, PC2, and herbivore damage (Table 2).

Tests of different evolutionary models showed that latex tannin, C:N, leaf tannin, and SLA followed Brownian-motion or Lambda models of evolution (Fig. 2). These traits also showed different disparities among closely related *Ficus* species in DTT plots (Fig. 3). The DTT plots provide further evidence of different evolutionary histories for leaf traits (Fig. 3). In late-divergent lineages, both latex tannin and SLA are marked by larger disparity (solid line) than expected by 999 neutral evolution simulations (Fig. 3a, d). A slight escalation in latex tannin concentration was also observed along phylogenetic distance (Appendix S1: Fig. S2a). In early-divergent lineages, limited disparities of latex tannin and SLA were observed. The disparity of C:N within lineages was smaller than expected under neutral evolution (Fig. 3b). Results of PERMANOVA showed escalation of C:N (Appendix S1: Fig. S2b). In contrast, leaf tannin displayed high disparity among early-divergent lineages but limited disparity among late-diverging lineages (Fig. 3c). Other traits followed a white-noise model (Fig. 2, Table 2), and high disparities of these traits were detected among late-divergent lineages (i.e., closely related fig species) (Fig. 3e-i).

Using three-way ANOVA, we also found that life form, season and year affected leaf traits (Appendix S1: Table S3). Among the traits, SLA, C:N and latex alkaloid content varied markedly between dry and wet seasons. Almost all of the traits studied differed among different life forms (Appendix S1: Table S3).

Covariation among traits

The observed hypervolume (H_{ob}) was significantly smaller than hypervolumes expected under the first three null models (H_1–H_3), while significantly larger than that expected under H_4 (Fig. 4). The trait hypervolume occupied by figs reflects that not all of the nine traits were normally distributed and that they were not independent from each other, converging towards a relatively small set of frequent trait combinations.

We detected four positive and three negative pairwise correlations among traits of these 36 *Ficus* species with average values across four sampling times (Table 3). Latex dry content was positively correlated with latex alkaloid content, but only when phylogenetic non-independence was accounted for (Pearson's r = −0.01, P = 0.97, PGLS r = 0.35, LR = 4.60, P = 0.03). Latex tannin content was also positively correlated with latex alkaloid content, but again only when phylogenetic non-independence was accounted for (Pearson's r = 0.31, P = 0.06, PGLS r = 0.36, LR = 4.93, P = 0.03). C:N was positively correlated with both toughness (PGLS r = 0.52, LR = 11.58, P = 0.001) and leaf tannin (PGLS r = 0.41, LR = 6.58, P = 0.01). Three negative correlations were also found, between latex dry content and SLA (PGLS r = −0.33, LR = 4.18, P = 0.04), latex dry content and leaf tannin content (but not when phylogenetic non-independence was accounted for; Pearson's r = −0.22, P = 0.19, PGLS r = −0.43, LR = 7.43, P = 0.01) and C:N and trichome density (PGLS r = −0.46, LR = 8.39, P = 0.003). When we considered values from each of the four sample times separately (n = 144), 5 positive and 11 negative pairwise correlations among traits of these 36 *Ficus* species were observed (Appendix S1: Table S4).

Principal components analysis of defenses showed that groups of species formed strong syndromes, including epiphytic figs with high C:N, leaf tannin content, and toughness, free-standing figs with high trichome density, latex alkaloid and latex tannin concentrations, and latex dry content, and hemi-epiphytic figs with high SLA and leaf water content (Fig. 5).

Plant traits that predict resistance to herbivores

We first analyzed the relationship between leaf traits and herbivore damage using mean values for each fig species for each of the four sampling times. Analysis of model residuals demonstrated a lack of phylogenetic

TABLE 2. Selected models of evolution and phylogenetic signal for individual *Ficus* traits measured by Blomberg's *K* and PIC ($n = 36$, mean values across four sampling times).

Trait	Units measured	Model (AIC$_c$)	*K*	PIC mean Observed	PIC mean Randomized	*P*
log(latex dry)	g/cm^2	white	0.23	11.79	8.99	0.89
sqrt(latex tannin)	g/L	white	0.49	92.54	148.57	**0.03***
log(latex alkaloid)	g/L	white	0.51	81.48	117.93	**0.05***
sqrt(leaf water)	%	white	0.28	8.78	7.97	0.73
log(SLA)	cm^2/g	lambda†	0.52	9.10	15.07	**0.02***
log(C:N)		BM	0.74	3.73	8.97	**0.001*****
log(toughness)	g	white	0.37	15.96	19.48	0.26
log(trichome density)	no./mm^2	white	0.39	76.16	96.93	0.25
log(leaf tannin)	%	BM	0.63	33.25	64.19	**0.001*****
sqrt(herbivore damage)	%	lambda‡	0.47	122.97	187.40	**0.04***
PC1		BM	0.84	109.69	279.41	**0.001*****
PC2		BM	0.89	70.67	205.80	**0.001*****

Notes: PIC, phylogenetically independent contrasts; SLA, specific leaf area; sqrt, square-root-transformed; BM, Brownian motion, i.e., the correlation structure among trait values is proportional to the extent of shared ancestry between species; white, white noise, a non-phylogenetic null model; lambda, lambda model, a more complex model of evolution with strong ($\lambda = 1$) to weak ($\lambda = 0$) phylogenetic covariation.

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

†$\lambda = 0.46$.

‡$\lambda = 0.41$.

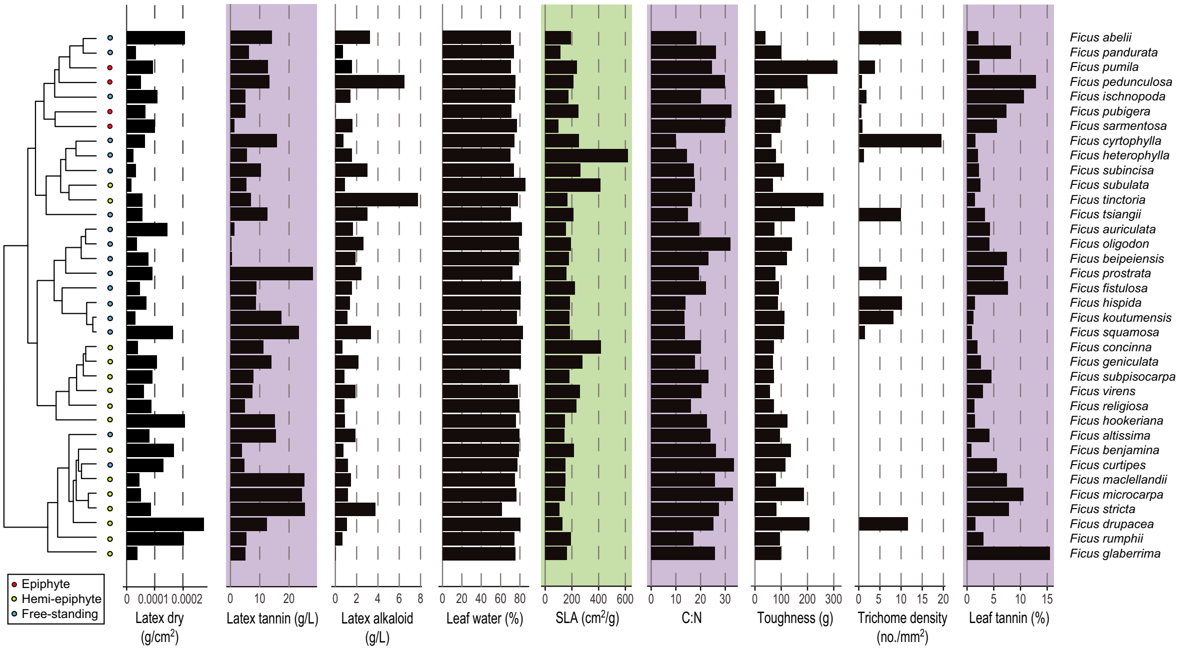

FIG. 2. Distribution of *Ficus* defenses across the phylogeny. Traits following Brownian-motion (purple), Lambda model (green), and white-noise (white) models are differentiated by background color. *Ficus* traits include latex dry (g/cm^2), latex tannin (g/L), latex alkaloid (g/L), leaf water (%), specific leaf area (SLA, cm^2/g), C:N, toughness (g), trichome density (no./mm^2) and leaf tannin (%) ($n = 36$, data are the mean values for four sampling times [two seasons in each of two years]).

signal (Appendix S1: Table S5), the results using the linear models were in broad agreement with those derived from GLMMs and PGLMMs, with strong negative relationships between herbivore damage and latex tannin and between damage and C:N remaining once temporal and phylogenetic effects had been filtered out (Table 4). Life form and leaf toughness also remained as significant explanatory variables in GLMMs. In addition, suites of traits predicted herbivore damage better than single traits, in both linear models and mixed-effects analyses

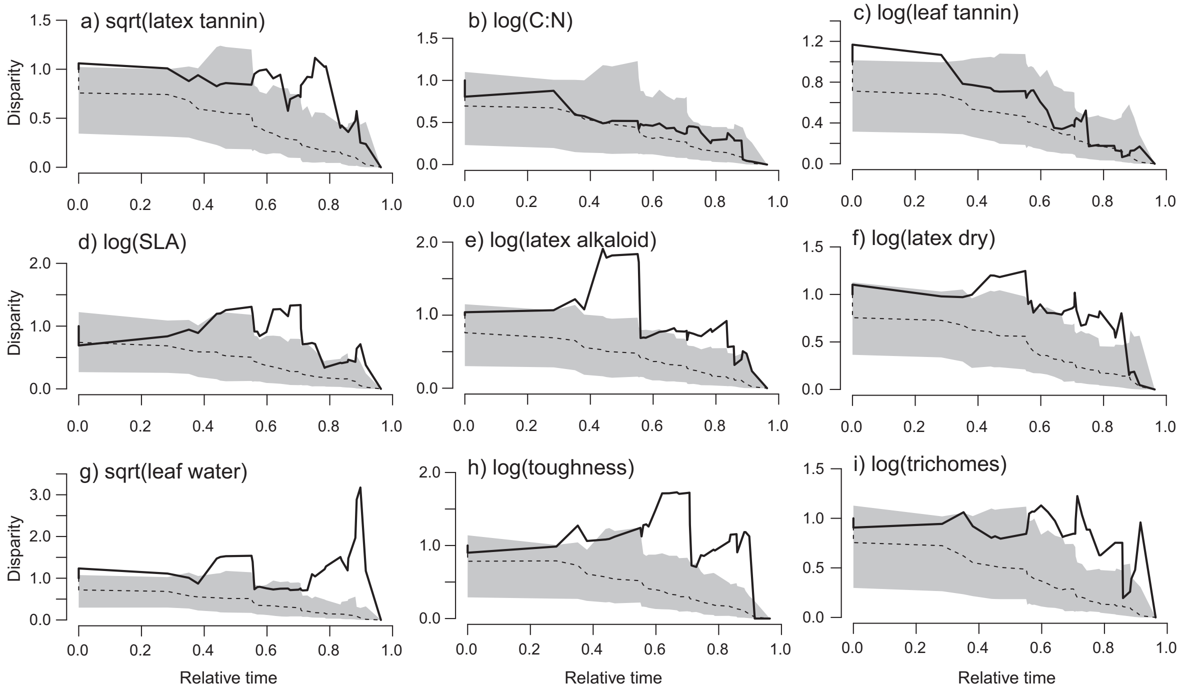

FIG. 3. Mean disparity through time (DTT) for traits with significant effects on herbivore damage (solid line). The dashed line indicates the median DTT based on 999 simulations of character evolution on the phylogeny of the studied *Ficus* species under Brownian motion. The gray-shaded area indicates the 95% confidence interval for the simulated data (n = 36, data are the mean values across four sampling times (two seasons in each of two years)). sqrt, square root.

(Table 5). Leaf traits explained a significant proportion of the variation in herbivore damage ($R^2 = 0.38$, $P < 0.001$), followed by variation among species not explained by the traits we measured ($R^2 = 0.16$, $P < 0.001$). Neither phylogenetic history of figs nor sampling times contributed significantly to explaining variation measured in herbivore damage (Table 1). Phylogenetic signal in model residuals was minimal; as such, the GLMM without a phylogenetic term remains the most powerful analysis.

When we analyzed the relationship between herbivore damage and leaf traits using mean values for each fig species across the four sampling times, high values of latex tannin content and C:N were detected to affect herbivore damage negatively, in both LM and PGLS analyses. Although latex alkaloid content was positively correlated with latex tannin content (high values of which are associated with low herbivory; Appendix S1: Table S6), high values of latex alkaloid content were associated with high herbivory. This might be explained by the importance of specialist herbivores adapted to *Ficus* alkaloids. Linear model analysis confirmed that latex alkaloid content was positively correlated across fig species with the abundance of the specialist herbivore *Asota paliura* ($R^2 = 0.12$, $P = 0.02$, $F = 5.99$, df = 1, 34). Values for PC1 (high C:N) (Appendix S1: Table S6) were predictors of resistance to herbivores in LM analysis, but not in PGLS analysis (Fig. 5, Appendix S1: Table S6).

Ficus–*herbivore interaction network*

The *Ficus*–herbivore network (Fig. 1) was composed of 1,285 insects from 65 morphospecies, with a total of 342 distinct interactions. Among insects, 1,207 (94%) individuals were leaf-chewers and 78 (6%) were leaf-suckers. Leaf-chewers (including 38 Lepidoptera, 20 Coleoptera, and 1 Orthoptera species) represented the principal herbivorous insects of *Ficus* species and inflicted most of the damage. In the communities, the majority of caterpillars were Erebidae (85% of individuals), followed by Noctuidae (10% of individuals) and Crambidae (3% of individuals). Furthermore, 50% of Erebidae individuals were from the genus *Asota*, which is believed to be able to sequester or detoxify alkaloids of *Ficus* species (Sourakov and Emmel 2001). The relatively polyphagous group Crambidae (Novotny et al. 2002) represented 4% of all polyphagous individuals, Erebidae represented 52% of all polyphagous individuals (Erebidae included 49% polyphagous individuals, 50% *Asota* and 1% others); the other generalists included Coleoptera and Noctuidae. Among all of the individual phytophagous insects, 54% (698 individuals, 48 species) fed on free-standing figs, 44% (564 individuals, 39 species) interacted with hemiepiphytic figs, and only 2% (23 individuals, 11 species) fed on epiphytic figs. The abundance of specialist and generalist herbivores differed significantly among fig life forms (Appendix S1: Fig. S4). Of the individual insects feeding

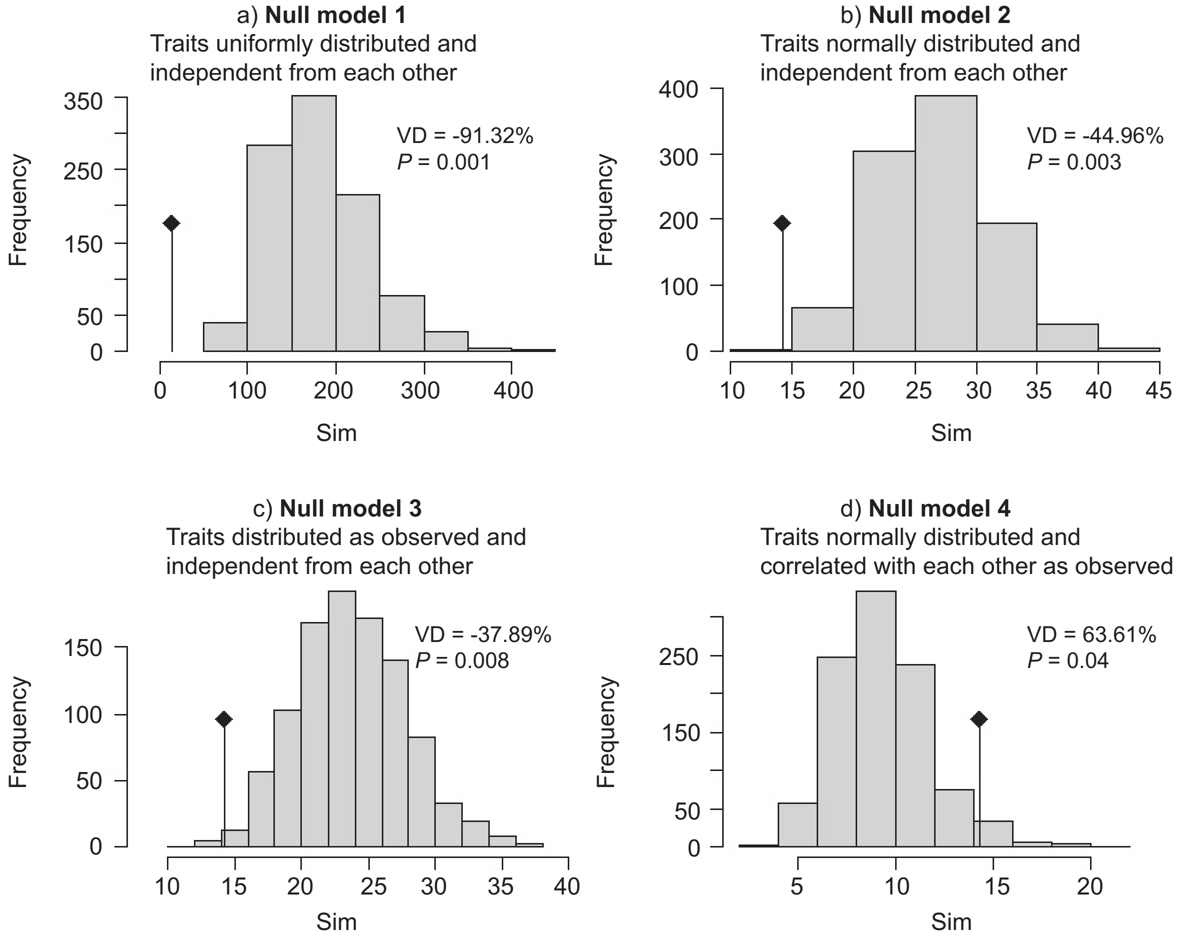

FIG. 4. The volume in trait space occupied by *Ficus* species is constrained compared to theoretical null models. A volume of H_{ob} is less than volume of (a) H_1, (b) H_2, and (c) H_3, but higher than (d) H_4. The hypervolumes are constructed on the basis of log- or square-root-transformed observed values of latex dry, latex tannin, latex alkaloid content, leaf water content, SLA, C:N, toughness, trichome density, and leaf tannin content (H_{ob} the observed hypervolume, indicated by solid diamond), or on the basis of four different null models of multivariate variation of those traits (H_1 to H_4). VD (volume difference) means the difference between H_{ob} and H_1 to H_4 (see *Methods*).

on epiphytic figs, 45% were specialists, which accounted for 30% and 34% of individuals feeding on hemi-epiphytic and free-standing figs, respectively. Several defensive traits were correlated with herbivore community structure (Fig. 6), including trichome density ($F_{1,\ 34} = 2.00$, $P = 0.005$), C:N ($F_{1,\ 34} = 2.08$, $P = 0.01$), leaf toughness ($F_{1,\ 34} = 2.29$, $P = 0.01$), leaf tannin content ($F_{1,\ 34} = 2.09$, $P = 0.02$) and latex alkaloid content ($F_{1,\ 34} = 1.94$, $P = 0.005$). Life form was not a significant predictor of insect herbivore community structure ($F_{2,\ 33} = 0.73$, $P = 0.91$, $R^2 = 0.04$).

Comparing the defensive traits of damaged vs. intact leaves

Levels of all nine defense traits of intact *Ficus* leaves were significantly and positively correlated with those in damaged leaves (Appendix S1: Fig. S3), with all traits showing very similar levels of defense in intact and damaged leaves. The order of species along defense levels was also the same whether leaves are damaged or intact. In addition, the slope of the relationship between defensive traits of intact and damaged leaves ranged from 0.75 to 1.01 (median value was 0.94) while intercepts ranged from −0.93 to 0.17 (median value was 0.06). Coefficients of determination (R^2) of the relationships ranged from 0.74 to 0.99, except for latex dry content ($R^2 = 0.58$). Thus, there was neither large upregulation of defenses in damaged leaves nor differences in “inducibility” among species.

DISCUSSION

Although the concept of “defense syndromes” has been proposed as an evolutionary outcome of arms races between plants and their herbivores for decades

TABLE 3. Correlations among leaf traits of 36 *Ficus* species using mean values across four sampling times (two seasons in each of two years) as measured in a common garden.

Traits	log (latex dry)	sqrt (latex tannin)	log(latex alkaloid)	sqrt (leaf water)	log (SLA)	log (C:N)	log (toughness)	log (trichome)	log (leaf tannin)
log(latex dry)		0.02	−0.01	−0.01	**−0.43****	0.06	−0.00	0.13	−0.22
sqrt(latex tannin)	0.12 (0.52)		0.31	−0.28	−0.12	−0.21	0.02	**0.35***	−0.11
log(latex alkaloid)	**0.35 (4.60)***	**0.36 (4.93)***		−0.03	−0.14	−0.19	0.07	0.12	−0.14
sqrt(leaf water)	0.13 (0.58)	−0.10 (0.38)	−0.01 (0.38)		0.04	−0.01	0.02	−0.17	−0.30
log(SLA)	**−0.33 (4.18)***	−0.05 (0.14)	−0.15 (0.86)	0.08 (0.22)		**−0.36***	−0.19	−0.03	−0.27
log(C:N)	−0.29 (3.14)	−0.31 (3.69)	−0.31 (3.69)	0.13 (0.60)	−0.02 (0.01)		**0.36***	**−0.48****	**0.62*****
log(toughness)	−0.13 (0.66)	−0.09 (0.28)	−0.09 (0.28)	0.23 (2.01)	−0.04 (0.06)	**0.52 (11.60)*****		0.01	−0.01
log(trichome)	0.01 (0.001)	0.20 (1.50)	0.20 (1.50)	−0.22 (1.87)	0.08 (0.26)	**−0.46 (8.39)****	−0.14 (0.68)		−0.31
log(leaf tannin)	**−0.43 (7.43)****	0.12 (0.01)	−0.02 (0.01)	−0.21 (1.69)	−0.14 (0.67)	**0.41 (6.75)****	−0.05 (0.10)	−0.26 (2.61)	

Notes: Shown are Spearman correlation coefficient (above the diagonal), phylogenetic generalized least squares (PGLS) coefficient (below the diagonal) and likelihood ratios (LR, also below the diagonal, in parentheses), calculated as twice the difference in likelihood ratio of the estimated model, and a model where trait covariance is set to zero (n = 36). Boldface type indicates significant difference.

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

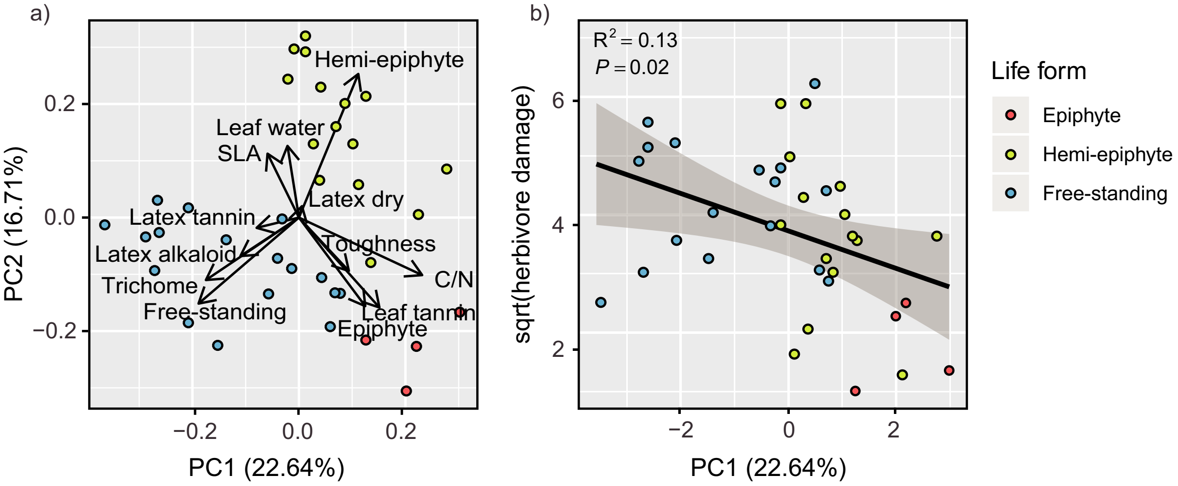

FIG. 5. Correlations between *Ficus* traits, as visualized by (a) the principal components analysis biplot and (b) regressions showing how PC1 predicts resistance to herbivores (herbivore damage was measured as a percentage). The individual points show the species' raw means across four sampling times while the line shows the regression slope from the linear mixed model, with the corresponding statistics shown (each point depicts the mean for a *Ficus* species across four sampling times, n = 36).

(Agrawal and Fishbein 2006), empirical evidence supporting the concept is rare. Using convex hull methods, we showed that only a small subset of all possible trait combinations occurred or were frequent, indicating strong inherent correlations among traits in *Ficus*. These syndromes grouped together fig species with different life forms. Furthermore, these traits combined predicted resistance better than did each trait individually in a common-garden setting where all plant individuals were freely accessible to the herbivore community. We also found strong phylogenetic signal in combination with escalation in defense-related traits, including latex tannin and C:N, suggestive of selection rather than drift. Other traits, in contrast, appeared to have more limited phylogenetic signal or tended to de-escalate. The study therefore suggests that an evolutionary arms race can drive the evolution of defensive syndromes in this tropical genus.

Evolution of Ficus *traits*

In our study, C:N, latex tannin content, leaf tannin content and SLA displayed strong phylogenetic signal among *Ficus* species. Previous studies have suggested the

TABLE 4. Results of LM, GLMM, and PGLMM analyses of the effects of traits on herbivore damage giving model coefficients and significance with fixed effects listed, and random effects being *Ficus* species, season and year for PGLMM (n = 144).

Fixed effect	Estimate	SE	t	z	P
LM					
log(C:N)	−2.24	0.47	−4.82		<0.001***
sqrt(latex tannin)	−0.39	0.10	−3.94		<0.001***
log(trichome density)	−0.36	0.16	−2.31		0.02*
Free-standing	1.33	0.43	3.09		0.01**
Hemi-epiphyte	1.06	0.45	2.38		0.02**
GLMM					
log(C:N)	−1.57	0.53		−2.97	<0.003***
sqrt(latex tannin)	−0.32	0.12		−2.60	0.009**
log(toughness)	−0.77	0.38		−2.03	0.04*
Free-standing	1.41	0.64		2.21	0.03*
PGLMM					
log(C:N)	−1.48	0.53		−2.78	0.01**
sqrt(latex tannin)	−0.31	0.12		−2.55	0.01**

Notes: Only traits for which results of the analysis were significant are shown. LM, linear models; LME, linear mixed-effects models; PGLMM, phylogenetic linear mixed models.
$^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

TABLE 5. Results of LM, GLMM, and PGLMM analyses giving model coefficients and significance with fixed effects listed, and random effects being *Ficus* species, season and year for PGLMM (n = 144).

Formula	R^2	P
LM: Herbivore damage ~ C:N	0.17	<0.001***
LM: Herbivore damage ~ Latex tannin	0.08	<0.001***
LM: Herbivore damage ~ Life form	0.18	<0.001***
LM: Herbivore damage ~ Trichome density	0.01	0.12
LM: Herbivore damage ~ C:N + Latex tannin + Life form + Trichome density	0.40	<0.001***
GLMM: Herbivore damage ~ C:N	0.11	<0.001***
GLMM: Herbivore damage ~ Latex tannin	0.04	0.05*
GLMM: Herbivore damage ~ Life form	0.04	0.04*
GLMM: Herbivore damage ~ Toughness	0.01	0.36
GLMM: Herbivore damage ~ C:N + Latex tannin + Life form + Toughness	0.24	<0.001***
PGLMM: Herbivore damage ~ C:N	0.06	0.02*
PGLMM: Herbivore damage ~ Latex tannin	0.06	0.02*
PGLMM: Herbivore damage ~ C:N + Latex tannin	0.11	<0.001***

Note: For PGLMMs, the additional random effect of phylogenetic covariance was included. Only traits for which results were significant are shown.
$^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

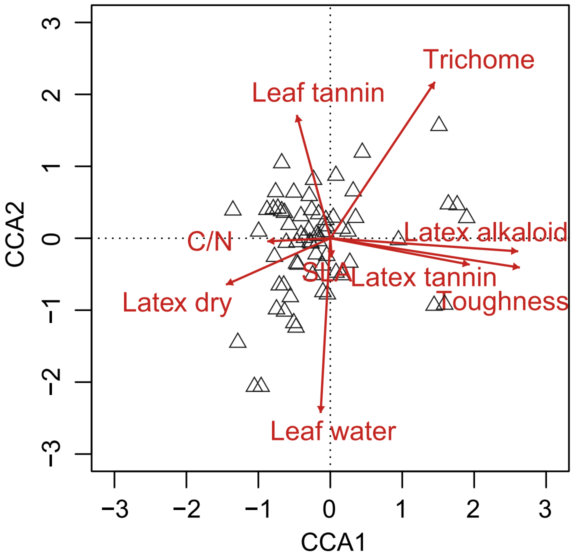

FIG. 6. Correlations between herbivore insect community structure and *Ficus* traits visualized by the Canonical Correlation Analysis biplot (point depicts herbivore insect species and lines represent direction and strength of correlations).

occurrence of macroevolutionary escalation (Farrell et al. 1991, Agrawal and Fishbein 2008, Becerra et al. 2009, Pearse and Hipp 2012) or divergence (Becerra 2007, Kursar et al. 2009, Salazar et al. 2016) of defensive traits. Whether these leaf traits displayed phylogenetic signal depended on the plant lineage studied (Agrawal et al. 2009*b*, Rasmann and Agrawal 2011, Nakadai et al. 2014, Eichenberg et al. 2015, Liu et al. 2015, Volf et al. 2018). The carbon/nitrogen ratio (C:N) is one of the most important indices of leaf quality for herbivores (Agrawal and Fishbein 2006, Mason and Donovan 2015). Our study reports C:N to undergo strong and significant escalation, suggesting that this trait could be particularly important in the evolutionary response of plants to selective pressures exerted by herbivores. A high C:N ratio in leaf tissue slows the development of insects that feed on them (Bryant et al. 1983). Leaf N has been reported to influence insect herbivory and low leaf N contents might negatively affect the preference and performance of insect herbivores (Minkenberg and Ottenheim 1990, Stamp and Casey 1993, Anderson et al. 2004, Kagata and Ohgushi 2012), particularly when combined with carbon-rich fiber and tannins. The importance of leaf N content to insect preference and performance is further indicated by the fact that in some plants, damage to leaves results in reallocation of nutrients, leading to a decrease in N content (Wold and Marquis 1997, Cornelissen and Fernandes 2001).

Secondary metabolites have been shown to have strong phylogenetic signal in some studies (Nakadai et al. 2014, Cárdenas et al. 2014), and we found such a signal for tannin content in latex. However, we did not find latex dry matter content to be evolutionarily conserved, in contrast to findings on *Asclepias* (Agrawal et al. 2009*c*). SLA also displayed a phylogenetic signal in our study. SLA is one of the traits related to resource acquisition (especially for light) and also an important index of leaf quality for herbivores. SLA has been found to be conserved in some plant lineages (Rasmann and Agrawal 2011, Liu et al. 2015) and divergent in others (Agrawal and Fishbein 2006, Agrawal et al. 2009*a*, Nakadai et al. 2014, Volf et al. 2018). Furthermore, our previous study demonstrated that *Ficus* saplings showed interspecific variability in compensatory regrowth after herbivore damage (Zhao and Chen 2012). This is consistent with the hypothesis that plants can either produce abundant defensive chemicals to prevent tissue consumption or invest energy into regrowth after being eaten; but, given energetic limitations, not both (Agrawal and Fishbein 2008, Agrawal et al. 2009*c*, Volf et al. 2018, but see Mesa et al. 2017).

Covariation of traits

Considering analyses of pairwise covariation of individual traits, we encountered four positive and three negative correlations when we considered species' mean values across the four sampling times ($n = 36$). These are fewer than we expected (and fewer than we found when all values from the four sample times were used [$n = 144$]), but these low numbers are in line with results of some other studies. For example, in a global investigation of relationships among four chemical and six physical defenses of 261 plant species, only five of the 45 pairwise correlations between defense traits were significant and three of these correlations were negative, indicating trade-offs (Moles et al. 2013). In another study, which examined potential defense syndromes in 24 species of milkweeds (*Asclepias* spp.) in a field experiment, analysis employing phylogenetically independent contrasts found few correlations between seven defensive traits. No bivariate trade-offs were detected, while two positive correlations were found, between trichome density and latex production and between C:N ratio and leaf toughness (Agrawal and Fishbein 2006). That relatively few correlations and trade-offs were demonstrated might be explained by two main reasons. First, theory that predicts trade-offs between defenses is based on allocation of resources, but different traits may place demands on different resources, and "exchange rates" between different resources are not clear. Traits that place demands on the same resource (e.g., different N-based defenses, or different C-based defenses) may be more likely to show negative correlations than traits using different resources (e.g., N-based vs. C-based defenses). Second, leaf traits that play roles in plant resistance against insect herbivores may have multiple additional functions. For example, trichomes confer protection against herbivores, but also contribute to drought resistance (Ohrui et al. 2007). Defenses may coexist because each has different additional functions. Similarly, defenses may coexist because they have different defensive functions, for example, deterring different types of herbivores or pathogens. Finally, defenses can act synergistically to reduce damage (Agrawal 2007). This could explain the occurrence of positive associations. Conversely, negative associations might be explained not only by resource-allocation trade-offs but also by antagonism (the opposite of synergy) between two kinds of defenses. Several interspecific comparative studies have reported negative correlations between distributions of tannins and alkaloids (Gartlan et al. 1980, Janzen and Waterman 1984, Silvertown and Dodd 1996, Mali and Borges 2003). These might be explained by the formation, when both are present, of insoluble alkaloid tannates that reduce the effectiveness of both kinds of defenses (Janzen and Waterman 1984, Mali and Borges 2003). However, we detected no negative associations between alkaloids and tannins in our study.

The convex hull method analysis indicated that the nine traits we studied are neither totally independent nor identically normally distributed, indicating that inherent correlations among different traits exist in figs. The results of PCA performed in our study (Fig. 5, Appendix S1: Table S7) also showed that species frequently converge on particular combinations of traits. This result was also found in other studies (Agrawal and Fishbein 2006, Travers-Martin and Mueller 2008). In particular, we found that *Ficus* species of different life forms were characterized by different combinations of defensive traits (Fig. 5), supporting the idea that leaf traits are driven by multiple ecological and evolutionary forces (Agrawal 2007).

Defense syndromes as predictors of resistance against herbivores

The defense-syndrome hypothesis predicts that trait combinations should provide better protection against herbivores than any single trait alone. However, relatively few studies have tested this prediction (Agrawal 2011). Although some studies have detected defense syndromes (Agrawal and Fishbein 2006, Travers-Martin and Mueller 2008, Pearse 2011, Moreira et al. 2016, Raffa et al. 2017), few of them have tested the relationship between these syndromes and resistance to herbivores (Agrawal and Fishbein 2006, Travers-Martin and Mueller 2008, Pearse 2011). In this study, suites of traits combined did indeed predict level of herbivory better than individual traits, suggesting that these suites of traits provide better protection than individual traits (Table 5). We identify two important considerations that should be taken into account in studies of defense syndromes. First, studies must be capable of detecting resistance conferred by suites of defensive traits not only against a particular herbivore, but against all herbivores. The function of defense is to reduce herbivory, regardless of its source. Many studies have focused on particular herbivores, sometimes specialists (Travers-Martin and Muller 2008, Haak et al. 2014), sometimes generalists (Mason et al. 2016). Second, defense syndromes are unlikely to exist in the form originally outlined because herbivores are just one selective pressure among many in nature that act on leaf traits. Defense syndromes are likely to reflect adaptation to the great diversity of environmental challenges, both abiotic and biotic, that plants face (Janzen 1980, Strauss et al. 2005). As demonstrated by our GLMM and PGLMM results, variation in the defense traits we studied explains only a part of interspecific variation in plant resistance to herbivores ($R^2 = 0.38$ for level of herbivory). There must exist other important traits affecting resistance that were not included in our study. Additional unmeasured defenses might include protease activity, oxidative activity and alkaloid diversity, which have been confirmed to be correlated with the community structure of herbivorous insects (Volf et al. 2018). In the present study, latex alkaloid content was positively correlated with herbivore damage (Appendix S1: Table S6), implying that latex alkaloid might be an ineffective trait. Intriguingly, alkaloid-rich *Ficus* species can host distinct insect communities (Volf et al. 2018), and the abundance across *Ficus* species of one *Asota* species studied here was positively correlated with latex alkaloid content.

Interestingly, there was variation among the three life forms of figs both in herbivore damage to their leaves (Appendix S1: Fig. S1, Table S3) and in the combinations of defensive traits of their leaves. Epiphytic figs suffered lower herbivore damage compared to hemi-epiphytic and free-standing *Ficus* species. In our study, leaves of epiphytic species possessed significantly higher C:N and leaf tannin content and were tougher than leaves of other figs (Fig. 5). Free-standing fig species displayed high trichome density, along with high concentrations of tannin and alkaloids in their latex, whereas hemi-epiphytic figs had higher leaf water content and SLA. Similar results were found for these two life forms in *Ficus* spp. in Papua New Guinea (Volf et al. 2018). These results imply that different life forms of *Ficus* have evolved different defensive strategies against the multiple selective pressures imposed by the biotic and abiotic environment (also see Agrawal and Fishbein 2006). Epiphytic figs, with no access to nutrients and water in soil, face limited resource availability compared to hemi-epiphytic and free-standing figs and appear to have the most effective (and probably costliest) defenses against herbivores, consistent with resource-availability theory (Coley et al. 1985, Fine et al. 2006). By comparison, hemi-epiphytic and free-standing figs appear to have less effective defenses and suffer more damage from herbivorous insects. Tolerance to herbivory (not measured here) might be an available strategy for these figs, congruent with the compensatory regrowth hypothesis (Strauss and Agrawal 1999, Stowe et al. 2000). We also detected linear and tight positive correlations, with slopes differing little from 1 and intercepts differing little from 0, between the defenses of intact and damaged leaves. This is highly suggestive of only small induced responses in our focal traits for the *Ficus* species we studied (we note that volatile organic compounds were not collected during our study). We recorded higher levels of herbivory within our common-garden setting than in natural populations (especially for *F. auriculata*), perhaps driven by higher densities of available hosts or a simpler landscape. While the magnitude of the effects measured may thus have been larger than in natural populations, it is doubtful as to whether this increased rate of herbivory influenced the general conclusions of our study.

Ficus*–herbivore interaction network*

Our study demonstrated that epiphytic figs interacted with fewer herbivorous insects than hemi-epiphytic and free-standing figs (Appendix S1: Fig. S4), and that herbivores of epiphytic figs tended to be more specialized. The diverse set of pressures imposed by species-rich communities of insect herbivores is believed to select for variability in plant defenses (Ehrlich and Raven 1964, Agrawal and Fishbein 2006). Correspondingly, the community structure of insect herbivores is driven by plant defenses (Volf et al. 2018). In our study, epiphytic figs had nutrient-poor leaves that were tough and rich in tannins while free-standing trees tended to have leaves covered in trichomes and full of alkaloid-rich latex. Our results imply that generalists might be deterred by the high tannin levels (or by other, unmeasured, defensive traits) of epiphytic figs, resulting in lower damage levels compared to hemi-epiphytic and free-standing figs. However, while levels of resistance varied, there was no overall difference in insect community structure between each life form. We surveyed herbivore communities for only one season. Longer-term investigation of the structure of the herbivore community might improve our understanding of the roles of different evolutionary processes in generating the interaction network between *Ficus* and insects. Our modest data set was, however, sufficient to reveal that traits with distinct evolutionary trajectories shaped insect herbivore communities. For example, both escalating (C:N) and divergent (leaf tannin) traits explained significant proportions of variance in insect community structure. In agreement with Volf et al. (2018), we also found a role for latex alkaloids and trichome density in predicting herbivore community composition.

In conclusion, our results provide some support for the concept of coordinated defense syndromes and demonstrate that one suite of traits (latex tannin content and C:N) was a better predictor of resistance against herbivore insects than were individual leaf traits taken singly. Latex tannin content and C:N both followed the Brownian-motion model of evolution. Although latex tannin showed some disparity among late-divergent lineages, whereas C:N showed consistent escalation along the phylogeny, we suggest that this trait combination could have been driven by evolutionary arms races with herbivores. *Ficus*, as one of the most species-rich pantropical plant genera (Lewinsohn et al. 2005), represents an excellent model system for exploring the assembly of rich insect–plant food webs. Focusing on this species-rich system may further shed light on the role of abiotic/biotic factors in generating the astonishing diversity of plant defense traits.

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Supporting Information

Additional supporting information may be found online at: http://onlinelibrary.wiley.com/doi/10.1002/ecm.1428/full

Data Availability

Data are publicly available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.5dv41ns42.