ECOLOGY LETTERS

Ecology Letters, (2018) **21**: 83–92 doi: 10.1111/ele.12875

LETTER

Community structure of insect herbivores is driven by conservatism, escalation and divergence of defensive traits in *Ficus*

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Abstract

Escalation (macroevolutionary increase) or divergence (disparity between relatives) in trait values are two frequent outcomes of the plant-herbivore arms race. We studied the defences and caterpillars associated with 21 sympatric New Guinean figs. Herbivore generalists were concentrated on hosts with low protease and oxidative activity. The distribution of specialists correlated with phylogeny, protease and trichomes. Additionally, highly specialised *Asota* moths used alkaloid rich plants. The evolution of proteases was conserved, alkaloid diversity has escalated across the studied species, oxidative activity has escalated within one clade, and trichomes have diverged across the phylogeny. Herbivore specificity correlated with their response to host defences: escalating traits largely affected generalists and divergent traits specialists; but the effect of escalating traits on extreme specialists was positive. In turn, the evolution of defences in *Ficus* can be driven towards both escalation and divergence in individual traits, in combination providing protection against a broad spectrum of herbivores.

Keywords

Alkaloids, Choreutidae, coevolution, cysteine protease, herbivore, Lepidoptera, New Guinea, polyphenols, Pyraloidea, trichomes.

Ecology Letters (2018) **21**: 83–92

INTRODUCTION

Insect-plant arms races have been suggested to support diversification and escalation of plant defences (Ehrlich & Raven 1964), resulting in a directional trend for increased anti-herbivore traits during the macroevolution of a lineage (Agrawal *et al.* 2008). In turn, traits should escalate across plant clades (more derived lineages should have more potent defences), with trait values positively correlating with phylogenetic distance from the root, and/or phylogenetic dissimilarity between species. Such an escalation of host-plant defences has been found in several plant genera (Agrawal *et al.* 2008; Becerra *et al.* 2009; Pearse & Hipp 2012).

However, a range of alternative trends exist (e.g. Kursar *et al.* 2009; Pearse & Hipp 2012; Cacho *et al.* 2015; Salazar *et al.* 2016). For example, a decrease in chemical complexity occurs in milkweed cardenolides, which are probably now ineffective against specialised herbivores (Agrawal *et al.* 2008). Divergent defences (traits more dissimilar between close relatives than expected under a conserved model of evolution) have been found in sympatric communities of closely related hosts. It has been suggested that insect herbivores impose divergent selection, resulting in increased chemical disparity (Becerra 2007; Kursar *et al.* 2009; Salazar *et al.* 2016). Such an increase in trait disparity between sympatric congeners should facilitate escape from shared herbivores with conservative host-use (Becerra 2007; Kursar *et al.* 2009; Salazar *et al.* 2016; Sedio *et al.* 2017).

The macroevolution of a given trait is likely to depend both on the ability of the trait to deter herbivores and its metabolic flexibility (Wink 2003). Consistently effective traits may be conserved, or even escalate over time, such that they have a large effect on non-adapted herbivores, while divergent traits are harder for specialists to circumvent. Generalist herbivores

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can consume multiple hosts, at the cost of being maladapted to potent defences (Bernays & Chapman 2007), while specialists often track host phylogeny and adapt to such defences. The composition of insect communities attacking the host is therefore key – assemblages of specialists should select mainly for divergent traits (e.g. Becerra 2007), whereas assemblages of generalists, sensitive to specialised defences, should impose selection for escalating traits.

In response to variability in herbivore pressure between guilds and across the specialisation continuum, plant defensive syndromes consist of suites of complementary traits, as found in *Asclepias* (Agrawal & Fishbein 2006). In *Asclepias* these syndromes are shaped by both shared evolutionary ancestry and herbivore driven adaptive convergence. Mixing and matching defences over evolutionary time can allow plants to evade the current community of herbivores (Agrawal & Fishbein 2006; Janz 2011). Such evolutionary processes should result in an oscillating equilibrium between diverging and escalating defences.

Rainforest assemblages of *Ficus* represent an excellent model system for exploring such evolutionary processes. This pantropical genus is extraordinarily speciose (over 750 species). The paleotropics are particularly diverse, with over 150 species found in Papua New Guinea (PNG), the global centre of *Ficus* diversity (Berg & Corner 2005; Cruaud *et al.* 2012). *Ficus* can comprise ~15% of all stems with DBH (diameter at breast height) ≥ 5 cm, in both primary and secondary lowland forests in PNG (Whitfeld *et al.* 2012). The genus *Ficus* also supports diverse insect communities, including many herbivores which are lineage specialists (Basset & Novotny 1999; Novotny *et al.* 2010).

Over the course of ~75 MY (Cruaud *et al.* 2012) *Ficus* has acquired a broad range of chemical and physical defences. These include 'universal' traits, such as polyphenols, terpenoids and trichomes. Most *Ficus* species also produce latex that serves as a physical defence, as well as a vessel for more taxonomically restricted chemical defences. These specialised defences include phenanthroindolizidine alkaloids (Damu *et al.* 2005) and cysteine proteases (Konno *et al.* 2004). Among these defences, cysteine proteases likely play a prominent role, as they interfere with insect digestion and increase larval mortality (Konno *et al.* 2004). These traits show considerable interspecific variation, making *Ficus* a promising model for testing evolutionary trends in host plant defences.

Here, we focus on 21 sympatric New Guinean rainforest *Ficus* species. This community approach allows us to relate *Ficus* traits to local insect communities. First, we identify the *Ficus* defences which correlate with communities of leaf-chewing larvae, and analyse whether these correlations hold consistently across herbivores with a range of host specificity. Second, we analyse the evolutionary patterns in these defences and test whether they are conserved, escalate over evolutionary time, or are divergent among closely related species. We predict that: (I) defences in this speciose system will show a range of evolutionary histories in response to different selective pressures; (II) generalist insect community structure will correlate mainly with escalating defences, while the structure of specialist insect communities will relate to diverging defences; and (III) traits with different anti-herbivore roles will be independent or positively correlated, and form distinctive defensive syndromes, combining various evolutionary histories (Agrawal & Fishbein 2006).

We suggest that insect ecology is a key element when interpreting the evolution of host-plant defences, as escalating and diverging defences likely have different correlations with specialist and generalist herbivores. Here, we relate communities of generalist and specialist insects to defensive traits. We expect the evolution of plant defensive traits to be varied, with few trade-offs and a range of macroevolutionary dynamics. It is important to recognise that insect-herbivore interactions are reciprocal, and while 'bottom up' effects can determine host-use by insect herbivores, insects themselves are a key selective pressure (Marquis *et al.* 2016). In summary, we do not expect that the defences of plants and their herbivorous assemblages could be explained by a single evolutionary mechanism in speciose systems, such as tropical rainforests.

METHODS

Ficus traits

We measured both specialised and generalised chemical and physical defences in *Ficus*: cysteine protease activity, alkaloid content, alkaloid diversity, polyphenol content, polyphenol diversity, polyphenol oxidative activity, polyphenol protein precipitation capacity, triterpene content, triterpene diversity, trichome density and trichome length. We also measured resource acquisition traits correlating with leaf quality: specific leaf area (SLA) and C:N (Fig. 1). The sampling was carried out within a matrix of primary and secondary forest in a 10 × 20 km area around Madang in Ohu and Baitabag villages (PNG), sampled also for insect herbivores by Novotny *et al.* (2010). We sampled the 19 *Ficus* species surveyed by Novotny *et al.* (2010) for insect herbivores, along with two additional species lacking detailed insect data (Table S1). We avoided trees with high rates of herbivory, signs of pathogen infection or physical damage and maintained > 10 m distance between trees, avoiding obviously clonal individuals. We sampled up to five individuals per species for all traits. The sampling included the subgenus *Sycomorus*, which has radiated in PNG and represents a large component of local *Ficus* diversity. The study also includes species from its sister sections, and more distant relatives, representing most sections of *Ficus* occurring in the Australasian region.

For the analysis of protease activity, we sampled latex by cutting the main vein of each leaf and letting latex flow into a 2 mL collection tube for 30 s. Protease activity was analysed using a modified version of the methods of Konno *et al.* (2004). Alkaloids and polyphenols were extracted using acetone and aqueous acetone from ca 0.5 g of the *Ficus* leaf tissue. Alkaloid quantification (area of peak/mg) was obtained with non-targeted UPLC-DAD-Orbitrap-MS analysis (Table S2). The main polyphenol sub-groups were quantified (as mg/g) with UPLC-QqQ-MS/MS as detailed in Engström *et al.* (2014, 2015). Furthermore, we measured polyphenol oxidative activity, following Salminen & Karonen (2011), and protein precipitation capacity, following Hagerman's RDA method (Hagerman & Butler 1978), as the two major

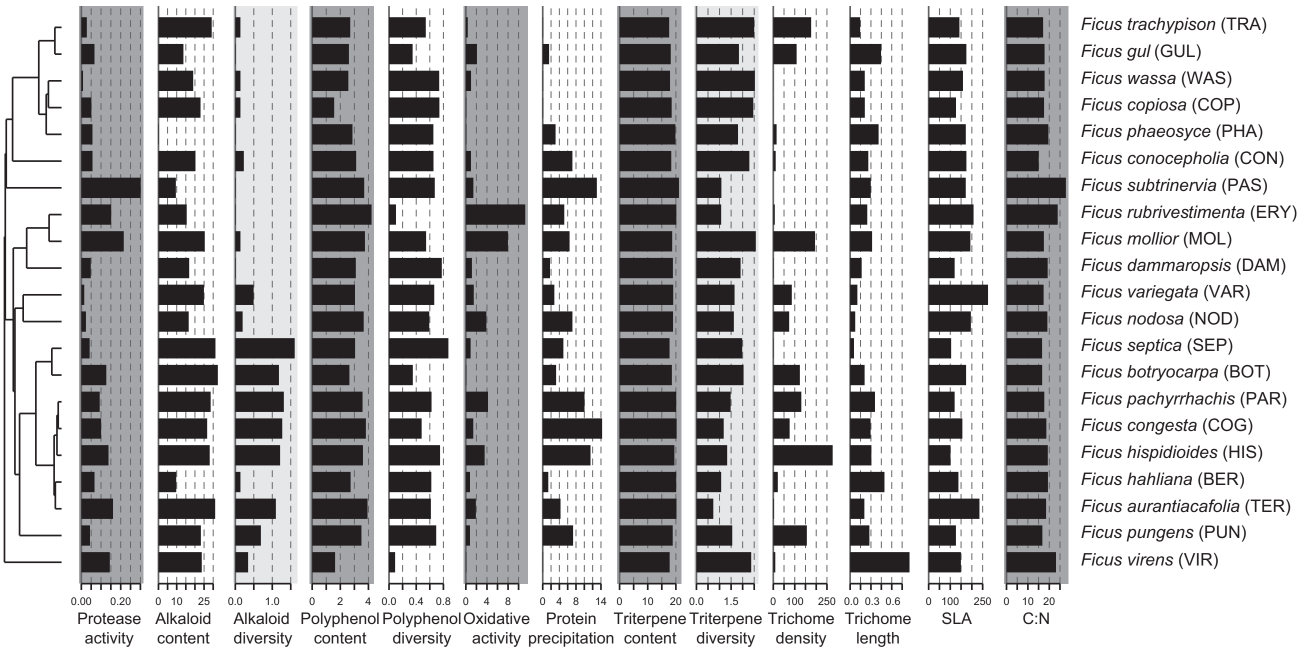

Figure 1 Distribution of *Ficus* defences across the phylogeny. Traits following Brownian motion (dark grey), Lambda model of evolution (light grey), and white noise (white) are differentiated by background colour. *Ficus* traits include protease activity in latex (ΔA280), alkaloid content (ln(peak area/mg)), alkaloid diversity (Shannon), polyphenol content (mg/g), polyphenol diversity (Shannon), polyphenol oxidative activity (mg/g), protein precipitation capacity (mg/g), triterpene content (ln(peak area/mg)), triterpene diversity (Shannon), trichome density (number of trichomes per 10 mm^2), trichome length (mm), C:N, and specific leaf area (cm^2/g).

functions of polyphenols in anti-herbivore protection. Low polarity terpenoids were extracted from ca 0.5 g of the *Ficus* leaf tissue using methanol. Terpenoid quantification (area of peak/mg) was obtained with HPLC-Orbitrap Q-Exactive HRMS equipped with atmospheric chemical ionisation (APCI) (Table S3). Chemical diversity was quantified by Shannon diversity indexes for alkaloids, polyphenols and triterpenes. Triterpene diversity was based on the content of individual compounds. Alkaloid and polyphenol diversities, for which more detailed structural data were available, were calculated based on the content of major structural groups to account for structural diversity, rather than for the number of compounds in a sample (see Table S4 for more details).

The total number of trichomes per 10 mm^2 and their average length was measured on five leaf discs per individual, avoiding the central vein. Values for dorsal and ventral sides of the discs were averaged. SLA was measured as the area per mass using twenty dried leaf discs which were cut avoiding the central vein. Total carbon and nitrogen content were determined by dry combustion using ca 0.45 g of homogenised dry leaf material.

See Appendix S1 for more details on the trait measurements and chemical analyses.

Insect data

The insect data were taken from Novotny *et al.* (2010) (Table S1). The data include only reared individuals, with host associations confirmed by feeding trials, sampled from 1500 m^2 of leaf area per plant species. We focused on leaf-chewing larvae (including 122 Lepidoptera and two Coleoptera species) as a guild that is well represented on our focal *Ficus* species, and which inflicts a large amount of damage. We conducted additional analyses to compare the two dominant microlepidopteran taxa, which represented the majority of caterpillars in the focal communities: Pyraloidea (31% of all caterpillar individuals), a relatively polyphagous group feeding on several plant taxa, and Choreutidae (45% of all caterpillar individuals), which are mostly specialists of Moraceae in our community (Novotny *et al.* 2002). We included recent taxonomic revisions for Choreutidae (Table S1). Singleton species were removed from all statistical analyses. The residual insect community comprised several (super) families, with Noctuoidea (11%) and Tortricidae (10%) being the most abundant. We note that 84% of all noctuoid individuals are in the brightly coloured genus *Asota* (largely restricted to *Ficus*), a specialist genus potentially capable of alkaloid sequestration (Sourakov & Emmel 2001). We separated *Asota* in a subset of our analyses.

Ficus phylogeny reconstruction

The host-plant phylogeny was estimated using four loci: ITS, ETS, G3PD and GBSSI. We used sequences from Cruaud *et al.* (2012) when available. We obtained the sequences of missing species using dried leaf tissue following Cruaud *et al.* (2012). The host-plant phylogeny was reconstructed using Bayesian inference as implemented in BEAST v2.1.3 (Drummond *et al.* 2012), with section level constraints taken from Cruaud *et al.* (2012). Furthermore, for section *Sycocarpus* we used constraints based on microsatellite data using Nei's distance neighbour joining trees, based on nine microsatellite loci previously published for the genus *Ficus* (Moe & Weiblen 2011; Garcia *et al*. 2012). See Appendix S1 for details.

Ficus traits and insect communities

To test the hypothesis that *Ficus* species form distinct groups with respect to their defensive traits, we clustered them using Ward's method with Euclidean distances as implemented in the 'pvclust 2.0' R package (Suzuki & Shimodaira 2015). The optimal number of clusters was selected using BIC (Bayesian information criterion). The key traits for defining these clusters were identified using a classification tree analysis in the R package 'rpart' (Therneau *et al*. 2017). All secondary metabolite contents were log transformed. The data were centred and standardised and the results were visualised using principal component analysis (PCA) in CANOCO 5 (Ter Braak & Smilauer 2012). Additionally, we analysed correlations between traits in a phylogenetic context using Phylogenetic Least Squares Regression (PGLS) in the R package 'caper' (Orme *et al*. 2013). PGLS analysis allowed us to identify whether there are any indications of trade-offs between the traits significantly correlated with insect community structure.

To test the hypothesis that defensive and resource acquisition traits correlated with insect community structure, we analysed the relationships of *Ficus* traits and phylogeny with larval leaf-chewer communities using canonical correspondence analysis (CCA). We used species means of traits as explanatory variables, and identified those with a significant correlation with insect communities by forward selection. Phylogenetic similarity is often an integrator for trait similarity. We therefore assessed the explanatory power of both phylogeny and its covariance with traits to explain the residual variance not captured by our traits. Specifically, we ran variance partitioning analysis with the selected *Ficus* traits and significant phylogenetic axes, derived from the ultrametric tree using principal coordinate analysis (PCoA), to identify the proportion of variability in insect data explained by traits, phylogeny, and their covariation. All insect data were log-transformed. We down weighted rare species and used adjusted explained variability (Ter Braak & Smilauer 2012). To test our hypothesis that host specialisation may determine which traits had explanatory power, we ran separate analyses for the whole larval leaf-chewer community, generalist Pyraloidea, and *Ficus* specialised Choreutidae.

The ability of methods relying on a limited number of eigenvectors to include complex phylogenetic structure and model trait evolution has been criticised (Freckleton *et al*. 2011). We therefore used two additional approaches to test whether traits affected insect diversity (i.e. presence of species) and abundance. First, we used both standard binomial Generalised Linear Mixed Models (GLMM) and binomial Phylogenetic Linear Mixed Models (PGLMM) (Ives & Helmus 2011) to correlate insect presence (response variable) with defensive traits (fixed explanatory variable), while including insect and *Ficus* species identities as random effects. We included phylogenetic covariation as an additional random effect in the PGLMM's. We used R package 'pez' (Pearse *et al*. 2015) to construct PGLMM's (models were fitted using restricted maximum likelihood). We excluded all species with less than ten individuals from our binomial mixed effect models to limit the effect of rare species on the analysis, and restricted this analysis to the whole leaf-chewer community.

Second, the relationships between plant traits and caterpillar abundance were tested using PGLS. We controlled for phylogenetic non-independence of *Ficus* species, but note that a trait's value in defending against herbivores is not diminished by it being phylogenetically conserved (Agrawal 2007). Because traits evolve in different ways we fitted the most appropriate branch length transformation. In cases where traits followed Brownian motion, we used the 'corBrownian' correlation structure in GLS models. In cases where more complicated branch length transformations were required, we selected the parameter value of the transformation using maximum likelihood as implemented in the R package 'caper' (Orme *et al*. 2013), using the transformation as selected by AICc. For traits where a non-phylogenetic white noise model fitted best, we used GLS models without any correlation structure. We had a strong *a priori* reason to expect a correlation between alkaloid diversity and *Asota* abundance, and conducted an additional PGLS analysis to test this hypothesis.

Evolution of *Ficus* traits

Initially, we tested for phylogenetic signal in our traits using Blomberg's K (a widely used metric) and a randomisation test based on Phylogenetic Independent Contrasts in the R package 'Phylosignal' (Keck *et al*. 2016). Phylogenetic signal is widely used in studies of trait distribution, and therefore provides connectivity, but it lacks the power to detect and distinguish between certain evolutionary processes. As such we test directly for divergence, trait conservatism, and finally escalation.

Herbivore pressure can be a key selective agent, and we tested the hypothesis that it has led to overall divergence in trait values in our community. While conserved traits (i) generally follow a model of Brownian motion and (ii) have a more or less constant rate of change across the phylogeny, divergent traits exhibit a dramatic increase in trait disparity at the tip. We therefore tested if individual traits followed a set of standard macroevolutionary models, by selecting and fitting models of evolution for each trait across the phylogeny. We fitted the following models: 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 (the data come from a normal distribution with no covariance structure among species), and Pagel's lambda – allowing a more complex model of evolution with strong (lambda = 1) to weak (lambda = 0) phylogenetic covariation. The models were implemented using the 'fitContinous' function in the R package 'Geiger' (Harmon *et al*. 2008). We used the default bounds for each model, and compared the models using their AICc weights. To further examine the evolution of individual traits through time (e.g. if they diverged at the tips or followed Brownian motion), we plotted the values of trait disparity through time (DTT) from the root to tips using the function 'dtt' in the R package 'Geiger' (Harmon *et al*. 2008). The advantage of DTT analyses is that they not only detect significant deviations from Brownian motion, but reveal the depth in the tree at which divergence occurs. 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.

To test the hypothesis that herbivores may drive some traits to increase in value across the *Ficus* phylogeny, we tested for escalation in trait values across the whole phylogeny and within subclades. We tested for correlation between phylogenetic distance among plant species and trait values using linear models. First, we used Permutational Multivariate Analysis of Variance (PERMANOVA) and a patristic distance matrix derived from the host phylogeny, as implemented in the function 'adonis' in the R package 'vegan' (Oksanen *et al.* 2017). We included the distance matrix as the response variable and the trait values as the explanatory variables, used 999 permutations and selected significant variables using forward selection. An increase in explanatory power with phylogenetic distance between species suggests overall escalation. Increases in explanatory power are detectable through increased sum of squares contributions at the species level, detecting local escalation within clades. Second, we used linear models to test for general directional changes in trait values from the root of the tree, by correlating Abouheif's distance (distance from the root) with trait values, as calculated in the R package 'adephylo' (Jombart *et al.* 2010).

RESULTS

Ficus traits and insect communities

Most *Ficus* traits showed high interspecific variability (Fig. 1, Table S4). Cluster analysis revealed three major clusters based on their traits: (i) high polyphenol content and polyphenol activities, (ii) high protease activity, and (iii) mixed defences with low polyphenols (Fig. 2, Fig. S1). These clusters were mirrored by insect communities, with species from clusters (i) and (ii) harbouring distinct assemblages (Fig. 2). Individual defences were generally independent once phylogenetic non-independence was controlled for by PGLS, and the only significant correlation between traits relevant to insect community structure was a negative correlation between alkaloid diversity and trichome length ($t_{19,1} = -2.56$, $P = 0.019$).

Multivariate analyses revealed that protease activity in latex, polyphenol oxidative activity, trichome length, and alkaloid diversity significantly correlated with overall community structure (Table 1, Fig. 2). Protease activity in latex and trichome density correlated with choreutid community structure, and protease activity in latex and polyphenol oxidative activity correlated with pyraloid community structure. Variance partitioning revealed that traits explained a significant amount of the variance in community structure for all comparisons apart from choreutids, while phylogeny was a consistently significant explanatory variable in all cases (Table 1, Fig. S2).

The results using binary occurrence of insect species were in broad agreement with the multivariate analyses (Table 2), with the strong negative correlation between protease activity and herbivore occurrence remaining once phylogenetic non-independence had been filtered out. Non-phylogenetic analyses also revealed a negative correlation between oxidative activity and herbivore occurrence that was not detected in PGLMM's. In contrast to our multivariate analyses, mixed effect models uncovered a positive relationship between both triterpene and polyphenol diversity and insect occurrence, with the latter correlation remaining in phylogenetically controlled analyses.

PGLS analyses for the whole larval leaf-chewer community showed that only protease activity had a significant negative relationship with larval leaf-chewer abundance ($t_{17,1} = -2.86$, $P = 0.011$). However, there was a strong positive correlation between the abundance of *Asota* individuals and alkaloid diversity ($t_{17,1} = 3.90$, $P = 0.001$).

Evolution of *Ficus* traits

The chemical traits having a significant correlation with insect communities, including protease activity, alkaloid diversity, and polyphenol oxidative activity showed phylogenetic signal when analysed using Blomberg's K and PICs (Table 3). They followed Brownian motion or Lambda models of evolution, and showed limited disparity among closely related *Ficus* species in DTT plots (Fig. 3). On the other hand, trichome density and length followed a white noise model of evolution and showed high disparity among closely related species of *Ficus* (Fig. 3, Table 3). The non-significant traits (according to CCA) followed various models of evolution (Fig. S3).

Among the traits that correlated with insect community structure, we found significant trait escalation in the case of alkaloid diversity ($F = 21.43$, $P < 0.001$, $R^2 = 0.49$) and polyphenol oxidative activity ($F = 4.43$, $P = 0.034$, $R^2 = 0.10$) in the PERMANOVA analyses. Alkaloid diversity escalated from the root towards the terminal clade of section *Sycocarpus*. Polyphenol oxidative activity escalated slightly within section *Sycidium* and significantly in *Adenosperma* (see Table S5 for details). None of the other traits showed local or general escalation. Tests of escalation using Abouheif's distance from root to terminal clades confirmed a strong positive correlation between alkaloid diversity and distance from the root ($F_{19,1} = 14.10$, $P = 0.001$, $R^2 = 0.32$) while more limited escalation of oxidative activity (restricted to two clades) was non-significant in a general context ($F_{19,1} = 0.001$, $P = 0.969$, $R^2 < 0.01$; Fig. S4). There was no significant correlation with distance from the root for any of the other traits.

DISCUSSION

Previous studies have suggested macroevolutionary escalation (Agrawal *et al.* 2008; Becerra *et al.* 2009; Pearse & Hipp 2012) or divergence (Becerra 2007; Kursar *et al.* 2009; Salazar *et al.* 2016) of defensive traits. Here, we propose (Hypothesis I) that defensive traits in large plant genera show a range of evolutionary histories, which are strongly dependent on the selective pressures exerted by the insects attacking them. In the case of the focal *Ficus* species, some traits were phylogenetically conserved, others escalated globally or within clades and others diverged between close relatives. Such variability in the evolutionary history of individual defences is expected in species-rich communities, reflecting the myriad selective pressures imposed

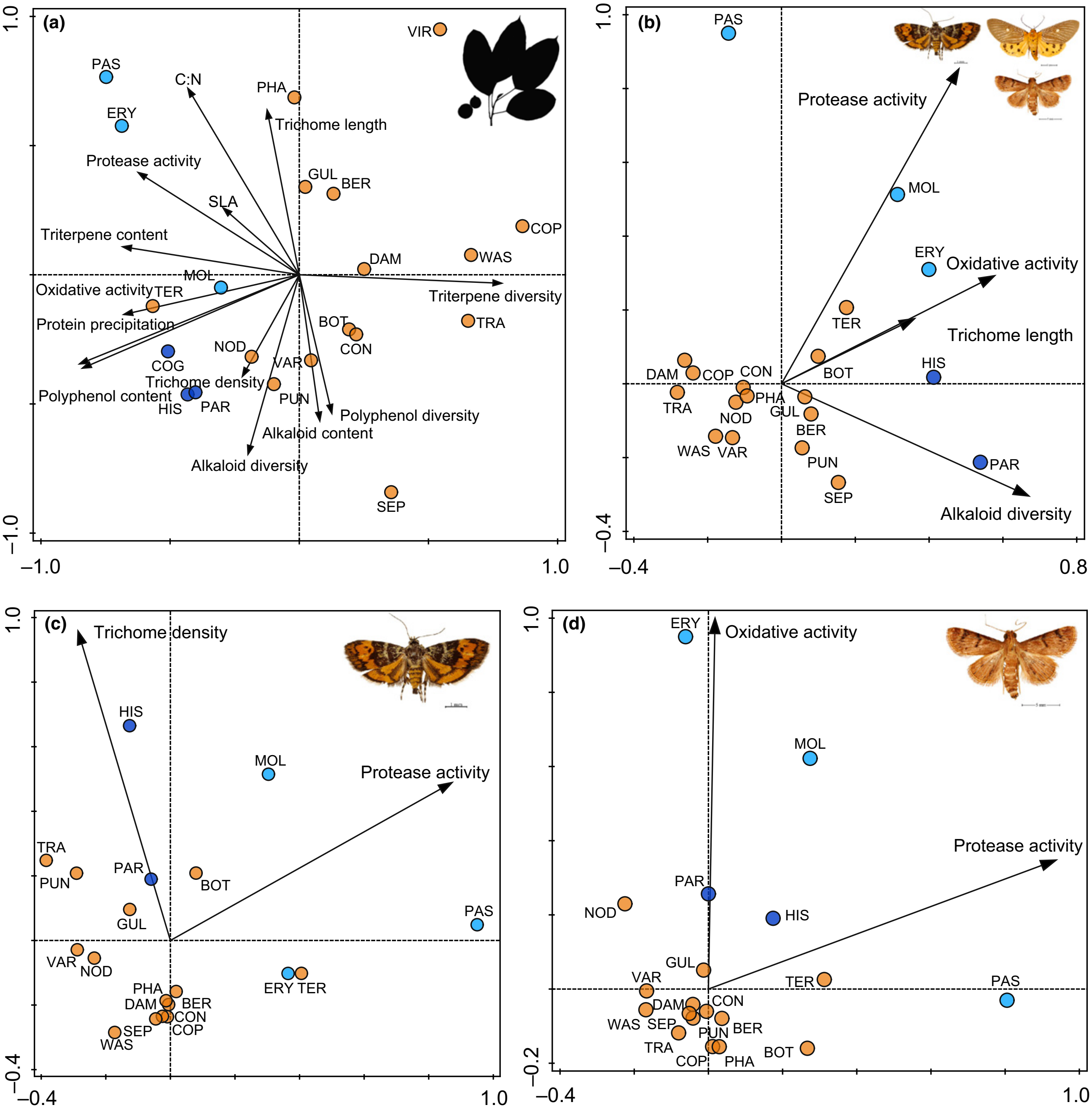

Figure 2 Correlation between *Ficus* traits (a) and response of the whole larval leaf-chewer community (b), Choreutidae (c), and Pyraloidea (d) to host-plant traits. The correlation between *Ficus* traits was visualised by a PCA biplot showing *Ficus* defences and individual *Ficus* species. First two PCA axes explained 47.9% of variability. The clusters of *Ficus* species with distinctive defences recovered using Ward's method with Euclidean distances are colour coded – (i) high polyphenol content and polyphenol activities (dark blue), (ii) high protease activity (light blue), and (iii) mixed defences with low polyphenols (orange). The response of insect communities to the host-plant traits was analysed using canonical correspondence analysis and visualised by biplots showing *Ficus* defences and communities associated with *Ficus* species (first two constrained axes are shown). The traits shown explained 15.9% of adjusted variability in case of whole leaf-chewer communities ($P < 0.001$, pseudo-$F = 1.8$), 12.3% in case of choreutids ($P < 0.001$, pseudo-$F = 2.3$), and 12.1% in case of pyraloids ($P < 0.001$, pseudo-$F = 2.2$). All singletons were removed from the analyses. See Fig. 1 for the *Ficus* species codes.

by diverse communities of insect herbivores (Agrawal & Fishbein 2006). It is likely that any individual defence is only effective against a subset of the herbivores in a given system (Koricheva *et al.* 2004; Volf *et al.* 2015). Our results show that the structure of generalist and specialist insect communities correlates with traits that have evolved in different ways.

We predicted (Hypothesis II) that generalist insect community structure would correlate mainly with escalating defences, while the structure of specialist insect communities would relate to divergent defences. Escalation not only results in trait dissimilarity increasing with phylogenetic distance, thus restricting generalists from shifting between unrelated hosts,

Table 1 Results of the canonical correspondence analyses for whole larval leaf-chewer community, Choreutidae and Pyraloidea

Response Variable	Whole Community	Choreutidae	Pyraloidea
Protease activity	pseudo-F = 2.0, P = 0.006	pseudo-F = 2.7, P < 0.001	pseudo-F = 2.6, P = 0.001
Polyphenol oxidative activity	pseudo-F = 1.5, P = 0.034	–	pseudo-F = 1.8, P = 0.029
Trichome length	pseudo-F = 1.6, P = 0.027	–	–
Trichome density	–	pseudo-F = 1.7, P = 0.022	–
Alkaloid diversity	pseudo-F = 1.8, P = 0.010	–	–
Whole Model, % Variance	pseudo-F = 1.8, P < 0.001, 15.9%	pseudo-F = 2.3, P < 0.001, 12.3%	pseudo-F = 2.2, P < 0.001, 12.1%
Variance Traits	10.3%, P = 0.004	1.4%, P = 0.310	7.2%, P = 0.001
Variance Phylogeny	10.0%, P = 0.005	8.4%, P = 0.006	16.2%, P < 0.001
Covariation	5.6%	10.9%	4.2%

The table shows effects of individual traits selected by forward selection as well as the statistics (including percentage of explained variability in the community data) for the overall model including these traits. Traits marked with '–' were not included in the respective models. The values below the horizontal line give results of variance partitioning analysis showing the significance and percentage of variability in the community explained by *Ficus* traits and phylogeny, including the percentage of the variance in the community structure explained by covariation between the two.

but also increases toxicity for non-specialised herbivores. This is the case in some plant genera, such as *Asclepias* or *Bursera* (Agrawal *et al.* 2008; Becerra *et al.* 2009), which harbour almost exclusively specialist herbivores. Here, we observed that generalist pyraloids (spread across many plant families) (Novotny *et al.* 2002, 2010) have distinct and often depauperate communities on hosts with high oxidative activity. These hosts are often derived species in clades with otherwise low oxidative activity, demonstrating the power of local escalation. The local escalation of traits is reminiscent of 'co-evolutionary hotspots' (Thompson 1994), and may demonstrate an early stage of the *escape and radiate* model of evolution proposed by Ehrlich & Raven (1964). Escalation in oxidative activity may 'free' these *Ficus* lineages from pyraloid herbivores, opening up a new adaptive zone.

However, specialised insects can adapt to host defences over evolutionary time, and in turn use host secondary metabolites to their own advantage (Agrawal & Fishbein 2008), for example as a protection against predators. In our study, alkaloid diversity escalated across the entire phylogeny and alkaloid rich plants hosted distinct insect communities. Alkaloid diversity was highly and positively correlated with the abundance of the specialist moth genus *Asota*, with alkaloid rich *Ficus pachyrhachis*, *Ficus septica* and *Ficus hispidoides* being the main hosts. The bright, presumably aposematic, coloration of *Asota* moth larvae and adults is suggestive of chemical sequestration (Sourakov & Emmel 2001). This mirrors the larval ecology of the specialist monarch butterflies (Nymphalidae) associated with *Asclepias*. Overall, our results confirm the importance of escalating host-plant defensive traits by empirically demonstrating their correlation with insect community structure as we illustrate both their generally negative correlation with generalist communities (polyphenols), as well as their positive correlation with specialists (alkaloids).

In contrast, the community structure of the *Ficus* specialist Choreutidae correlated with trichome density, a trait that showed high disparity among closely related *Ficus* species. As suggested above, any defensive strategy will decrease in efficiency as specialised herbivores accumulate with time (Janz 2011). This trend is likely to be especially pronounced when defences show phylogenetic predictability, such as in the case of cardenolides in milkweeds (Agrawal *et al.* 2008). In such a

Table 2 The results of GLMM (above the horizintal line) and PGLMM (below the horizontal line) analyses giving model coefficients and significance with fixed effects listed, and random effects being *Ficus* species and herbivore species for GLMMs

Fixed effect	Estimate	Standard error	z-value	P-value
Protease activity	−3.927	1.919	−2.046	0.041
Triterpene diversity	0.526	0.268	1.965	0.049
Polyphenol diversity	1.902	0.827	2.301	0.021
Oxidative activity	−0.109	0.051	−2.152	0.031
Protease activity	−5.956	2.723	−2.187	0.029
Polyphenol diversity	1.783	0.813	2.192	0.028

For PGLMMs the additional random effect of phylogenetic covariance was included. Only significant results are shown.

GLMM, Generalised Linear Mixed Models; PGLMM, Phylogenetic Linear Mixed Models.

Table 3 Selected models of evolution (Brownian motion, Lambda and white noise) and phylogenetic signal for individual *Ficus* traits measured by Blomberg's K and PIC

	Model (AIcc)	K	PIC observed mean	PIC randomised mean	PIC P
Protease activity	BM	0.703	0.2	0.4	**0.017***
Alkaloid content	White	0.312	5081.1	5528.4	0.471
Alkaloid diversity	Lambda (0.66)	0.779	9.1	23.2	**0.014***
Polyphenol content	BM	0.632	17.0	38.0	**0.013***
Polyphenol diversity	White	0.387	2.4	3.2	0.299
Oxidative activity	BM	0.725	237.8	602.1	0.066
Protein precipitation	White	0.456	896.1	1472.3	0.092
Triterpene content	BM	0.673	31.9	76.4	**0.009***
Triterpene diversity	Lambda (0.47)	0.543	12.6	23.7	**0.028***
Trichome density	White	0.251	590757.6	504354.1	0.730
Trichome length	White	0.508	152279.6	262148.8	0.193
SLA	White	0.309	130152.3	144310.7	0.465
C:N	BM	0.819	245.4	630.4	**0.027***

Lambda values are given for the traits following the Lambda model of evolution.

SLA, specific leaf area.

*Traits showing significant phylogenetic signal are in bold.

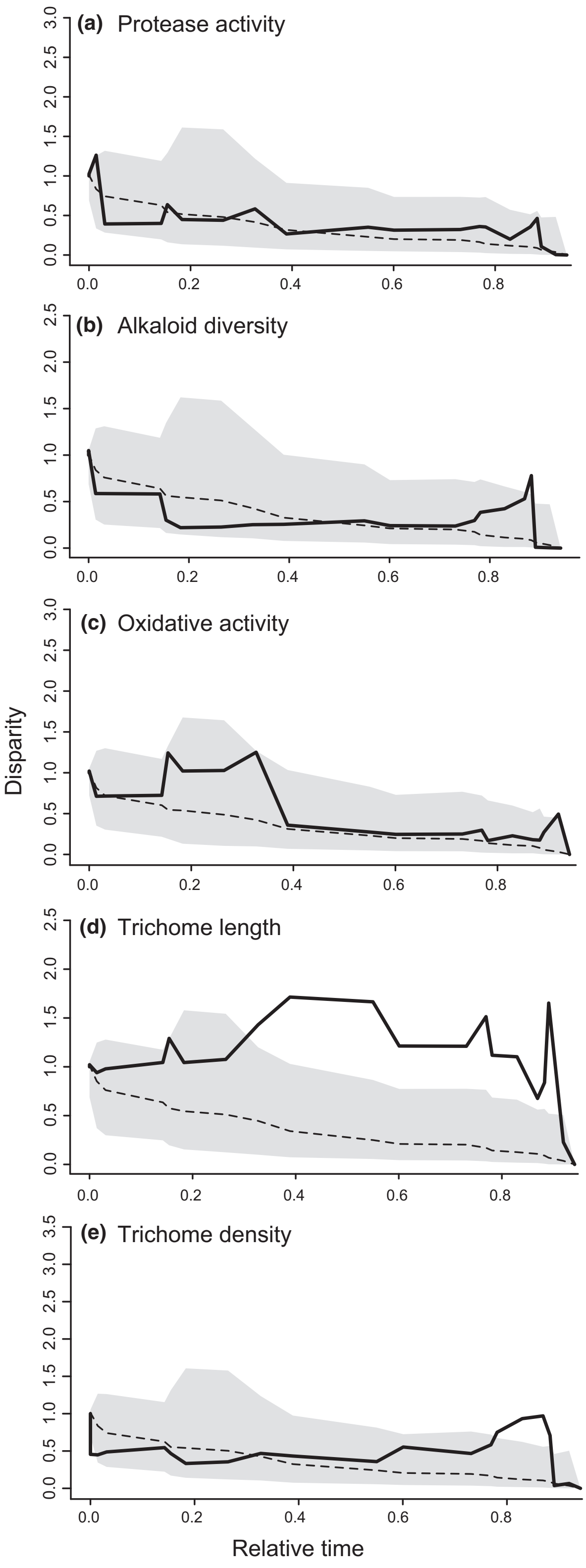

Figure 3 Mean disparity through time (DTT) for traits with significant effects on insect communities (solid line). Plots show disparity in protease activity (a), alkaloid diversity (b), oxidative activity (c), trichome length (d), and trichome density (e). The dashed line indicates the median DTT based on 999 simulations of character evolution on the phylogeny of studied *Ficus* species under Brownian motion. The grey shaded area indicates the 95% confidence interval for the simulated data.

situation, the ability to mix and match between a pool of conserved and divergent defensive traits, which are harder to overcome for specialised herbivores, may be beneficial (Janz 2011). This might be the case for Choreutidae that are *Ficus* specialists, with 63% of local species and 81% of individuals feeding exclusively on *Ficus*. Choreutidae radiated ~70 million years ago, shortly after the divergence of *Ficus* (Cruaud *et al.* 2012; Rota *et al.* 2016), which could lead to sequential coevolution between the two. Indeed, choreutid community structure was highly dependent on host *Ficus* phylogeny, and most correlations to defensive traits resulted from covariation between traits and phylogeny. Divergent defences may be beneficial to overcome the phylogenetic conservatism of specialised herbivores, such as Choreutidae here, *Eois* on *Piper*, or *Blepharida* on *Bursera* (e.g. Becerra 2007; Salazar *et al.* 2016). Likewise, divergent volatile profiles reduced herbivory in *Piper* (Massad *et al.* 2017).

Interestingly, phylogenetically conserved protease activity was the only trait with a direct negative correlation with larval leaf-chewer abundance. Experimental evidence suggests that protease activity is very efficient at protecting leaves from a broad suite of insects, deterring them from feeding and reducing their growth rates, probably without synergy with other traits (Konno *et al.* 2004). Our data from natural communities suggest that cysteine proteases are an important form of defence for the studied *Ficus* species, which may explain their conserved evolution.

We observed three main defensive syndromes in *Ficus*, each of them supporting different insect communities. In line with our expectations (Hypothesis III), there were only a few negative correlations between defence traits, suggesting that trade-offs in anti-herbivore defence are uncommon (Agrawal & Fishbein 2006). Defensive syndromes comprising a combination of traits with different effects on herbivores are likely to maintain efficient protection against insects (Koricheva *et al.* 2004; Agrawal & Fishbein 2006; Volf *et al.* 2015). For example, synergy between latex production and other physical defences may promote anti-herbivore protection in milkweeds (Agrawal & Fishbein 2006). Our results suggest that defensive syndromes can consist of traits following different evolutionary trajectories, possibly making adaptation even harder for herbivores. This would shape the evolution of plant defensive traits into a dynamic system, with traits undergoing periods of diversification, divergence and sometimes decline (Agrawal *et al.* 2008; Janz 2011). This cyclical process and the multiple selective pressures involved likely act to erode phylogenetic signal in defensive traits in some systems (e. g. Kursar *et al.* 2009; Pearse & Hipp 2012; Cacho *et al.* 2015; Salazar *et al.* 2016).

The diversification of host plant defences due to herbivore pressure is, in turn, likely to promote the diversity of insect herbivores themselves, resulting in reciprocal diversification of plant defences and herbivores (Ehrlich & Raven 1964). It has been shown that chemical diversity may be both driven by insect diversity and be one of the mechanisms promoting it, as chemical diversity prevents the dominance of any one insect group in the herbivore community (Richards *et al.* 2015; Salazar *et al.* 2016). This is also illustrated by the positive relationship between polyphenol and triterpene diversity and occurence of insects found here. Plants that possess diverse defensive traits, such as *Ficus*, are likely to harbour herbivores with various life histories, promoting overall diversity in local communities.

Here, we have taken a community approach that has allowed us to demonstrate that escalating traits primarily affect generalist herbivores, whereas diverging defences affect specialists; this difference influences the overall community structure of insect herbivores across different *Ficus* species. This means that insect-plant food webs are assembled at least partly through coevolutionary dynamics, contributing to changes in regional species pools and interactions (Lewinsohn *et al.* 2005). Species rich pantropical plant genera, such as *Ficus*, *Piper*, or *Psychotria,* possessing a diverse array of anti-herbivore defences, often with different phylogenetic dynamics, are ideal models for studying the assembly of rich insect-plant food webs (Lewinsohn *et al.* 2005). Focusing on these systems may allow us to further improve our understanding of the role of different evolutionary processes in generating the astonishing diversity of herbivorous insects on plants.

ACKNOWLEDGEMENTS

We thank the staff of the New Guinea Binatang Research Centre in Papua New Guinea. STS acknowledges funding from a USB Postdoc project reg.no. CZ.1.07/2.3.00/30.0006 (funded by the European Social Fund and the Czech State Budget) and Grant Agency of the Czech Republic 15-24571S. MV acknowledges funding from the Grant Agency of the University of South Bohemia GAJU 156/2013/P and GAJU 103-016/2015/P. VN acknowledges the European Science Foundation grant 669609, Darwin Initiative project no. 22-002, and Grant Agency of the Czech Republic 17-23862S. We acknowledge US National Science Foundation DEB 9407297. JPS acknowledges funding from the Academy of Finland (grant no 258992), and the help of Saku Valkamaa, Atte Tuominen and Anne Koivuniemi in the chemical analyses. We thank the Papua New Guinea Forest Research Institute, in particular Kipiro Damas, for assistance granting export permits. We also thank again those acknowledged in Novotny *et al.* (2010), especially colleagues at the Natural History Museum, London, for taxonomic assistance. We thank Petr Klimeš and Philip T. Butterill for constructive criticism of the manuscript and Will Pearse and Petr Šmilauer for analytical advice. Access to computing and storage facilities owned by parties and projects contributing to the National Grid Infrastructure MetaCentrum provided under the programme 'Projects of Large Research, Development, and Innovations Infrastructures' (CESNET LM2015042), is greatly appreciated. In addition, we thank the editors and anonymous reviewers for their valuable comments, which greatly improved the manuscript.

AUTHORSHIP

MV and STS conceived the study, MV and STS performed the statistical analyses and wrote the first draft of the manuscript, MV collected the new sequence and microsatellite data and built the phylogenetic tree, STS, MS and BI collected the leaf tissue, SEM led the barcoding and species delimitation of Lepidoptera, GA and SW collected the cysteine data, PS and MM collected the triterpene data and conducted metabolite identification, JPS, JL and JK collected the polyphenol and alkaloid data and conducted metabolite identification, JZ optimised the PCR conditions for microsatellite analysis and conducted the genotyping of the microsatellite data, JR led the taxonomic revision of Choreutidae, GDW collected most of the plant sequence data and contributed to phylogeny estimation, YB and VN collected the insect data and VN helped conceive the study and led many aspects of the field work. All authors commented on a first draft of the manuscript and contributed substantially to the text.

DATA ACCESSIBILITY STATEMENT

The insect, chemical and trait data supporting the results are available in the supplementary materials (Tables S1, S2, S3, and S4). The sequences used for reconstructing the *Ficus* phylogeny are available in EMBL database: http://www.ebi.ac.uk/ena/data/view/LT907940-LT907943 and LT907946-LT907950. The data on DNA barcodes used for the insect identification are available in BOLD database (DOI: dx.doi.org/10.5883/DS-PNGLOWLP).

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SUPPORTING INFORMATION

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Editor, Ted Turlings
Manuscript received 9 August 2017
First decision made 18 September 2017
Manuscript accepted 10 October 2017