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

Martin Volf[+*1,2], Simon T Segar[+1,2], Scott E Miller[3], Brus Isua[4], Mentap Sisol[4], Gibson Aubona[4], Petr Šimek[2], Martin Moos[2], Juuso Laitila[5], Jorma Kim[5], Jan Zima Jr[2,6], Jadranka Rota[7], George D Weiblen[8], Stewart Wossa[9], Juha-Pekka Salminen[5], Yves Basset[1,10,11] and Vojtech Novotny[1,2]

[1]University of South Bohemia in Ceske Budejovice, Faculty of Science, Branisovska 31, 37005 Ceske Budejovice, Czech Republic; [2]Biology Centre, The Czech Academy of Sciences, Branisovska 31, 37005 Ceske Budejovice, Czech Republic; [3]National Museum of Natural History, Smithsonian Institution, 10th St. & Constitution Ave. NW, Washington, 20560 DC, USA; [4]New Guinea Binatang Research Center, P.O. Box 604, Madang, Papua New Guinea; [5]Natural Chemistry Research Group, Department of Chemistry, University of Turku, FI-20500 Turku, Finland; [6]Institute of Botany, Czech Academy of Sciences, Dukelska 135, Trebon 37982, Czech Republic; [7]Lund University, Department of Biology, Sölvegatan 37, 223 62 Lund, Sweden; [8]Bell Museum and Department of Plant & Microbial Biology, University of Minnesota, 250 Biological Science Center, 1445 Gortner Avenue, Saint Paul, 55108 Minnesota, USA, [9]Centre for Natural Resources Research and Development, University of Goroka, Goroka, P.O Box 1078, Eastern Highland Province, Papua New Guinea; [10] Smithsonian Tropical Research Institute, Apartado 0843-03092, Balboa, Ancon, Panamá; [11]Maestria de Entomologia, Universidad de Panama, 080814 Panama City, Republic of Panama.

[+]Joint first authors

*Corresponding author: Martin Volf, volf@entu.cas.cz, tel. +420 387775038, Biology Centre, The Czech Academy of Sciences, Branisovska 31, 37005 Ceske Budejovice, Czech Republic

Running title: Evolution of *Ficus* defenses

Statement of 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 optimized 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 data supporting the results are available in the supplementary materials (Tables S1, S2, S3, and S4). The sequences used for reconstructing the *Ficus* phylogeny were submitted to EMBL and their accession numbers are included at the end of the article.

Key words: alkaloids, Choreutidae, coevolution, cysteine protease, herbivore, Lepidoptera, New Guinea, polyphenols, Pyraloidea, trichomes

Type of article: Letters

Number or words in the abstract: 150
Number of words in the main text: 4754
Number of references: 50
Number of figures: 3
Number of tables: 3

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 defenses 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 to phylogeny, protease and trichomes. Additionally, highly specialized *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 defenses: 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 defenses in *Ficus* can be driven towards both escalation and divergence in individual traits, in combination providing protection against a broad spectrum of herbivores.

Introduction

Insect-plant arms races have been suggested to support diversification and escalation of plant defenses (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 defenses), with trait values positively correlating to phylogenetic distance from the root, and/or phylogenetic dissimilarity between species. Such an escalation of host-plant defenses 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 specialized herbivores (Agrawal *et al.* 2008). Divergent defenses (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 can consume multiple hosts, at the cost of being maladapted to potent defenses (Bernays & Chapman 2007), while specialists often track host phylogeny and adapt to such

93 defenses. The composition of insect communities attacking the host is therefore key –
94 assemblages of specialists should select mainly for divergent traits (e.g. Becerra 2007),
95 whereas assemblages of generalists, sensitive to specialized defenses, should impose selection
96 for escalating traits.

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

105 Rainforest assemblages of *Ficus* represent an excellent model system for exploring such
106 evolutionary processes. This pantropical genus is extraordinarily speciose (over 800 species).
107 The paleotropics are particularly diverse, with over 150 species found in Papua New Guinea
108 (PNG), the global center of *Ficus* diversity (Berg & Corner 2005; Cruaud *et al.* 2012). *Ficus*
109 can comprise ~15% of all stems with DBH ≥ 5 cm, in both primary and secondary lowland
110 forests in PNG (Whitfeld *et al.* 2012). The genus *Ficus* also supports diverse insect
111 communities, including many herbivores which are lineage specialists (Basset & Novotny
112 1999; Novotny *et al.* 2010).

113 Over the course of ~75 MY (Cruaud et al., 2012) *Ficus* has acquired a broad range of chemical
114 and physical defenses. These include 'universal' traits, such as polyphenols, terpenoids, and
115 trichomes. Most *Ficus* species also produce latex that serves as a physical defense, as well as
116 vessel for more taxonomically restricted chemical defenses. These specialized defenses

include phenanthroindolizidine alkaloids (Damu *et al.* 2005) and cysteine proteases (Konno *et al.* 2004). Among these defenses, 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 defenses.

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* defenses which correlate with communities of leaf-chewing larvae, and analyze whether these correlations hold consistently across herbivores with a range of host specificity. Second, we analyze the evolutionary patterns in these defenses and test whether they are conserved, escalate over evolutionary time, or are divergent among closely related species. We predict that: I) defenses 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 defenses, while the structure of specialist insect communities will relate to diverging defenses; 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 defenses, as escalating and diverging defenses 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 recognize 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 defenses of plants and their herbivorous assemblages

142 could be explained by a single evolutionary mechanism in speciose systems, such as tropical
143 rainforests.

144

145 **Methods**

146 *Ficus traits*

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

163 For the analysis of protease activity, we sampled latex by cutting the main vein of each leaf
164 and letting latex flow into a 2 ml collection tube for 30 seconds. Protease activity was analyzed
165 using a modified version of the methods of Konno *et al.* (2004). Alkaloids and polyphenols

were extracted using acetone and aqueous acetone from ca 0.5g 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.* (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 functions of polyphenols in anti-herbivore protection. Low polarity terpenoids were extracted from ca 0.5g 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 ionization (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 homogenized dry leaf material.

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

Insect data

189 The insect data were taken from Novotny *et al.* (2010) (Table S1). The data include only
190 reared individuals, with host associations confirmed by feeding trials, sampled from 1,500 m^2
191 of leaf area per plant species. We focused on leaf-chewing larvae (including 122 Lepidoptera
192 and two Coleoptera species) as a guild that is well represented on our focal *Ficus* species, and
193 which inflicts a large amount of damage. We conducted additional analyses to compare the
194 two dominant microlepidopteran taxa, which represented the majority of caterpillars in the
195 focal communities: Pyraloidea (31% of all caterpillar individuals), a relatively polyphagous
196 group feeding on several plant taxa, and Choreutidae (45% of all caterpillar individuals),
197 which are mostly specialists of Moraceae in our community (Novotny *et al.* 2002). We
198 included recent taxonomic revisions for Choreutidae (Table S1). Singleton species were
199 removed from all statistical analyses. The residual insect community comprised several
200 (super)families, with Noctuoidea (11%) and Tortricidae (10%) being the most abundant. We
201 note that 84% of all noctuid individuals are in the brightly colored genus *Asota* (largely
202 restricted to *Ficus*), a specialist genus potentially capable of alkaloid sequestration (Sourakov
203 & Emmel 2001). We separated *Asota* in a subset of our analyses.

204 *Ficus phylogeny reconstruction*

205 The host-plant phylogeny was estimated using four loci: ITS, ETS, G3PD, and GBSSI. We
206 used sequences from Cruaud *et al.* (2012) when available. We obtained the sequences of
207 missing species using dried leaf tissue following Cruaud *et al.* (2012). The host-plant
208 phylogeny was reconstructed using Bayesian inference as implemented in BEAST v2.1.3
209 (Drummond *et al.* 2012), with section level constraints taken from Cruaud *et al.* (2012).
210 Furthermore, for section *Sycocarpus* we used constraints based on microsatellite data using
211 Nei's distance neighbor joining trees, based on nine microsatellite loci previously published
212 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. 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 centered and standardized and the results were visualized using PCA in CANOCO 5 (Ter Braak & Smilauer 2012). Additionally, we analyzed correlations between traits in a phylogenetic context using 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 to insect community structure.

To test the hypothesis that defensive and resource acquisition traits correlated with insect community structure, we analyzed 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 specialization may determine which traits had explanatory power, we ran separate

238 analyses for the whole larval leaf-chewer community, generalist Pyraloidea, and *Ficus*
239 specialized Choreutidae.

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

252 Second, the relationships between plant traits and caterpillar abundance were tested using
253 Phylogenetic Least Squares Regression (PGLS). We controlled for phylogenetic non-
254 independence of *Ficus* species, but note that a trait's value in defending against herbivores is
255 not diminished by it being phylogenetically conserved (Agrawal 2007). Because traits evolve
256 in different ways we fitted the most appropriate branch length transformation. In cases where
257 traits followed Brownian motion, we used the 'corBrownian' correlation structure in GLS
258 models. In cases where more complicated branch length transformations were required, we
259 selected the parameter value of the transformation using maximum likelihood as implemented
260 in the R package 'caper' (Orme *et al.* 2013), using the transformation as selected by AICc.
261 For traits where a non-phylogenetic white noise model fitted best, we used GLS models
262 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. All insect data were log-transformed.

Evolution of Ficus traits

Initially, we tested for phylogenetic signal in our traits using Blomberg's K (a widely used metric) and a randomization 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 defenses with low polyphenols (Fig. 2, Fig. S1). These clusters were mirrored by insect communities, with species from clusters i) and ii) harboring distinct assemblages (Fig. 2). Individual defenses 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 latex 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 analyzed 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 defenses is expected in species-rich communities, reflecting the myriad selective pressures imposed by diverse communities of insect herbivores (Agrawal & Fishbein 2006). It is likely that any individual defense 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 defenses, while the structure of specialist insect communities would relate to divergent defenses. Escalation not only results in trait dissimilarity increasing with phylogenetic distance, thus restricting generalists from shifting between unrelated hosts, but also increases toxicity for non-specialized herbivores. This is the case in some plant genera, such as *Asclepias* or *Bursera* (Agrawal *et al.* 2008; Becerra *et al.* 2009), which harbor almost exclusively specialist herbivores. Here we observed that generalist pyraloids (spread across many plant families) (Novotny *et al.* 2002; Novotny *et al.* 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

384 evolution proposed by Ehrlich & Raven (1964). Escalation in oxidative activity may 'free'
385 these *Ficus* lineages from pyraloid herbivores, opening up a new adaptive zone.

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

399 In contrast, the community structure of the *Ficus* specialist Choreutidae correlated with
400 trichome density, a trait that showed high disparity among closely related *Ficus* species. As
401 suggested above, any defensive strategy will decrease in efficiency as specialized herbivores
402 accumulate with time (Janz 2011). This trend is likely to be especially pronounced when
403 defenses show phylogenetic predictability, such as in the case of cardenolides in milkweeds
404 (Agrawal *et al.* 2008). In such a situation, the ability to mix and match between a pool of
405 conserved and divergent defensive traits, which are harder to overcome for specialized
406 herbivores, may be beneficial (Janz 2011). This might be the case for Choreutidae that are
407 *Ficus* specialists, with 63% of local species and 81% of individuals feeding exclusively on
408 *Ficus*. Choreutidae radiated ~70 million years ago, shortly after the divergence of *Ficus*

409 (Cruaud *et al.* 2012; Rota *et al.* 2016), which could lead to sequential coevolution between
410 the two. Indeed, choreutid community structure was highly dependent on host *Ficus*
411 phylogeny, and most correlations to defensive traits resulted from covariation between traits
412 and phylogeny. Divergent defenses may be beneficial to overcome the phylogenetic
413 conservatism of specialized herbivores, such as Choreutidae here, *Eois* on *Piper*, or
414 *Blepharida* on *Bursera* (e.g. Becerra 2007; Salazar *et al.* 2016). Likewise, divergent volatile
415 profiles reduced herbivory in *Piper* (Massad *et al.* 2017).

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

423 We observed three main defensive syndromes in *Ficus*, each of them supporting different
424 insect communities. In line with our expectations (Hypothesis III), there were only a few
425 negative correlations between defense traits, suggesting that trade-offs in anti-herbivore
426 defense are uncommon (Agrawal & Fishbein 2006). Defensive syndromes comprising a
427 combination of traits with different effects on herbivores are likely to maintain efficient
428 protection against insects (Koricheva *et al.* 2004; Agrawal & Fishbein 2006; Volf *et al.* 2015).
429 For example, synergy between latex production and other physical defenses may promote
430 anti-herbivore protection in milkweeds (Agrawal & Fishbein 2006). Our results suggest that
431 defensive syndromes can consist of traits following different evolutionary trajectories,
432 possibly making adaptation even harder for herbivores. This would shape the evolution of
433 plant defensive traits into a dynamic system, with traits undergoing periods of diversification,

434 divergence and sometimes decline (Agrawal *et al.* 2008; Janz 2011). This cyclical process
435 and the multiple selective pressures involved likely act to erode phylogenetic signal in
436 defensive traits in some systems (e. g. Kursar *et al.* 2009; Pearse & Hipp 2012; Cacho *et al.*
437 2015; Salazar *et al.* 2016).

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

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

458

459 **Acknowledgements**

460 We thank the staff of the New Guinea Binatang Research Centre in Papua New Guinea. STS
461 acknowledges funding from a USB Postdoc project reg.no. CZ.1.07/2.3.00/30.0006 (funded
462 by the European Social Fund and the Czech State Budget) and Grant Agency of the Czech
463 Republic 15-24571S. MV acknowledges funding from the Grant Agency of the University of
464 South Bohemia GAJU 156/2013/P and GAJU 103-016/2015/P. VN acknowledges the ESF
465 grant 669609, Darwin Initiative project no. 22-002, and Grant Agency of the Czech Republic
466 14-36098G. We acknowledge US National Science Foundation DEB 9407297. JPS
467 acknowledges funding from the Academy of Finland (grant no 258992), and the help of Saku
468 Valkamaa, Atte Tuominen and Anne Koivuniemi in the chemical analyses. We thank the
469 Papua New Guinea Forest Research Institute, in particular Kipiro Damas, for assistance
470 granting export permits. We also thank again those acknowledged in Novotny et al. (2010),
471 especially colleagues at the Natural History Museum, London, for taxonomic assistance. We
472 than Petr Klimeš and Philip T. Butterill for a constructive criticism of the manuscript. Access
473 to computing and storage facilities owned by parties and projects contributing to the National
474 Grid Infrastructure MetaCentrum provided under the programme "Projects of Large Research,
475 Development, and Innovations Infrastructures" (CESNET LM2015042), is greatly
476 appreciated. In addition, we thank the editors and anonymous reviewers for their valuable
477 comments, which improved the manuscript.

478

479 **References**

480 1.

481 Agrawal, A.A. (2007). Macroevolution of plant defense strategies. *Trends in Ecology &*
482 *Evolution*, 22, 103-109.
483 2.

484 Agrawal, A.A. & Fishbein, M. (2006). Plant defense syndromes. *Ecology*, 87, S132-S149.
485 3.

486 Agrawal, A.A. & Fishbein, M. (2008). Phylogenetic escalation and decline of plant defense
487 strategies. *Proceedings of the National Academy of Sciences of the United States of*
488 *America*, 105, 10057-10060.
489 4.

490 Agrawal, A.A., Salminen, J.P. & Fishbein, M. (2008). Phylogenetic trends in phenolic
491 metabolism of milkweeds (*Asclepias*): evidence for escalation. *Evolution*, 63, 663-
492 673.
493 5.

494 Basset, Y. & Novotny, V. (1999). Species richness of insect herbivore communities on *Ficus*
495 in Papua New Guinea. *Biological Journal of the Linnean Society*, 67, 477-499.
496 6.

497 Becerra, J.X. (2007). The impact of herbivore-plant coevolution on plant community
498 structure. *Proceedings of the National Academy of Sciences of the United States of*
499 *America*, 104, 7483-7488.
500 7.

501 Becerra, J.X., Noge, K. & Venable, D.L. (2009). Macroevolutionary chemical escalation in
502 an ancient plant-herbivore arms race. *Proceedings of the National Academy of*
503 *Sciences of the United States of America*, 106, 18062-18066.

504 8.

505 Berg, C. & Corner, E. (2005). *Moraceae (Ficus). Flora Malesiana, Series I (Seed plants)*.
506 National Herbarium of the Netherlands, Leiden.

507 9.

508 Bernays, E.A. & Chapman, R.F. (2007). *Host-plant Selection by Phytophagous Insects*.
509 Springer US, New York.

510 10.

511 Cacho, N.I., Kliebenstein, D.J. & Strauss, S.Y. (2015). Macroevolutionary patterns of
512 glucosinolate defense and tests of defense-escalation and resource availability
513 hypotheses. *New Phytologist*, 208, 915-927.

514 11.

515 Cruaud, A., Ronsted, N., Chantarasuwan, B., Chou, L.S., Clement, W.L., Couloux, A. *et al.*
516 (2012). An extreme case of plant-insect codiversification: Figs and fig-pollinating
517 wasps. *Systematic Biology*, 61, 1029-1047.

518 12.

519 Damu, A.G., Kuo, P.C., Shi, L.S., Li, C.Y., Kuoh, C.S., Wu, P.L. *et al.* (2005).
520 Phenanthroindolizidine alkaloids from the stems of *Ficus septica*. *Journal of Natural*
521 *Products*, 68, 1071-1075.

522 13.

523 Drummond, A.J., Suchard, M.A., Xie, D. & Rambaut, A. (2012). Bayesian phylogenetics with
524 BEAUti and the BEAST 1.7. *Molecular Biology and Evolution*, 29, 1969-1973.

525 14.

526 Ehrlich, P.R. & Raven, P.H. (1964). Butterflies and plants - a study in coevolution. *Evolution*,
527 18, 586-608.
528 15.

529 Engström, M.T., Palijarvi, M., Fryganas, C., Grabber, J.H., Mueller-Harvey, I. & Salminen,
530 J.-P. (2014). Rapid qualitative and quantitative analyses of proanthocyanidin
531 oligomers and polymers by UPLC-MS/MS. *Journal of Agricultural and Food*
532 *Chemistry*, 62, 3390-3399.
533 16.

534 Engström, M.T., Pälijärvi, M. & Salminen, J.-P. (2015). Rapid fingerprint analysis of plant
535 extracts for ellagitannins, gallic acid, and quinic acid derivatives and quercetin-,
536 kaempferol- and myricetin-based flavonol glycosides by UPLC-QqQ-MS/MS.
537 *Journal of Agricultural and Food Chemistry*, 63, 4068-4079.
538 17.

539 Freckleton, R.P., Cooper, N. & Jetz, W. (2011). Comparative methods as a statistical fix: the
540 dangers of ignoring an evolutionary model. *The American Naturalist*, 178, E10-E17.
541 18.

542 Garcia, M., Bain, A., Tzeng, H.-Y., Peng, Y.-Q., Chou, L.-S. & Kjellberg, F. (2012). Portable
543 microsatellite primers for *Ficus* (Moraceae). *American Journal of Botany*, 99, E187-
544 E192.
545 19.

546 Hagerman, A.E. & Butler, L.G. (1978). Protein precipitation method for the quantitative
547 determination of tannins. *Journal of Agricultural and Food Chemistry*, 26, 809-812.
548 20.

549 Harmon, L.J., Weir, J.T., Brock, C.D., Glor, R.E. & Challenger, W. (2008). GEIGER:
550 investigating evolutionary radiations. *Bioinformatics*, 24, 129-131.
551 21.

552 Ives, A.R. & Helmus, M.R. (2011). Generalized linear mixed models for phylogenetic
553 analyses of community structure. *Ecological Monographs*, 81, 511-525.
554 22.

555 Janz, N. (2011). Ehrlich and Raven revisited: mechanisms underlying codiversification of
556 plants and enemies. In: *Annual Review of Ecology, Evolution, and Systematics*, pp.
557 71-89.
558 23.

559 Jombart, T., Balloux, F. & Dray, S. (2010). Adephylo: new tools for investigating the
560 phylogenetic signal in biological traits. *Bioinformatics*, 26, 1907-1909.
561 24.

562 Keck, F., Rimet, F., Bouchez, A. & Franc, A. (2016). phylosignal: an R package to measure,
563 test, and explore the phylogenetic signal. *Ecology and Evolution*, 6, 2774-2780.
564 25.

565 Konno, K., Hirayama, C., Nakamura, M., Tateishi, K., Tamura, Y., Hattori, M. *et al.* (2004).
566 Papain protects papaya trees from herbivorous insects: role of cysteine proteases in
567 latex. *Plant Journal*, 37, 370-378.
568 26.

569 Koricheva, J., Nykanen, H. & Gianoli, E. (2004). Meta-analysis of trade-offs among plant
570 antiherbivore defenses: Are plants jacks-of-all-trades, masters of all? *American*
571 *Naturalist*, 163, 64-75.

572 27.

573 Kursar, T.A., Dexter, K.G., Lokvam, J., Pennington, R.T., Richardson, J.E., Weber, M.G. *et*
574 *al.* (2009). The evolution of antiherbivore defenses and their contribution to species
575 coexistence in the tropical tree genus *Inga*. *Proceedings of the National Academy of*
576 *Sciences of the United States of America*, 106, 18073-18078.

577 28.

578 Lewinsohn, T.M., Novotny, V. & Basset, Y. (2005). Insects on plants: diversity of herbivore
579 assemblages revisited. *Annual Review of Ecology, Evolution, and Systematics*, 36,
580 597-620.

581 29.

582 Marquis, R.J., Salazar, D., Baer, C., Reinhardt, J., Priest, G. & Barnett, K. (2016). Ode to
583 Ehrlich and Raven or how herbivorous insects might drive plant speciation. *Ecology*,
584 97, 2939-2951.

585 30.

586 Massad, T.J., Martins de Moraes, M., Philbin, C., Oliveira, C., Cebrian Torrejon, G., Fumiko
587 Yamaguchi, L. *et al.* (2017). Similarity in volatile communities leads to increased
588 herbivory and greater tropical forest diversity. *Ecology*, 98, 1750-1756.

589 31.

590 Moe, A.M. & Weiblen, G.D. (2011). Development and characterization of microsatellite loci
591 in dioecious figs (*Ficus*, Moraceae). *American Journal of Botany*, 98, e25-e27.

592 32.

593 Novotny, V., Basset, Y., Miller, S.E., Drozd, P. & Cizek, L. (2002). Host specialization of
594 leaf-chewing insects in a New Guinea rainforest. *Journal of Animal Ecology*, 71, 400-
595 412.
596 33.

597 Novotny, V., Miller, S.E., Baje, L., Balagawi, S., Basset, Y., Cizek, L. *et al.* (2010). Guild-
598 specific patterns of species richness and host specialization in plant-herbivore food
599 webs from a tropical forest. *Journal of Animal Ecology*, 79, 1193-1203.
600 34.

601 Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'hara, R. *et al.* (2017).
602 vegan: Community Ecology Package. R package version 2.4-3. https://CRAN.R-
603 project.org/package=vegan.
604 35.

605 Orme, D., Freckleton, R., Thomas, G., Petzoldt, T., Fritz, S., Isaac, N. *et al.* (2013). caper:
606 Comparative Analyses of Phylogenetics and Evolution in R. R package version 0.5.2.
607 https://CRAN.R-project.org/package=caper.
608 36.

609 Pearse, I.S. & Hipp, A.L. (2012). Global patterns of leaf defenses in oak species. *Evolution*,
610 66, 2272-2286.
611 37.

612 Pearse, W.D., Cadotte, M.W., Cavender-Bares, J., Ives, A.R., Tucker, C.M., Walker, S.C. *et*
613 *al.* (2015). pez: phylogenetics for the environmental sciences. *Bioinformatics*, 31,
614 2888-2890.
615 38.

616 Richards, L.A., Dyer, L.A., Forister, M.L., Smilanich, A.M., Dodson, C.D., Leonard, M.D. *et*
617 *al.* (2015). Phytochemical diversity drives plant–insect community diversity.
618 *Proceedings of the National Academy of Sciences of the United States of America*,
619 112, 10973-10978.
620 39.

621 Rota, J., Peña, C. & Miller, S.E. (2016). The importance of long-distance dispersal and
622 establishment events in small insects: historical biogeography of metalmark moths
623 (Lepidoptera, Choreutidae). *Journal of Biogeography*, 43, 1254-1265.
624 40.

625 Salazar, D., Jaramillo, A. & Marquis, R.J. (2016). The impact of plant chemical diversity on
626 plant–herbivore interactions at the community level. *Oecologia*, 181, 1199-1208.
627 41.

628 Salminen, J.P. & Karonen, M. (2011). Chemical ecology of tannins and other phenolics: we
629 need a change in approach. *Functional Ecology*, 25, 325-338.
630 42.

631 Sedio, B.E., Rojas Echeverri, J.C., Boya, P., Cristopher, A. & Wright, S.J. (2017). Sources of
632 variation in foliar secondary chemistry in a tropical forest tree community. *Ecology*,
633 98, 616-623.
634 43.

635 Sourakov, A. & Emmel, T.C. (2001). On the toxic diet of day-flying moths in the Solomon
636 Islands (Lepidoptera: Arctiidae). *Tropical Lepidoptera Research*, 12, 5-6.
637 44.

638 Suzuki, R. & Shimodaira, H. (2015). pvclust: Hierarchical Clustering with P-Values via
639 Multiscale Bootstrap Resampling. R package version 2.0-0. https://CRAN.R-
640 project.org/package=pvclust.
641 45.

642 Ter Braak, C.J. & Smilauer, P. (2012). *Canoco Reference Manual and User's Guide:*
643 *Software for Ordination (version 5.0)*. Microcomputer power, Ithaca.
644 46.

645 Therneau, T., Atkinson, B. & Ripley, B. (2017). rpart: Recursive Partitioning and Regression
646 Trees. R package version 4.1-11. https://CRAN.R-project.org/package=rpart.
647 47.

648 Thompson, J.N. (1994). *The Coevolutionary Process*. University of Chicago Press, Chicago.
649 48.

650 Volf, M., Hrcek, J., Julkunen-Tiitto, R. & Novotny, V. (2015). To each its own: differential
651 response of specialist and generalist herbivores to plant defence in willows. *Journal*
652 *of Animal Ecology*, 84, 1123-1132.
653 49.

654 Whitfeld, T.J.S., Novotny, V., Miller, S.E., Hrcek, J., Klimes, P. & Weiblen, G.D. (2012).
655 Predicting tropical insect herbivore abundance from host plant traits and phylogeny.
656 *Ecology*, 93, S211-S222.
657 50.

658 Wink, M. (2003). Evolution of secondary metabolites from an ecological and molecular
659 phylogenetic perspective. *Phytochemistry*, 64, 3-19.

660

Data Accessibility

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.

Supporting Information

Additional Supporting Information may be downloaded via the online version of this article at Wiley Online Library (www.ecologyletters.com).

As a service to our authors and readers, this journal provides supporting information supplied by the authors. Such materials are peer-reviewed and may be re-organized for online delivery, but are not copy-edited or typeset. Technical support issues arising from supporting information (other than missing files) should be addressed to the authors.

Tables

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

Response Variable	Whole Community	Choreutidae	Pyraloidea
Protease Latex	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%

Table 2. The results of GLMM and PGLMM analyses giving model coefficients and significance with fixed effects listed, and random effects being *Ficus* species and herbivore species for GLMMs. For PGLMMs the additional random effect of phylogenetic covariance was included. Only significant results are shown.

Fixed Effect	Estimate	Standard Error	z-value	p-value
Protease Latex	-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
Fixed Effect	Estimate	Standard Error	z-value	p-value
Protease Latex	-5.956	2.723	-2.187	0.029
Polyphenol Diversity	1.783	0.813	2.192	0.028

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. Traits showing significant phylogenetic signal are in bold and marked with *. Lambda values are given for the traits following the Lambda model of evolution.

	Model (AIcc)	**K**	**PIC observed mean**	**PIC randomized mean**	**PIC p**
Protease in latex	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***

Figures

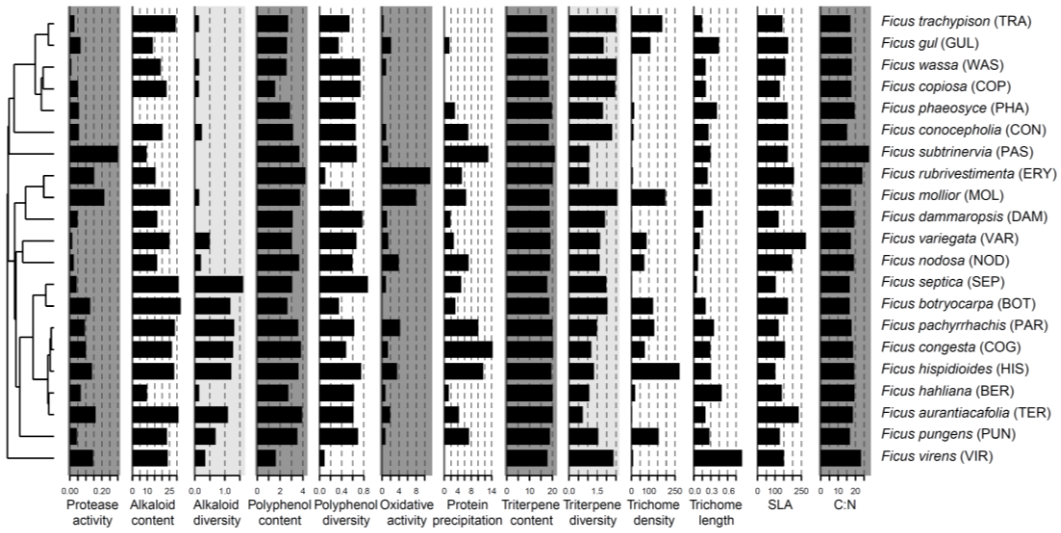


Figure 1. Distribution of *Ficus* defenses across the phylogeny. Traits following Brownian motion (dark grey), Lambda model of evolution (light grey), and white noise (white) are differentiated by background color. *Ficus* traits include protease activity in latex (ΔA280), alkaloid content (ln(peak area/mg)), 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 SLA (cm^2/g).

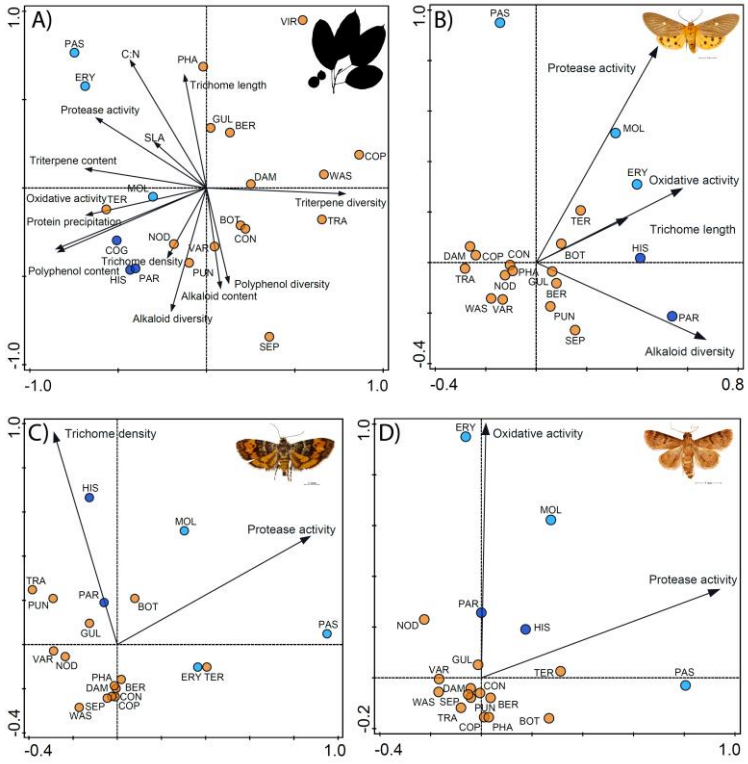


Figure 2. Correlation between *Ficus* traits (A) and response of the whole larval leaf-chewer community (B), Choreutidae (C), and Pyraloidea (D) to host-plant traits. The correlation between *Ficus* traits was visualized by a PCA biplot showing *Ficus* defenses and individual *Ficus* species. First two PCA axes explained 47.9% of variability. The clusters of *Ficus* species with distinctive defenses recovered using Ward´s method with Euclidean distances are color coded – i) high polyphenol content and polyphenol activities (dark blue), ii) high protease activity (light blue), and iii) mixed defenses with low polyphenols (orange). The response of insect communities to the host-plant traits was analyzed using CCA and visualized by biplots showing *Ficus* defenses 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 Figure 1 for the *Ficus* species codes.

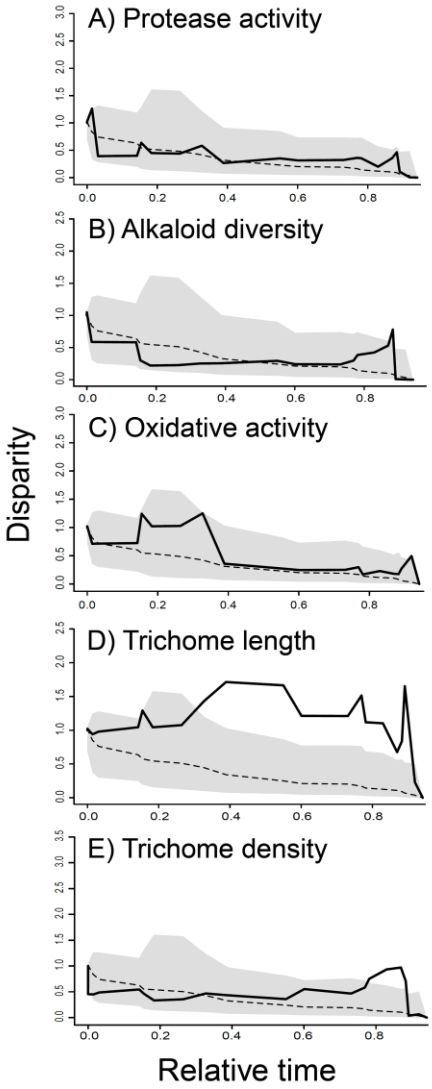


Figure 3. Mean disparity through time (DTT) for traits with significant effects on insect communities (solid line). Plots show disparity in protease activity (A), alkaloid diversity (B), oxidative activity (C), trichome length (D), and trichome density (E). The dashed line indicates the median DTT based 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.

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

Martin Volf, Simon T Segar, Scott E Miller, Brus Isua, Mentap Sisol, Gibson Aubona, Petr Šimek, Martin Moos, Juuso Laitila, Jorma Kim, Jan Zima Jr, Jadranka Rota, George D Weiblen, Stewart Wossa, Juha-Pekka Salminen, Yves Basset and Vojtech Novotny

Appendix S1

Chemical Analysis

For the analysis of protease activity, we sampled latex by cutting the main stem of each leaf and letting latex flow into a 2 ml collection tube for 30 seconds. All latex samples were stored on ice in the field and were not allowed to exceed a temperature of 2 ˚C before being stored at -20 ˚C prior to their analysis. Protease activity was analyzed using the methods of Konno *et al.* (2004) and Agrawal *et al.* (2008) by measuring post-reaction absorption at 280 nm. We modified the methods to deal with solidified latex by adding 50 ul of sodium phosphate buffer to the crude latex and centrifuging for 3000 rpm for 10 minutes at 4˚C, the supernatants were centrifuged again at 3500 rpm for 30 minutes at 4 ˚C. The gums were discarded and 20 µl of latex supernatant was used for the reaction and another 20 µl were used for the control (terminated immediately with trichloroacetic acid as described in Konno et al., 2004).

For the analysis of alkaloids, polyphenols, triterpenes, C:N, and physical traits, we collected two 4.5 cm^2 leaf discs per leaf from 20 young, but fully expanded leaves for each individual, avoiding the central vein (1 g of dry weight in total on average). Half of these leaf discs (0.5 g) were used for the analysis of polyphenols and alkaloids, while the other half (0.5 g) was used for analysis of other traits.

Leaf discs for alkaloid and polyphenol analysis were stored in 40 mL HLPC grade acetone. The storage acetone was transferred to an empty 50 ml Falcon tube and evaporated under N_2. Leaf material was transferred into a new IKA Ultra Turrax Dispenser tube and homogenized and extracted in 50 ml of acetone/water (80:20, v/v). The extract was combined with the evaporated storage acetone extract and the volume of the combined extract was reduced to under 50 ml with N_2. The extract was transferred to a 50 ml flask and volume adjusted to 50 ml by acetone. This extract, containing alkaloids and phenolics was split, with 10 ml being taken for polyphenol analysis and the remaining 40 ml being freeze-dried and used for alkaloid analysis. For the analysis of alkaloids the dried extract was dissolved in 10 ml of 5% HCl, vortexed and transferred into a 15 ml Falcon tube and centrifuged (9000 rpm, 10 min) before being transferred to a 10 ml clear vial, 8 ml of the sample was taken and pH adjusted to 10 with 25% NH_3. The alkaline solution was extracted in a 50 ml extraction funnel with an equal volume of $CHCl_3$. The chloroform solution was dried under nitrogen and dissolved into ethanol, filtered with a 0.2 um PTFE filter and analyzed by UPLC-DAD-Orbitrap MS at the positive ion mode. Acetone was evaporated from the polyphenol extract under N_2, freeze-dried, dissolved in water and filtered with a 0.2 um PTFE filter and analyzed by UPLC-DAD-QqQ-MS/MS.

The UPLC-DAD-Orbitrap MS system for alkaloids consisted of a binary solvent manager, sample manager, column (Acquity UPLC BEH Phenyl, 30 mm × 2.1 mm i.d., 1.7 µm, Waters

Corporation), and photodiode array detector (Acquity UPLC®, Waters Corporation, Milford, MA, USA) coupled to a hybrid quadrupole-Orbitrap mass spectrometer (Q Exactive™, Thermo Fisher Scientific GmbH, Bremen, Germany). The detected alkaloids were assigned to their structural sub-groups by comparing their UV spectral data and MS^2 fragmentation patterns with literature data (Bruneton *et al.* 1983; Baumgartner *et al.* 1990; Xiang *et al.* 2002; Cui *et al.* 2004) and by constructing their molecular formulas from the exact masses obtained with the high-resolution Orbitrap mass spectrometer. The substitution pattern was deduced from the molecular formula (Table S2). Alkaloid quantification (as area of peaks/mg) was obtained with non-targeted Orbitrap analysis. To control for the possible fluctuations in the MS performance, a *Ficus septica* extract was analysed every ten samples and the relative area of ficuseptine was monitored with an extracted ion chromatogram with Orbitrap-MS. Liquid chromatography was performed using a flow rate of 650 µL/min, injection volume of 5 µL, and a gradient mixture of 0.1% (v/v) formic acid in water (solvent A), and acetonitrile (solvent B). The gradient conditions: 0 min, 97% A + 3% B; 0.1 min, 97% A + 3% B; 3.0 min, 55% A + 45% B; 5.0 min, 10% A + 90% B; 7.0 min, 10% A + 90% B; 7.1 min, 97% A + 3% B; 7.2 min, 97% A + 3% B; total analysis time, 7.2 min. MS experiments were carried out on a Q Exactive using a heated ESI source (H-ESI II, Thermo Fisher Scientific GmbH) operated in positive ion mode. For full mass scan the resolving power was at 70,000; automatic gain control (AGC) target was at 3×10^6 ions; maximum injection time (IT) was at 200 ms; the scan range was from 150 to 1200 *m/z*. Ion source condition: spray voltage +4.0 kV; capillary temperature 380°C; Sheath gas (N_2) at 60 (arbitrary units), Aux gas at 20, Spare gas at 0; S-Lens RF level at 60. The data were processed with the Thermo Xcalibur Qual Browser software (Version 3.0.63, Thermo Fisher Scientific). Pierce® LTQ Velos ESI Positive Ion Calibration Solution (Thermo Fisher Scientific Inc., Waltham, MA, USA) was used for the calibration of the detector.

The main polyphenol sub-groups were quantified (as mg/g) by UPLC-DAD-QqQ-MS/MS with the methods of Engström *et al.* (Engström *et al.* 2014; 2015) as described e.g. in Malisch et al. (2016). Furthermore, we measured polyphenol oxidative activity, following Salminen & Karonen (2011), and protein precipitation capacity, following Hagerman's RDA method (Hagerman & Butler 1978). These two activity assays quantify two major functions of polyphenols in anti-herbivore protection.

The leaf discs for measuring other traits were air dried and first used for measuring trichomes and SLA. Then they were homogenized and 50 mg of the powder was used for the analysis of triterpenes while the rest (0.45 g) was used for C:N analysis. To analyze low polar terpenes, approximately 50 mg of dried powdered sample was ground with 1 ml of methanol in a TissueLyser LT (Dynex Technologies, Bustehrad, Czech Republic) at 30 Hz for 2 min. After centrifugation (10,000 rpm) at 8 °C for 10 min, a 100 µl of the supernatant´s aliquot was mixed with 200 µl of methanol containing 0.1% formic acid. Terpenoids were measured on a Dionex Ultimate 3000 LC system coupled to a hybrid quadrupole-Orbitrap mass spectrometer Q Exactive Plus (Thermo Fisher Scientific, San Jose, CA, USA). A reversed phase Kinetex C18 100A HPLC column, 150mm × 2.1 mm i.d., 2.6µm (Phenomenex, Torrance, CA, USA) at 35°C was used for chromatographic separation. Liquid chromatography was performed using flow rate 200 µL/min, autosampler temperature 15 °C and injection volume of 5 µL; using gradient mixture of 0.1% (v/v) formic acid in 2-propanol (solvent A), 0.1% (v/v) formic acid in mixture 2-propanol and methanol (99:1, v/v) (solvent B) and 0.1% (v/v) formic acid in water (solvent C). The gradient conditions used were: 0 min, 0% A + 85% B + 15% C; 12.0 min, 29% A + 70% B + 1% C; 18.5 min, 29% A

+ 70% B + 1% C; 19.0 min, 0 % A + 85% B + 18% C; 25.0 min, 0% A + 85% B + 15%; total analysis time, 30 min.

The non-targeted HPLC-HRMS experiments of terpenoids were carried out in a positive atmospheric pressure chemical ionization mode (APCI) and using a full mass scan (*m/z* 250 – 625) combined with a data dependent MS^2 scanning. The Orbitrap settings were: a full mass scan with the resolving power at 70,000; automatic gain control (AGC) target at 3×10^6 ions; maximum ion injection time (IT) was at 100 ms. The data dependent MS^2 scanning conditions: resolving power at 17 500; automatic gain control (AGC) target at 2×10^5 ions; maximum ion injection time (IT), 100 ms; the isolation window width, 3 Da and the normalized collision energy, 32, TopN 1. The ion source conditions: spray voltage 3.0 kV; capillary temperature 250 °C; sheath gas, 25; auxiliary gas, 5; spare gas, 1; an auxiliary gas heater temperature, 250 °C; S-lense level, 60 (arbitrary units by vendor); external lock mass. Hexakis(2,2-difluoroethoxy)phosphazene (621.0211 Da) was used as post column enrichment of the mobile phase (flow, 1µL/min; concentration at 25 µmol/L). The acquired raw HRMS data were processed by the in-house Metabolite Mapper software after initial characterization of terpenoids on the basis of their exact masses and their comparison with literature (Kitajima *et al.* 1999; Kuo & Chaiang 1999; Kuo & Lin 2004; Feleke & Brehane 2005; Chiang *et al.* 2005; Poumale *et al.* 2008; Rathee *et al.* 2015), (Table S3). The proportion of each detected analyte in the sample set was evaluated as area of peaks/mg. For statistical analysis, the annotated metabolite data matrix was reduced by processing only those metabolites which were detected at least in 50% of the samples employed in the study.

We calculated the Shannon diversity index for alkaloids, polyphenols, and triterpenes. In the case of triterpenes, the diversity was calculated based on the content of individual compounds. In the case of alkaloids and polyphenols, where more detailed structural data were available, the diversity was calculated based on the content of major structural groups to account for structural diversity rather than for the number of compounds in a sample.

Analysis of trichomes, SLA, and C:N

The total number of trichomes per 10 mm^2 and their average length was measured on five leaf discs per individual using ImageJ (ver.1.48) and avoiding the central vein. Values for dorsal and ventral sides of the discs were averaged. In addition we measured two resource acquisition traits correlating with leaf quality which are known to affect insect herbivores – specific leaf area (SLA) and C:N. SLA was measured for each individual using twenty 4.5 cm^2 dried leaf discs which were cut avoiding the central vein. SLA was calculated as the area per mass of these discs. Total carbon and nitrogen content was determined by dry combustion with a CHNS Elemental Analyzer vario MICRO cube (Elementar Analysensysteme GmbH, Germany) using dried and homogenized leaf material.

Ficus Phylogeny Reconstruction

Host-plant phylogeny was reconstructed using four loci: ITS, ETS, G3PD, and GBSSI. We used sequences from Cruaud *et al.* (2012) when available. For species not included in the analysis of Cruaud *et al.* (2012), silica gel dried leaf discs were used to obtain host-plant DNA. We used published procedures, reaction conditions and primer sequences for DNA extraction and PCR amplification (Mason-Gamer *et al.* 1998; Cronn *et al.* 2002; Ronsted *et al.* 2008). Sequences were

assembled and edited using Geneious 5.4 (Drummond *et al.* 2011). The host-plant phylogeny was reconstructed using Bayesian inference as implemented in BEAST v2.1.3 (Drummond *et al.* 2012). The following substitution models were used for individual loci: ITS: GTR+I+G, ETS: HKY+I+G, G3PD: GTR+I+G, GBSSI: HKY+I+G and were selected according to BIC using jModelTest 2 (Darriba *et al.* 2012). We used section level constraints as detailed by Cruaud *et al.* (2012). Sampling was carried out every 10^3 generations for 10^7 generations, the first 10% of all generations were discarded as 'burnin' and the results were summarized with a maximum clade credibility tree. Furthermore, for section *Sycocarpus* we used constraints based on microsatellite data, as this section has undergone a rapid radiation in PNG. We selected nine microsatellite loci previously published for the genus *Ficus* (Moe & Weiblen 2011; Garcia *et al.* 2012), which were amplified in three multiplex sets. The phylogenetic relationships between the species in section *Sycocarpus* were visualized by plotting neighbor joining trees using Nei's distance as implemented in BAPS v5.4 (Corander *et al.* 2004). We used the 'clustering of groups of individuals' method, assigning the five individuals from each species to a group and setting k to 20 to derive the distance matrix.

References

1.

Agrawal, A.A., Lajeunesse, M.J. & Fishbein, M. (2008). Evolution of latex and its constituent defensive chemistry in milkweeds (*Asclepias*): a phylogenetic test of plant defense escalation. *Entomologia Experimentalis et Applicata*, 128, 126-138.

2.

Baumgartner, B., Erdelmeier, C.A., Wright, A.D., Rali, T. & Sticher, O. (1990). An antimicrobial alkaloid fromFicus septica. *Phytochemistry*, 29, 3327-3330.

3.

Bruneton, J., Shamma, M., Minard, R.D., Freyer, A.J. & Guinaudeau, H. (1983). Novel biogenetic pathways from (+)-reticuline. Three dimeric alkaloids:(+)-vanuatine,(+)-vateamine, and (+)-malekulatine. *The Journal of Organic Chemistry*, 48, 3957-3960.

4.

Corander, J., Waldmann, P., Marttinen, P. & Sillanpää, M.J. (2004). BAPS 2: enhanced possibilities for the analysis of genetic population structure. *Bioinformatics*, 20, 2363-2369.

5.

Cronn, R.C., Small, R.L., Haselkorn, T. & Wendel, J.F. (2002). Rapid diversification of the cotton genus (*Gossypium* : *Malvaceae*) revealed by analysis of sixteen nuclear and chloroplast genes. *American Journal of Botany*, 89, 707-725.

6.

Cruaud, A., Ronsted, N., Chantarasuwan, B., Chou, L.S., Clement, W.L., Couloux, A. *et al.* (2012). An extreme case of plant-insect codiversification: Figs and fig-pollinating wasps. *Systematic Biology*, 61, 1029-1047.

7.

Cui, L., Abliz, Z., Xia, M., Zhao, L., Gao, S., He, W. *et al.* (2004). On-line identification of phenanthroindolizidine alkaloids in a crude extract from Tylophora atrofolliculata by liquid chromatography combined with tandem mass spectrometry. *Rapid Communications in Mass Spectrometry*, 18, 184-190.

Darriba, D., Taboada, G.L., Doallo, R. & Posada, D. (2012). jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods*, 9, 772-772.

Drummond, A.J., Ashton, B., Buxton, S., Cheung, M., Cooper, A., Duran, C. *et al.* (2011). Geneious v5.4.

Drummond, A.J., Suchard, M.A., Xie, D. & Rambaut, A. (2012). Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Molecular Biology and Evolution*, 29, 1969-1973.

Engström, M.T., Palijarvi, M., Fryganas, C., Grabber, J.H., Mueller-Harvey, I. & Salminen, J.-P. (2014). Rapid qualitative and quantitative analyses of proanthocyanidin oligomers and polymers by UPLC-MS/MS. *Journal of Agricultural and Food Chemistry*, 62, 3390-3399.

Engström, M.T., Pälijärvi, M. & Salminen, J.-P. (2015). Rapid fingerprint analysis of plant extracts for ellagitannins, gallic acid, and quinic acid derivatives and quercetin-, kaempferol- and myricetin-based flavonol glycosides by UPLC-QqQ-MS/MS. *Journal of Agricultural and Food Chemistry*, 63, 4068-4079.

Feleke, S. & Brehane, A. (2005). Triterpene compounds from the latex of Ficus sur I. *Bulletin of the Chemical Society of Ethiopia*, 19, 307-310.

Garcia, M., Bain, A., Tzeng, H.-Y., Peng, Y.-Q., Chou, L.-S. & Kjellberg, F. (2012). Portable microsatellite primers for *Ficus* (Moraceae). *American Journal of Botany*, 99, E187-E192.

Hagerman, A.E. & Butler, L.G. (1978). Protein precipitation method for the quantitative determination of tannins. *Journal of Agricultural and Food Chemistry*, 26, 809-812.

Chiang, Y.-M., Chang, J.-Y., Kuo, C.-C., Chang, C.-Y. & Kuo, Y.-H. (2005). Cytotoxic triterpenes from the aerial roots of Ficus microcarpa. *Phytochemistry*, 66, 495-501.

Kitajima, J., Kimizuka, K. & Tanaka, Y. (1999). New dammarane-type acetylated triterpenoids and their related compounds of Ficus pumila fruit. *Chemical & Pharmaceutical Bulletin*, 47, 1138-1140.

Konno, K., Hirayama, C., Nakamura, M., Tateishi, K., Tamura, Y., Hattori, M. *et al.* (2004). Papain protects papaya trees from herbivorous insects: role of cysteine proteases in latex. *Plant Journal*, 37, 370-378.
19.

Kuo, Y.-H. & Chaiang, Y.-M. (1999). Five new taraxastane-type triterpenes from the aerial roots of Ficus microcarpa. *Chemical & Pharmaceutical Bulletin*, 47, 498-500.
20.

Kuo, Y.H. & Lin, H.Y. (2004). Two novel triterpenes from the leaves of Ficus microcarpa. *Helvetica Chimica Acta*, 87, 1071-1076.
21.

Malisch, C.S., Salminen, J.-P., Kölliker, R., Engström, M.T., Suter, D., Studer, B. *et al.* (2016). Drought effects on proanthocyanidins in sainfoin (Onobrychis viciifolia Scop.) are dependent on the plant's ontogenetic stage. *Journal of Agricultural and Food Chemistry*, 64, 9307-9316.
22.

Mason-Gamer, R.J., Weil, C.F. & Kellogg, E.A. (1998). Granule-bound starch synthase: structure, function, and phylogenetic utility. *Molecular Biology and Evolution*, 15, 1658-1673.
23.

Moe, A.M. & Weiblen, G.D. (2011). Development and characterization of microsatellite loci in dioecious figs (*Ficus*, Moraceae). *American Journal of Botany*, 98, e25-e27.
24.

Poumale, H.M., Kengap, R.T., Tchouankeu, J.C., Keumedjio, F., Laatsch, H. & Ngadjui, B.T. (2008). Pentacyclic triterpenes and other constituents from Ficus cordata (Moraceae). *Zeitschrift fuer Naturforschung B*, 63, 1335-1338.
25.

Rathee, D., Rathee, S., Rathee, P., Deep, A., Anandjiwala, S. & Rathee, D. (2015). HPTLC densitometric quantification of stigmasterol and lupeol from Ficus religiosa. *Arabian Journal of Chemistry*, 8, 366-371.
26.

Ronsted, N., Weiblen, G., Clement, W., Zerega, N. & Savolainen, V. (2008). Reconstructing the phylogeny of figs (Ficus, Moraceae) to reveal the history of the fig pollination mutualism. *Symbiosis (Rehovot)*, 45, 45.
27.

Salminen, J.P. & Karonen, M. (2011). Chemical ecology of tannins and other phenolics: we need a change in approach. *Functional Ecology*, 25, 325-338.
28.

Xiang, Y., Abliz, Z., Li, L.j., Huang, X.s. & Yu, S.s. (2002). Study of structural characteristic features of phenanthriondolizidine alkaloids by fast atom bombardment with tandem mass spectrometry. *Rapid Communications in Mass Spectrometry*, 16, 1668-1674.

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

Martin Volf, Simon T Segar, Scott E Miller, Brus Isua, Mentap Sisol, Gibson Aubona, Petr Šimek, Martin Moos, Juuso Laitila, Jorma Kim, Jan Zima Jr, Jadranka Rota, George D Weiblen, Stewart Wossa, Juha-Pekka Salminen, Yves Basset and Vojtech Novotny

Supplementary Figures (Fig. S1, Fig. S2, Fig. S3, Fig. S4)

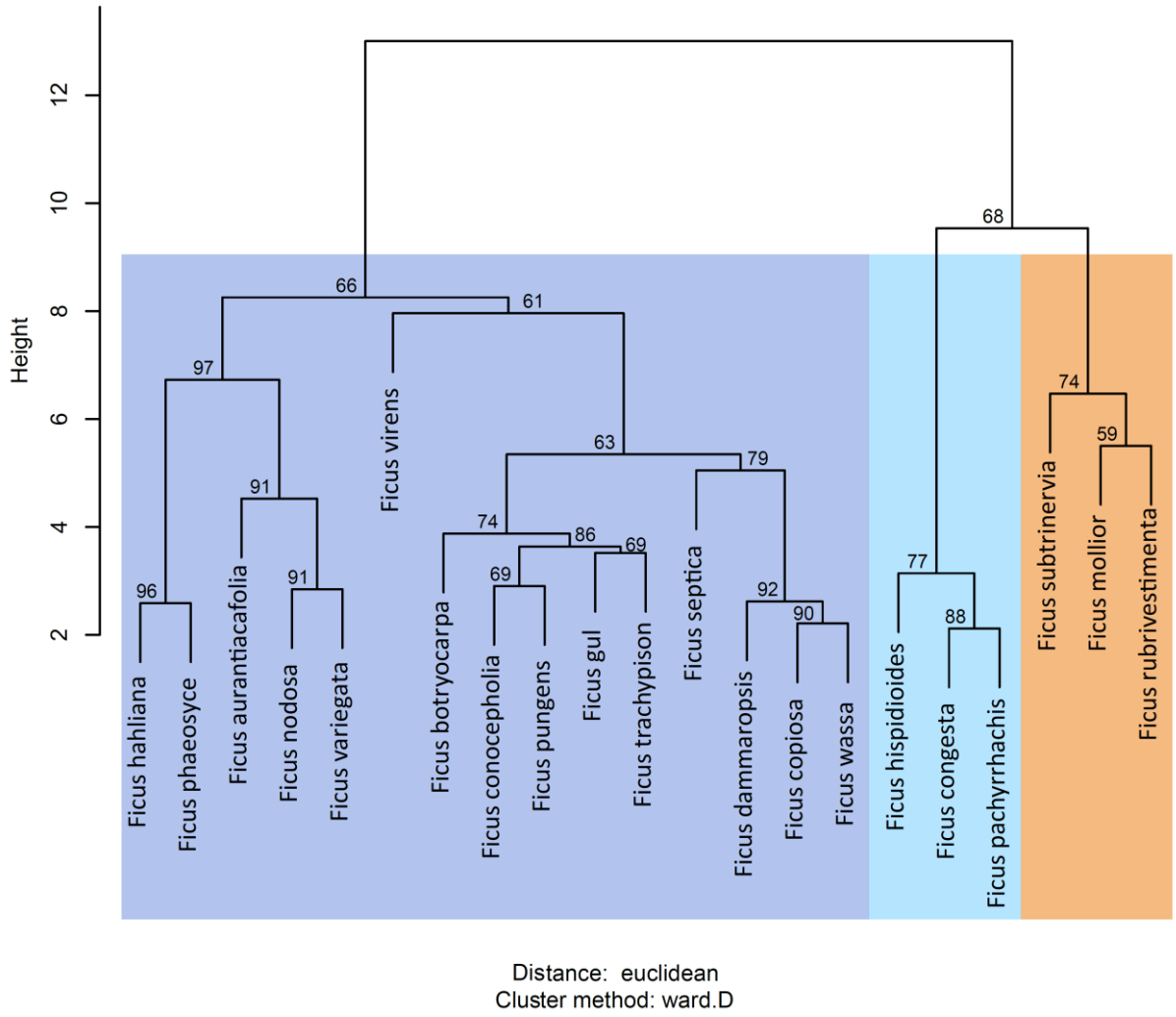


Figure S1. The similarity of *Ficus* species based on their defenses as analyzed by cluster analysis using Ward´s method with Euclidean distances. The optimal number of clusters was selected based on BIC. Clusters are color coded – i) high polyphenols (dark blue), ii) high protease (light blue), and iii) mixed defenses (orange). The numbers show bootstrap support.

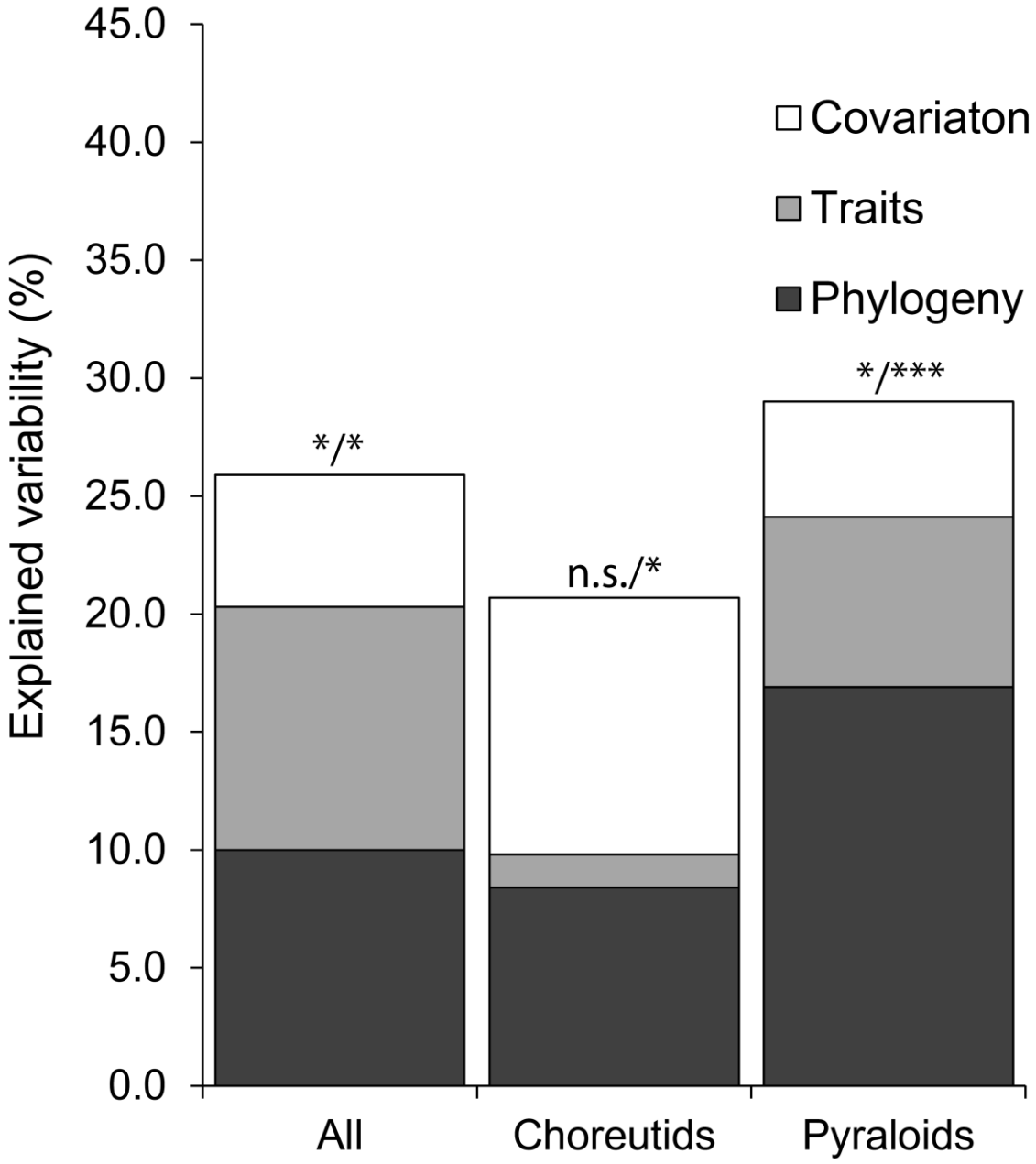


Figure S2. The variability in composition of insect communities explained by *Ficus* traits, phylogeny, and their covariation. The significance of effects of traits / phylogeny is marked above the columns – $p>0.05$ n.s., $p<0.05$ *, $p<0.001$ ***.

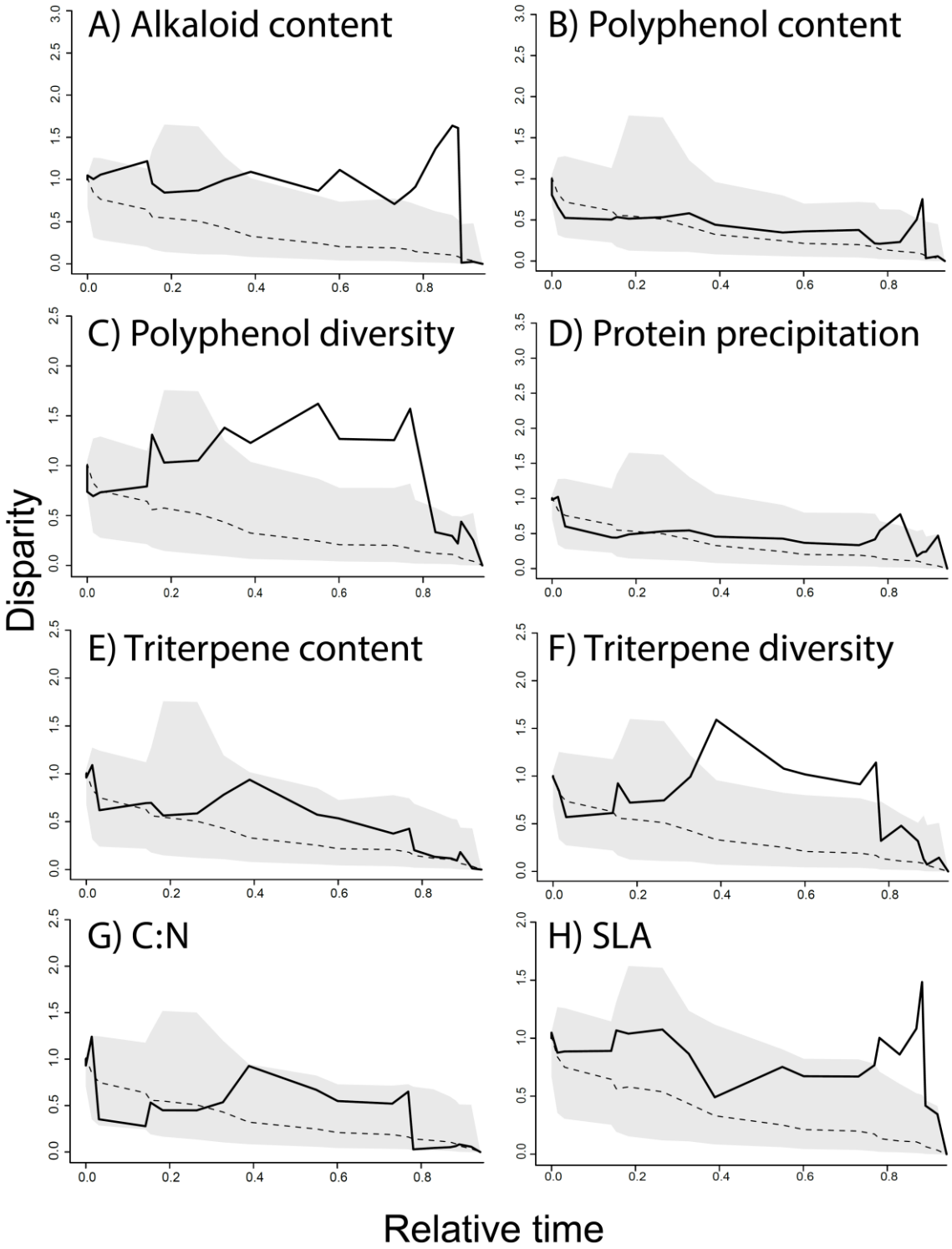


Figure S3. Mean disparity through time (DTT) for traits with no significant effects on insect community structure (solid line). Plots show disparity in alkaloid content (A), polyphenol content (B), polyphenol diversity (C), polyphenol protein precipitation capacity (D), triterpene content (E), triterpene diversity (F), C:N (G), and SLA (H). 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.

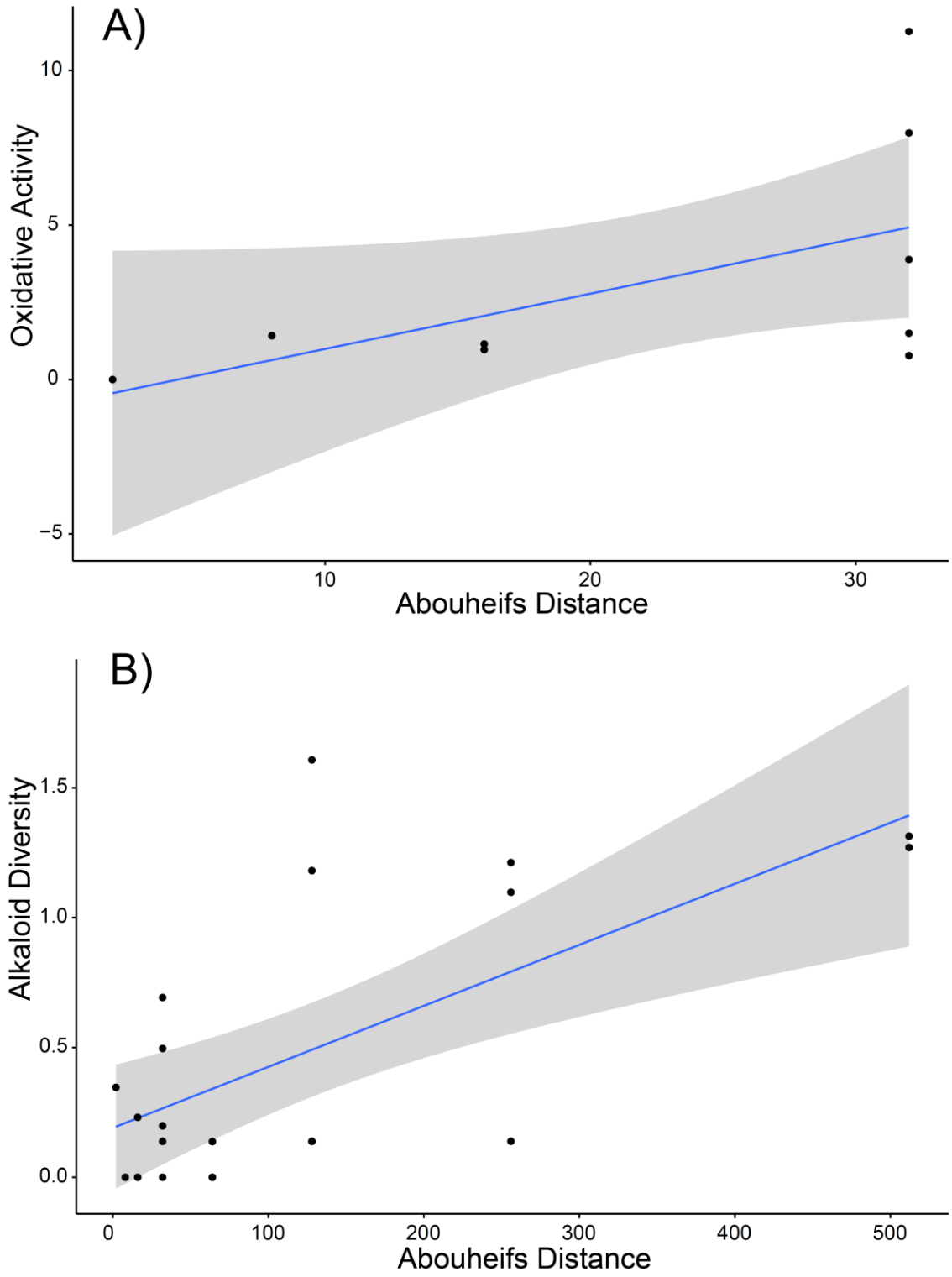


Figure S4. Scatter plots showing the directional increase in polyphenol oxidative activity (A) and alkaloid diversity (B) with distance from the root (Abouheif's distance). Oxidative activity increased only in more ancestral clades ($F=0.001_{19,1}$, $p=0.969$, $R^2<0.01$) while alkaloid diversity (Shannon group diversity) increased across the whole phylogeny ($F=14.101_{19,1}$, $p=0.001$, $R^2=0.32$).

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

Martin Volf, Simon T Segar, Scott E Miller, Brus Isua, Mentap Sisol, Gibson Aubona, Petr Šimek, Martin Moos, Juuso Laitila, Jorma Kim, Jan Zima Jr, Jadranka Rota, George D Weiblen, Stewart Wossa, Juha-Pekka Salminen, Yves Basset and Vojtech Novotny

Table S1. Insect data used in the analyses. The data were take from Novotny *et al.* (2010). Choreutid data were refined using barcode data, which became avaiable after 2010. The table shows number of individuals sampled on individual *Ficus* species (see Fig. 1 for species abbreviations).

Order	Family	Genus	Species	Sp. Code	BER	BOT	CON	COP	DAM	ERY	GUL	HIS	MOL	NOD	PAR	PAS	PHA	PUN	SEP	TER	TRA	VAR	WAS	Total
Col.	Chrysomelidae	*Cadmus*	*acalyphae*	CHRY218	0	0	0	0	0	0	0	27	0	0	0	0	0	0	0	0	0	0	0	27
Col.	Chrysomelidae	*Cadmus*	*sp. nr. acalyphae*	CHRY219	33	0	0	0	0	0	0	26	0	0	32	0	0	0	0	0	0	0	0	91
Lep.	Arctiidae	*Darantasia*	*caerulescens*	ARCT002	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1
Lep.	Bombycidae	*Elachyophthalma*	*cf. kebeae*	DREP008	0	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7
Lep.	Crambidae	*Authaeretis*	*eridora*	CRAM028	0	0	0	1	0	0	0	5	0	0	0	0	0	0	0	0	0	0	0	6
Lep.	Crambidae	*Cotachena*	*histricalis*	CRAM014	0	0	1	0	0	0	0	0	0	4	0	0	0	0	2	0	2	0	1	10
Lep.	Crambidae	*Cydalima*	*marginalis*	PYRA005	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1
Lep.	Crambidae	*Dracaenura*	*albonigralis*	CRAM025	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1
Lep.	Crambidae	*Glyphodes*	*caesalis*	CRAM030	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4
Lep.	Crambidae	*Glyphodes*	*doleschalii*	CRAM016	0	0	0	0	0	0	0	0	0	0	0	7	0	0	0	0	0	0	0	7
Lep.	Crambidae	*Glyphodes*	*eurygania*	CRAM017	0	0	0	0	0	0	0	0	0	0	0	30	0	0	0	0	0	0	0	30
Lep.	Crambidae	*Glyphodes*	*margaritaria*	CRAM003	5	12	16	4	0	7	13	0	5	18	0	4	7	2	27	11	4	33	7	175
Lep.	Crambidae	*Glyphodes*	*sp. cf. stolalis*	CRAM008	0	0	0	0	0	0	0	0	2	3	1	0	0	2	0	0	0	29	3	40
Lep.	Crambidae	*Haritalodes*	*adjunctalis*	CRAM012	21	13	25	3	11	0	0	4	0	23	0	0	12	5	8	1	13	23	17	179
Lep.	Crambidae	*Herpetogramma*	*platycapna*	CRAM029	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	4
Lep.	Crambidae	*Herpetogramma*	*sp. in bipunctalis complex*	CRAM018	0	0	0	0	1	0	0	0	0	4	0	0	0	2	5	0	0	0	0	12
Lep.	Crambidae	*Herpetogramma (s.l.)*		PYRA016	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Lep.	Crambidae	*Hyalobathra*	*miniosalis*	PYRA020	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1
Lep.	Crambidae	*Meekiaria*		CRAM044	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1
Lep.	Crambidae	*Meroctena*	*staintonii*	CRAM033	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	2
Lep.	Crambidae	*Notarcha*	*sp. nr. quaternalis*	CRAM026	0	0	0	0	0	0	0	1	0	0	0	0	2	0	0	0	2	0	0	5
Lep.	Crambidae	*Parotis*	*hilaralis*	CRAM050	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Lep.	Crambidae	*Parotis*	*sp. nr. marginata*	GEOM001	0	3	4	0	0	0	0	3	1	2	0	0	0	0	0	0	0	1	0	14
Lep.	Crambidae	*Pleuroptya*	*sabinusalis*	CRAM011	0	3	0	1	1	0	0	0	0	0	0	0	1	1	0	1	0	0	0	8
Lep.	Crambidae	*Pycnarmon*	*argenticincta*	CRAM034	0	0	0	0	0	0	0	0	0	3	0	0	0	0	2	0	0	0	0	5
Lep.	Crambidae	*Pycnarmon*	*jaguaralis*	CRAM023	0	0	0	0	0	0	0	0	1	2	0	0	0	0	0	0	0	0	1	4
Lep.	Crambidae	*Pycnarmon*	*sp. nr. dryocentra*	CRAM010	0	0	1	0	0	0	0	3	1	2	0	0	0	0	0	0	1	0	0	8

Order	Family	Genus	Species	Sp. Code	BER	BOT	CON	COP	DAM	ERY	GUL	HIS	MOL	NOD	PAR	PAS	PHA	PUN	SEP	TER	TRA	VAR	WAS	Total
Lep.	Crambidae	*Syntomodera*	*sp. nr. thoasalis*	CRAM020	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	2
Lep.	Crambidae	*Tabidia*	*insanalis*	PYRA022	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	2
Lep.	Crambidae	*Talanga*	*nr. sexpunctalis*	CRAM006	3	2	3	17	4	0	12	2	0	3	0	1	9	1	2	0	3	5	14	81
Lep.	Crambidae	*Talanga*	*deliciosa*	CRAM005	5	0	8	3	3	0	101	1	5	5	0	2	24	6	2	0	16	5	1	187
Lep.	Crambidae	*Talanga*	*excelsalis*	CRAM002	3	2	3	83	2	1	0	0	0	3	0	2	4	2	4	0	37	3	21	170
Lep.	Crambidae	*Talanga*	*polyzonalis*	CRAM009	1	1	9	12	2	0	0	0	0	1	0	0	0	0	0	0	4	0	9	39
Lep.	Crambidae			CRAM075	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Lep.	Erebidae	*Asota*	*carica*	NOCT010	0	1	0	0	0	0	0	10	0	0	90	0	0	0	0	0	0	0	0	101
Lep.	Erebidae	*Asota*	*eusemioides*	NOCT004	0	1	0	0	0	0	0	10	0	0	26	1	0	0	0	0	0	0	0	38
Lep.	Erebidae	*Asota*	*heliconia*	NOCT002	0	9	2	0	1	0	0	34	0	0	2	0	0	3	30	0	0	4	1	86
Lep.	Erebidae	*Asota*	*orbona*	NOCT003	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
Lep.	Erebidae	*Asota*	*plana*	NOCT009	0	0	0	0	0	0	0	0	0	8	27	0	0	0	0	0	0	0	0	35
Lep.	Erebidae	*Homodes*	*iomolybda*	THYR009	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	2
Lep.	Erebidae	*Mecistoptera*		XXXX092	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1
Lep.	Erebidae	*Mocis*	*trifasciata*	NOCT079	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1
Lep.	Erebidae	*Ophyx*	*bilinea*	NOCT076	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1
Lep.	Erebidae	*Ophyx*	*crinipes*	NOCT099	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1
Lep.	Erebidae	*Rusicada*	*revocans*	NOCT011	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Lep.	Gelechiidae			TORT055	0	0	3	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4
Lep.	Geometridae	*Ectropis*	*bhurmitra*	GEOM015	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1
Lep.	Geometridae	*Gymnoscelis*	*lavella*	TORT056	0	0	0	0	1	0	0	3	0	0	0	0	2	0	0	0	0	0	0	6
Lep.	Geometridae	*Scopula*	*amala*	GEOM051	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1
Lep.	Choreutidae	*Brenthia*		JR34	0	0	14	11	0	10	0	0	1	0	0	1	1	0	0	12	0	0	1	51
Lep.	Choreutidae	*Brenthia*		JR34a	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	2
Lep.	Choreutidae	*Brenthia*		JR34b	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	1	0	0	0	3
Lep.	Choreutidae	*Brenthia*		JR34c	0	0	0	5	0	3	2	0	1	0	2	1	0	0	0	2	0	0	1	17
Lep.	Choreutidae	*Brenthia*		JR37	0	0	0	0	32	0	0	0	2	0	0	0	0	0	0	0	0	0	0	34
Lep.	Choreutidae	*Brenthia*		JR54	8	5	4	0	2	0	1	18	7	1	8	0	0	20	0	0	0	5	0	79
Lep.	Choreutidae	*Brenthia*		JR55	0	0	0	0	0	0	0	0	0	0	22	0	0	0	0	0	0	0	0	22
Lep.	Choreutidae	*Brenthia*		JR62	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	2
Lep.	Choreutidae	*Brenthia*		JR66	0	0	0	0	0	0	0	0	0	0	0	0	0	7	0	2	0	0	0	9
Lep.	Choreutidae	*Brenthia*		JR67	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	4
Lep.	Choreutidae	*Brenthia*		CHOR003	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	2

Order	Family	Genus	Species	Sp. Code	BER	BOT	CON	COP	DAM	ERY	GUL	HIS	MOL	NOD	PAR	PAS	PHA	PUN	SEP	TER	TRA	VAR	WAS	Total
Lep.	Choreutidae	*Brenthia*		CHOR016	0	0	0	0	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10
Lep.	Choreutidae	*Brenthia*		Bren.sp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1
Lep.	Choreutidae	*Choreutis*	*argoxantha*	Chor.arg.	0	0	0	0	0	0	0	0	2	0	1	0	0	0	0	0	0	0	0	3
Lep.	Choreutidae	*Choreutis*	*basalis*	TORT012	3	6	4	0	2	1	0	5	1	2	6	0	1	1	37	0	0	0	1	70
Lep.	Choreutidae	*Choreutis*	*cf. anthorma*	TORT005	56	36	188	22	29	1	1	22	2	17	4	2	129	29	16	11	35	42	9	651
Lep.	Choreutidae	*Choreutis*	*cf. limonias*	Chor.lim.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
Lep.	Choreutidae	*Choreutis*	*chi*	TORT013	0	0	0	1	0	0	0	0	0	0	0	0	2	0	0	0	0	0	12	15
Lep.	Choreutidae	*Choreutis*		JR05	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	2
Lep.	Choreutidae	*Choreutis*	*lutescens*	TORT006	3	4	4	28	2	0	0	3	0	7	0	0	6	1	1	0	2	8	16	85
Lep.	Choreutidae	*Choreutis*		TORT018	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	2
Lep.	Choreutidae	*Choreutis*		CHOR011	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	1	0	0	0	5
Lep.	Choreutidae	*Choreutis*		JR11	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Lep.	Choreutidae	*Niveas*	*kone*	TORT015	0	2	0	0	0	0	0	0	0	14	0	0	1	1	0	0	0	43	1	62
Lep.	Choreutidae	*Saphta*	*sp. cf exanthista & divitiosa*	TORT009	0	6	0	0	0	3	0	0	2	0	0	0	1	0	8	1	0	0	0	21
Lep.	Choreutidae	*Saptha*		JR1	0	0	0	0	0	1	0	0	1	0	1	11	0	0	0	0	0	0	0	14
Lep.	Choreutidae	*Saptha*	*libanota*	TORT016	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	3
Lep.	Immidae	*Moca*	*congrualis*	TORT071	0	0	0	1	0	0	0	0	0	2	0	0	0	0	0	0	0	1	1	5
Lep.	Limacodidae			LIMA002	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1
Lep.	Limacodidae			LIMA008	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	2
Lep.	Limacodidae			LIMA001	4	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	5
Lep.	Lycaenidae	*Philiris*	*moira*	LYCA001	1	0	0	1	0	0	0	21	4	1	10	0	0	4	0	0	4	0	0	46
Lep.	Lycaenidae	*Philiris*	*ziska*	LYCA005	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
Lep.	Lymantriidae	*Euproctis*		LYMA003	1	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	3	1	2	9
Lep.	Lymantriidae	*Arctornis*	*sp. nr. intacta*	LYMA007	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	3
Lep.	Lymantriidae	*Artaxa*		LYMA054	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2
Lep.	Lymantriidae	*Lymantria*	*novaguineensis*	LYMA070	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1
Lep.	Lymantriidae	*Nygmiini*		LYMA038	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	2
Lep.	Lymantriidae	*Olene*	*nr. mendosa*	LYMA039	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
Lep.	Lymantriidae	*Orgyia*		LYMA010	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1
Lep.	Lymantriidae	*Orgyia sp.*		LYMA050	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1
Lep.	Lymantriidae	*Orvasca*		LYMA051	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1
Lep.	Lymantriidae	*Orvasca*		LYMA002	0	0	0	1	1	0	0	0	0	0	0	0	2	0	0	0	0	0	3	7
Lep.	Lymantriidae	*Orvasca*		LYMA004	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	1	0	3

Order	Family	Genus	Species	Sp. Code	BER	BOT	CON	COP	DAM	ERY	GUL	HIS	MOL	NOD	PAR	PAS	PHA	PUN	SEP	TER	TRA	VAR	WAS	Total
Lep.	Lymantriidae	*Somena*	*alba*	LYMA060	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Lep.	Lymantriidae	*Teia*	*nr. but not dewara*	LYMA001	0	0	0	0	0	0	0	0	0	2	0	0	0	1	0	0	0	2	1	6
Lep.	Lymantriidae			LYMA009	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
Lep.	Nymphalidae	*Cyrestis*	*acilia*	NYMP002	2	2	3	4	1	0	0	0	0	0	0	5	4	0	7	1	3	5	23	60
Lep.	Nymphalidae	*Euploea*	*algea*	NYMP006	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
Lep.	Nymphalidae	*Euploea*	*leucostictos*	NYMP001	2	1	3	4	1	0	0	1	0	0	0	0	3	0	0	0	1	0	32	48
Lep.	Peleopodidae	*Acria*	*sciogramma*	TORT120	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1
Lep.	Psychidae	*Eumeta*	*variegata*	PSYC001	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1
Lep.	Psychidae	*Hyalarcta*	*sp. nr. nigrescens*	PSYC004	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1
Lep.	Psychidae			PSYC002	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
Lep.	Pyralidae	*Pseudocera*	*trissosticha*	TORT041	1	0	1	0	0	0	0	0	0	0	0	0	2	0	1	0	3	0	2	10
Lep.	Pyralidae			PYRA036	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1
Lep.	Sphingidae			SPHI002	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	2
Lep.	Sphingidae			SPHI003	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	2
Lep.	Sphingidae			SPHI001	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2	1	4
Lep.	Thyrididae	*Mellea*	*ordinaria*	THYR001	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	2
Lep.	Thyrididae	*Striglina*	*asinina*	NOCT048	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Lep.	Tortricidae	*Adoxophyes*	*fasciculana*	TORT034	0	1	0	0	0	0	0	0	0	2	0	0	1	0	0	0	1	5	2	12
Lep.	Tortricidae	*Adoxophyes*		TORT044	0	0	0	0	2	0	0	0	0	2	0	5	2	0	0	0	1	1	0	13
Lep.	Tortricidae	*Adoxophyes*		TORT066	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	2	0	0	0	4
Lep.	Tortricidae	*Adoxophyes*	*templana complex*	TORT008	1	4	1	0	0	0	0	0	0	4	1	1	2	0	0	0	0	5	5	24
Lep.	Tortricidae	*Adoxophyes*	*thoracica*	TORT022	0	5	2	3	2	0	0	2	0	2	0	1	5	0	1	0	0	4	3	30
Lep.	Tortricidae	*Adoxophyes*	*tripselia*	TORT037	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Lep.	Tortricidae	*Ancylophyes*		XXXX114	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	2
Lep.	Tortricidae	*Dudua*	*n. sp. nr. aprobola*	TORT143	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1
Lep.	Tortricidae	*Homona*	*aestivana*	TORT085	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	2
Lep.	Tortricidae	*Homona*	*mermerodes*	TORT040	1	4	0	6	1	0	0	2	0	9	0	0	4	4	2	0	0	6	2	41
Lep.	Tortricidae	*Homona*	*trachyptera*	TORT067	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	2
Lep.	Tortricidae	*Isotenes*	*sp. nr. but not miserana*	TORT061	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1
Lep.	Tortricidae	*Sorolopha*	*epichares*	TORT026	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Lep.	Tortricidae	*Xenothictis*	*gnetivora*	TORT039	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	1	0	3

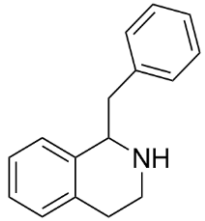
Community structure of insect herbivores is driven by conservatism, escalation and divergence of defensive traits in *Ficus* hosts

Martin Volf, Simon T Segar, Scott E Miller, Brus Isua, Mentap Sisol, Gibson Aubona, Petr Šimek, Martin Moos, Juuso Laitila, Jorma Kim, Jan Zima Jr, Jadranka Rota, George D Weiblen, Stewart Wossa, Juha-Pekka Salminen, Yves Basset and Vojtech Novotny

Table S2. Alkaloid metabolites screened in the study.

Number	Compound type	Molecular formula	Substitution pattern	Exact mass (Da)	Reference	Reference species	Structure of the carbon skeleton
1	Trimethoxy-phenantroindolizidine	$C_{23}H_{25}NO_3$	$3\times OCH_3$	363,1834	Baumgartner et al. (1990)	*F. septica*	
2	Hydroxy-trimethoxy-phenantroindolizidine	$C_{23}H_{25}NO_4$	$3\times OCH_3$ + OH	379,1784	Xiang et al. (2002)	*T. atrofolliculata*	
3	Tetramethoxy-phenantroindolizidine	$C_{24}H_{27}NO_4$	$4\times OCH_3$	393,194	Ueda et al. (2009)	*F. septica*	
4	Dihydroxy-dimethoxy-phenantroindolizidine	$C_{22}H_{23}NO_4$	$2\times OCH_3$ + $2\times$OH	365,1627	Xiang et al. (2002)	*T. atrofolliculata*	
5	Hydroxy-tetramethoxy-phenantroindolizidine	$C_{24}H_{27}NO_5$	$4\times OCH_3$ + OH	409,1889	Damu et al. (2005)	*F. septica*	
6	Pentamethoxy-phenantroindolizidine	$C_{25}H_{29}NO_5$	$5\times OCH_3$	423,2046	Damu et al. (2009)	*F. septica*	
7	Dihydroxy-trimethoxy-phenantroindolizidine	$C_{23}H_{25}NO_5$	$3\times OCH_3$ + $2\times$OH	395,1733	Cui et al. (2004)	*T. atrofolliculata*	
8	Trihydroxy-dimethoxy-phenantroindolizidine	$C_{22}H_{23}NO_5$	$2\times OCH_3$ + 3 OH	381,1576	Xiang et al. (2002)	*T. atrofolliculata*	
9	Hydroxy-methoxy-seco-phenantroindolizidine	$C_{21}H_{23}NO_2$	OCH_3 + OH	321,1729			
10	Dimethoxy-seco-phenantroindolizidine	$C_{22}H_{25}NO_2$	$2\times OCH_3$	335,1885			
11	Hydroxy-dimethoxy-seco-phenantroindolizidine	$C_{22}H_{25}NO_3$	$2\times OCH_3$ + OH	351,1834	Staerk et al. (2002)	*C. vincetoxicum*	
12	Trimethoxy-seco-phenantroindolizidine	$C_{23}H_{27}NO_3$	$3\times OCH_3$	365,1991	Staerk et al. (2002)	*C. vincetoxicum*	
13	Hydroxy-trimethoxy-seco-phenantroindolizidine	$C_{23}H_{27}NO_4$	$3\times OCH_3$ + OH	381,1940	Lee et al. (2011)	*T. ovata*	
14	Tetramethoxy-seco-phenantroindolizidine	$C_{24}H_{29}NO_4$	$4\times OCH_3$	395,2097	Lee et al. (2011)	*T. ovata*	
15	Hydroxy-methoxy-dehydro-seco-phenantroindolizidine	$C_{21}H_{20}NO_2^+$	OCH_3 + OH	318,1494			
16	Dimethoxy-dehydro-seco-phenantroindolizidine	$C_{22}H_{22}NO_2^+$	$2\times OCH_3$	332,1645	Baumgartner et al. (1990)	*F. septica*	
17	Hydroxy-dimethoxy-dehydro-seco-phenantroindolizidine	$C_{22}H_{22}NO_3^+$	$2\times OCH_3$ + OH	348,1594			
18	Trimethoxy-dehydro-seco-phenantroindolizidine	$C_{23}H_{24}NO_3^+$	$3\times OCH_3$	362,1751			
19	Hydroxy-trimethoxy-dehydro-seco-phenantroindolizidine	$C_{23}H_{24}NO_4^+$	$3\times OCH_3$ + OH	378,1700			
20	Tetramethoxy-dehydro-seco-phenantroindolizidine	$C_{24}H_{26}NO_4^+$	$4\times OCH_3$	392,1856	Ueda et al. (2009)	*F. septica*	
21	Dihydroxy-dimethoxy-dehydro-seco-phenantroindolizidine	$C_{22}H_{22}NO_4^+$	$2\times OCH_3$ + $2\times$OH	364,1549			
22	Hydroxy-dimethoxy-N-methyl-tetrahydrobenzylisoquinoline	$C_{19}H_{23}NO_3$	$2\times OCH_3$ + OH + Me	313,1678	Jeong et al. (2012)	*C. ternata*	

23	Dihydroxy-methoxy-N-methyl-tetrahydrobenzylisoquinoline	$C_{19}H_{23}NO_4$	$2\times OCH_3 + 2\times OH + Me$	329,1627	Khan et al. (1993)	*F. pachyrrachis*
24	Hydroxy-trimethoxy-N-methyl-tetrahydrobenzylisoquinoline	$C20H_{25}NO_4$	$3\times OCH_3 + OH + Me$	343,1784	Jeong et al. (2012)	*C. ternata*
25	Dihydroxy-trimethoxy-N-methyl-tetrahydrobenzylisoquinoline	$C_{20}H_{25}NO_5$	$3\times OCH_3 + 2\times OH + Me$	359,1733		
26	Hydroxy-tetramethoxy-N-methyl-tetrahydrobenzylisoquinoline	$C_{21}H_{27}NO_5$	$4\times OCH_3 + OH + Me$	373,1889		
27	Pentamethoxy-N-methyl-tetrahydrobenzylisoquinoline	$C_{22}H_{29}NO_5$	$5\times OCH_3 + Me$	387,2046		
28	Trihydroxy-tetramethoxy-tetrahydrobenzylisoquinoline	$C_{21}H_{27}NO_7$	$4\ OCH_3 + 3\ OH$	405,1788		
29	Ficuseptamine A or B	$C_{15}H_{23}NO_3$	-	265,1678	Ueda et al. (2009)	*F. septica*



References

Baumgartner, B., Erdelmeier, C.A.J., Wright, A.D., Rali, P., Sticher, O. (1990). An antimicrobial alkaloid from *Ficus septica*. Phytochemistry, 29, 3327-3330.

Cui, L., Abliz, Z., Xia. M., Zhao, L., Gao, S., He, W., Xiang, Y., Liang, F., Yu, S. (2004). On-line identification of phenantroindolizidine alkaloids in a crude extract from *Tylophora atrofolliculata* by liquid chromatography combined with tandem mass spectrometry. Rapid communications in mass spectrometry, 18, 187-190.

Damu, A.G., Kuo, P.C., Shi, L.S., Li, C.Y., Kuoh, C.S., Wu, P.L., Wu, T.S. (2005). Phenantroindolizidine alkaloids from the stems of *Ficus septica*. Journal of Natural Products, 68, 1071-1075.

Damu, A.G., Kuo, P.C., Shi, L.S., Li, C.Y., Su, C.R., Wu, T.S. (2009). Cytotoxic phenantroindolizidine alkaloids from the roots of Ficus septica. Planta medica, 75, 1152-1156.

Jeong, E.K., Lee, S.Y., Yu, S.M., Park, N.H., Lee, H.S., Yim, Y.H., Hwang, G.S., Cheong, C., Jung, J.H., Hong, J. (2012) Identificaiton of structurally diverse alkaloids in *Corydalis* species by liquid chromatography/electrospray ionization tandem mass spectrometry. Rapid Communications in Mass Spectrometry, 26, 1661-1674.

Khan, I.A., Rali, T., Sticher, O., (1993) Alkaloids from *Ficus pachyrhachis*. Planta Medica, 59, 286.

Lee, Y.Z., Huang, C.W., Yang, C.W., Hsu, H.Y., Kang, I.J., Chao, Y.S., Chen, I.S., Chang, H.Y., Lee, S.J. (2011). Isolation and biological activities of phenantroindolizidine and septicinealkaloids from the Formosan *Tylophora ovata*. Planta Medica, 77, 1932-1938.

Staerk, D., Lykkeberg, A.K., Christensen, J., Budnik, B.A., Abe, F., Jaroszewski, J.W. (2002). In vitro cytotoxic activity of phenantroindolizidine alkaloids from *Cynanchum vincetoxicum* and *Tylophora ranakae* drug-sensitive and multidrug-resistant cancer cells. Journal of Natural Products, 65, 1299-1302.

Ueda, J.Y., Takagi, M., Kazuo, S.Y. (2009). Aminocaprophenone- and pyrrolidine-type alkaloids from the leaves of Ficus septica. Journal of natural products, 72, 2181-2183.

Xiang, Y., Abliz, Z., Li, L.J., Huang, X.S., Yu, S.S. (2002). Study of structural characteristic features of phenantroindolizidine alkaloids by fast atom bombardment with tandem mass spectrometry. Rapid Communications in Mass Spectrometry, 16, 1668-1674.

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

Martin Volf, Simon T Segar, Scott E Miller, Brus Isua, Mentap Sisol, Gibson Aubona, Petr Šimek, Martin Moos, Juuso Laitila, Jorma Kim, Jan Zima Jr, Jadranka Rota, George D Weiblen, Stewart Wossa, Juha-Pekka Salminen, Yves Basset and Vojtech Novotny

Table S3. Terpenoid metabolites screened in the study.

Compound name	Compound type	Molecular formula	MW	MM	$[M+H]^+$	$[M-H_2O+H]^+$	Absorption Data	Stuructural Data	Reference	Reference species
Stigmasterol	Pentacyclic Triterpene	$C_{29}H_{48}0$	412,69	412,3705	413,3778	395,3672		Yes	Rathee et al. (2011)	*F. religiosa*
Lupeol	Pentacyclic Triterpene	$C_{30}H_{50}O$	426,72	426,3862	427,3935	409,3829		Yes	Rathee et al. (2011)	*F. religiosa*
8,26-cyclo-urs-21-en-3β ,20β -diol (ursane type)	Pentacyclic Triterpene	$C_{30}H_{49}O_2$	441,37	441,3733	442,3806	424,3700	Yes		Poumale et al. (2008)	*F. cordata*
3β -acetoxy-8,26-cyclo-ursan-20β -ol	Pentacyclic Triterpene	$C_{32}H_{52}O_3$	484,00	484,3916	485,3989	467,3883	Yes		Poumale et al. (2008)	*F. cordata*
3-friedelanone	Pentacyclic Triterpene	$C_{30}H_{50}O$	426,72	426,3862	427,3935	409,3829		Yes	Poumale et al. (2008)	*F. cordata*
oleanolic acid	Pentacyclic Triterpene	$C_{30}H_{48}O_3$	456,70	456,3603	457,3676	439,3570		Yes	Poumale et al. (2008)	*F. cordata*
betulinic acid	Pentacyclic Triterpene	$C_{30}H_{48}O_3$	456,70	456,3603	457,3676	439,3570		Yes	Poumale et al. (2008)	*F. cordata*
lupeol acetate	Pentacyclic Triterpene	$C_{32}H_{52}O_2$	468,75	468,3967	469,4040	451,3934			Poumale et al. (2008)	*F. cordata*
α- and β -amyrine	Pentacyclic Triterpene	$C_{30}H_{50}O$	426,72	426,3862	427,3935	409,3829			Poumale et al. (2008)	*F. cordata*
3,5,7,4-tetrahydroxyflavane		$C_{15}H_{14}O_5$	274,00	274,0841	275,0914	257,0808			Poumale et al. (2008)	*F. cordata*
3,5,7,3,4-pentahydroxyflavane		$C_{15}H_{14}O_6$	290,27	290,0790	291,0863	273,0757			Poumale et al. (2008)	*F. cordata*
27-nor-3b -hydroxy-25-oxocycloartane	Cyclopropyl Triterpene	$C_{29}H_{48}O_2$	428,69	428,3654	429,3727	411,3621	Yes	Yes	Chiang et al. (2001)	*F. microcarpa*
(22E)-25,26,27-trinor-3b -hydroxycycloart-22-en-24-al	Cyclopropyl Triterpene	$C_{27}H_{42}O_2$	398,62	398,3185	399,3258	381,3152	Yes	Yes	Chiang et al. (2001)	*F. microcarpa*
3b -acetoxy-15a-hydroxy-13,27-cyclours-11-ene	Cyclopropyl Triterpene	$C_{32}H_{50}O_3$	482,74	482,3760	483,3833	465,3727	Yes	Yes	Chiang et al. (2001)	*F. microcarpa*
3b-acetoxy-12a-formyloxy-13,27-cycloursan-11a-ol	Cyclopropyl Triterpene	$C_{33}H_{52}O_5$	528,76	528,3815	529,3888	511,3782	Yes	Yes	Chiang et al. (2001)	*F. microcarpa*
3b-acetoxy-12,19-dioxo-13(18)-oleanene		$C_{32}H_{48}O_4$	496,36	496,3553	497,3626	479,3520	Yes	Yes	Chiang et al. (2005)	*F. microcarpa*
3b-acetoxy-19(29)-taraxasten-20a-ol		$C_{32}H_{52}O_3$	484,39	484,3916	485,3989	467,3883	Yes	Yes	Chiang et al. (2005)	*F. microcarpa*
3b-acetoxy-21a,22a-epoxytaraxastan-20a-ol		$C_{32}H_{52}O_4$	500,38	500,3866	501,3939	483,3833	Yes	Yes	Chiang et al. (2005)	*F. microcarpa*
3,22-dioxo-20-taraxastene		$C_{30}H_{46}O_2$	438,35	438,3498	439,3571	421,3465	Yes	Yes	Chiang et al. (2005)	*F. microcarpa*
3b-acetoxy-11a,12a-epoxy-16-oxo-14-taraxerene		$C_{32}H_{48}O_4$	496,35	496,3553	497,3626	479,3520	Yes	Yes	Chiang et al. (2005)	*F. microcarpa*
3b-acetoxy-25-methoxylanosta-8,23-diene		$C_{33}H_{54}O_3$	498,41	498,4073	499,4146	481,4040			Chiang et al. (2005)	*F. microcarpa*
oleanolic acid	Pentacyclic Triterpene	$C_{30}H_{48}O_3$	456,70	456,3603	457,3676	439,3570		Yes	Chiang et al. (2005)	*F. cordata*
acetylbetulinic acid		$C_{32}H_{50}O_4$	498,74	498,3709	499,3782	481,3676		Yes	Chiang et al. (2005)	*F. microcarpa*

Compound name	Compound type	Composition	MW	MM	$[M+H]^+$	$[M-H_2O+H]^+$	Absorption Data	Stuructural Data	Reference	Source
betulonic acid		$C_{30}H_{46}O_3$	454,68	544,3447	545,3520	527,3414		Yes	Chiang et al. (2005)	*F. microcarpa*
acetylursolic acid		$C_{32}H_{50}O_4$	498,74	498,3709	499,3782	481,3676		Yes	Chiang et al. (2005)	*F. microcarpa*
ursonic acid		$C_{30}H_{46}O_3$	454,68	454,3447	455,3520	437,3414		Yes	Chiang et al. (2005)	*F. microcarpa*
ursolic acid		$C_{30}H_{48}O_3$	456,70	456,3603	457,3676	439,3570		Yes	Chiang et al. (2005)	*F. microcarpa*
3-oxofriedelan-28-oic acid		$C_{30}H_{48}O_3$	456,70	456,3603	457,3676	439,3570		Yes	Chiang et al. (2005)	*F. microcarpa*
acetate-a-amyrin		$C_{32}H_{52}O_2$	468,00	468,3967	469,4040	451,3934			Feleke and Brehane (2005)	*F. sur*
acetate-b-amyrin		$C_{32}H_{52}O_2$	468,00	468,3967	469,4040	451,3934			Feleke and Brehane (2005)	*F. sur*
3b -acetoxy-22,23,24,25,26,27-hexanordammaran-20-one	Dammarane Type Acetylated Triterp.	$C_{26}H_{42}O_3$	402,00	402,3134	403,3207	385,3101		Yes	Kitajima et al. (1999)	*F. pumilla*
3b -acetoxy-20,21,22,23,24,25,26,27-octanordammaran-17b -ol	Dammarane Type Acetylated Triterp.	$C_{24}H_{40}O_3$	376,00	376,2977	377,3050	359,2944		Yes	Kitajima et al. (1999)	*F. pumilla*
3b -acetoxy-(20R,22E,24RS)-20,24-dimethoxydammaran-22-en-25-ol	Dammarane Type Acetylated Triterp.	$C_{34}H_{58}O_5$	546,82	546,4284	547,4357	529,4251		Yes	Kitajima et al. (1999)	*F. pumilla*
3b -acetoxy-(20S,22E,24RS)-20,24-dimethoxydammaran-22-en-25-ol	Dammarane Type Acetylated Triterp.	$C_{34}H_{58}O_5$	546,82	546,4284	547,4357	529,4251		Yes	Kitajima et al. (1999)	*F. pumilla*
29(20-19)abeolupane-3,20-dione	Lupane Type Triterpene	$C_{30}H_{48}O_2$	440,70	440,3654	441,3727	423,3621			Kuo and Lin (2004)	*F. microcarpa*
19,20-secoursane-3,19,20-trione	Ursane Type Triterpene	$C_{30}H_{48}O_3$	456,70	456,3603	457,3676	439,3570			Kuo and Lin (2004)	*F. microcarpa*
lupenone		$C_{30}H_{48}O$	424,70	424,3705	425,3778	407,3672			Kuo and Lin (2004)	*F. microcarpa*
a-amyrone		$C_{30}H_{48}O$	424,70	424,3705	425,3778	407,3672			Kuo and Lin (2004)	*F. microcarpa*
20(30)-taraxastene-3b ,21a-diol	Taraxastane Type Triterpenes	$C_{30}H_{50}O_2$	442,72	442,3811	443,3884	425,3778	Yes	Yes	Kuo and Chiang (1999)	*F. microcarpa*
20a,21a-epoxytaraxastan-3b -ol	Taraxastane Type Triterpenes	$C_{30}H_{50}O_2$	442,00	442,3811	443,3884	425,3778	Yes	Yes	Kuo and Chiang (1999)	*F. microcarpa*
20-taraxastene-3b ,22b -diol	Taraxastane Type Triterpenes	$C_{30}H_{50}O_2$	442,72	442,3811	443,3884	425,3778	Yes	Yes	Kuo and Chiang (1999)	*F. microcarpa*
and 3b -acetoxy-20-taraxasten-22-	Taraxastane Type Triterpenes	$C_{32}H_{50}O_3$	482,74	482,3760	483,3833	465,3727	Yes	Yes	Kuo and Chiang (1999)	*F. microcarpa*
20-taraxasten-3b -ol (pseudo-Taraxasterol)		$C_{30}H_{50}O$	426,72	426,3862	427,3935	409,3829			Kuo and Chiang (1999)	*F. microcarpa*
3b -acetoxy-11a-methoxy-12-ursene	Ursane Type Triterpene	$C_{33}H_{54}O_3$	498,78	498,4073	499,4146	481,4040	Yes		Kuo and Chiang (2000)	*F. microcarpa*
3b -acetoxy-11a-ethoxy-12-ursene	Ursane Type Triterpene	$C_{34}H_{56}O_3$	512,81	512,4229	513,4302	495,4196	Yes		Kuo and Chiang (2000)	*F. microcarpa*
3b -acetoxy-11a-hydroperoxy-12-ursene	Ursane Type Triterpene	$C_{32}H_{52}O_4$	500,75	500,3866	501,3939	483,3833	Yes		Kuo and Chiang (2000)	*F. microcarpa*
3b -hydroxy-11a-hydroperoxy-12-ursene	Ursane Type Triterpene	$C_{30}H_{50}O_3$	458,72	458,3760	459,3833	441,3727	Yes		Kuo and Chiang (2000)	*F. microcarpa*
3b -acetoxy-11a-ethoxy-12-oleanene	Oleanane Type Triterpene	$C_{34}H_{56}O_3$	512,81	512,4229	513,4302	495,4196	Yes		Kuo and Chiang (2000)	*F. microcarpa*
3b -acetoxy-11a-hydroperoxy-12-oleanene	Oleanane Type Triterpene	$C_{32}H_{52}O_4$	500,75	500,3860	501,3933	483,3827	Yes		Kuo and Chiang (2000)	*F. microcarpa*

References

Chiang, Y.-M., Chang, J.-Y., Kuo, C.-C., Chang, C.-Y. & Kuo, Y.-H. (2005). Cytotoxic triterpenes from the aerial roots of Ficus microcarpa. *Phytochemistry*, 66, 495-501.

Feleke, S. & Brehane, A. (2005). Triterpene compounds from the latex of Ficus sur I. *Bulletin of the Chemical Society of Ethiopia*, 19, 307-310.

Kitajima, J., Kimizuka, K. & Tanaka, Y. (1999). New dammarane-type acetylated triterpenoids and their related compounds of Ficus pumila fruit. *Chemical & pharmaceutical bulletin*, 47, 1138-1140.

Kuo, Y.-H. & Chaiang, Y.-M. (1999). Five new taraxastane-type triterpenes from the aerial roots of Ficus microcarpa. *Chemical & pharmaceutical bulletin*, 47, 498-500.

Kuo, Y.H. & Lin, H.Y. (2004). Two novel triterpenes from the leaves of Ficus microcarpa. *Helvetica chimica acta*, 87, 1071-1076.

Poumale, H.M., Kengap, R.T., Tchouankeu, J.C., Keumedjio, F., Laatsch, H. & Ngadjui, B.T. (2008). Pentacyclic triterpenes and other constituents from Ficus cordata (Moraceae). *Zeitschrift fuer Naturforschung B*, 63, 1335-1338.

Rathee, D., Rathee, S., Rathee, P., Deep, A., Anandjiwala, S. & Rathee, D. (2015). HPTLC densitometric quantification of stigmasterol and lupeol from Ficus religiosa. *Arabian Journal of Chemistry*, 8, 366-371.

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

Martin Volf, Simon T Segar, Scott E Miller, Brus Isua, Mentap Sisol, Gibson Aubona, Petr Šimek, Martin Moos, Juuso Laitila, Jorma Kim, Jan Zima Jr, Jadranka Rota, George D Weiblen, Stewart Wossa, Juha-Pekka Salminen, Yves Basset and Vojtech Novotny

Table S4. Species traits – protease activity (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, SLA (cm^2/g). Polyphenol diversity was based on the content galloyl derivatives, hexahydroxydiphenoyl derivatives, procyanidins, prodelphinidins, quinic acid derivatives, quercetin derivatives, kaempferol derivatives, and myricetin derivatives. Alkaloid diversity was based on the content of phenanthroindolizidines, *seco*-phenanthroindolizidines, dehydro-*seco*-phenanthroindolizidines, tetrahydrobenzylisoquinolines, and ficuseptamines.

Species	Protease activity	Alkaloid content	Alkaloid diversity	Polyphenol content	Polyphenol diversity	Oxidative activity	Protein prec.	Triterpene content	Triterpene diversity	Trichome density	Trichome length	C:N	SLA
F. aurantiacafolia	0.161±0.113	31.09±0.43	1.10±0.00	49.91±0.20	0.62±0.50	1.90±1.36	4.28±8.57	20.22±0.30	0.73±0.16	3.56±4.75	0.21±0.14	18.5±0.7	235.1±26.7
F. botryocarpa	0.127±0.108	32.57±0.44	1.18±0.30	13.15±1.94	0.35±0.36	0	3.22±7.20	18.58±0.25	2.04±0.21	123.92±17.33	0.22±0.11	16.7±2.1	173.3±42.6
F. congesta	0.100±0.176	26.70±1.28	1.27±0.16	44.6±0.54	0.48±0.28	1.40±1.15	14.28±10.39	20.27±0.44	1.18±0.26	76.23±80.53	0.30±0.18	18.6±1.1	156.1±25.0
F. conocepholia	0.057±0.050	20.29±9.99	0.23±0.36	21.87±3.22	0.65±0.47	0.97±1.20	7.13±11.18	18.36±0.18	2.29±0.28	11.24±6.18	0.27±0.35	15.1±1.5	174.8±29.5
F. copiosa	0.051±0.038	23.07±1.97	0.14±0.31	3.76±0.11	0.74±0.51	0	0	18.51±1.72	2.46±0.80	0.44±0.30	0.22±0.12	17.6±2.1	126.6±28.2
F. dammaropsis	0.048±0.054	16.85±11.26	0	21.20±0.51	0.78±0.28	1.15±1.34	1.75±2.61	18.95±0.33	1.91±0.31	2.20±2.89	0.17±0.16	19.3±0.4	120.4±46.2
F. gul	0.067±0.074	13.69±12.52	0	12.60±1.59	0.35±0.43	2.10±0.92	1.54±3.43	18.18±0.85	1.84±0.83	108.75±41.69	0.46±0.28	17.8±2.2	174.6±41.3
F. hahliana	0.067±0.057	9.74±13.34	0.14±0.31	14.33±3.31	0.62±0.39	0.78±1.10	1.34±2.99	20.12±0.24	1.07±0.20	21.12±11.41	0.51±0.31	19.4±1.6	137.8±26.5
F. hispidioides	0.138±0.175	28.11±0.85	1.21±0.16	35.97±0.58	0.75±0.60	3.56±1.44	11.49±13.06	19.47±0.39	1.33±0.12	276.68±80.07	0.32±0.16	19.3±2.8	101.2±13.4
F. mollior	0.215±0.167	25.39±1.87	0.14±0.31	41.52±5.55	0.55±0.53	7.98±14.99	6.45±9.17	18.69±0.10	2.57±0.11	194.48±33.81	0.33±0.19	17.5±0.5	193.2±16.3
F. nodosa	0.023±0.014	16.58±11.39	0.20±0.34	37.86±0.16	0.59±0.43	3.89±2.13	7.14±5.27	18.98±0.82	1.62±0.38	73.97±36.68	0.08±0.05	19.1±2.1	195.4±67.1
F. pachyrrhachis	0.093±0.040	28.61±1.36	1.31±0.14	34.87±0.43	0.63±0.48	4.20±2.62	10.05±6.72	20.12±0.26	1.48±0.21	130.60±44.18	0.37±0.24	17.7±1.0	120.3±30.4
F. phaeosyce	0.057±0.037	0	0	16.46±1.25	0.65±0.57	0	3.09±2.76	19.84±0.61	1.80±0.28	15.50±10.98	0.42±0.38	19.6±1.1	172.0±9.6
F. pungens	0.044±0.025	23.25±1.49	0.69±0.57	32.12±0.21	0.69±0.21	0.78±0.90	7.30±5.39	18.88±0.86	1.55±0.16	156.40±73.43	0.29±0.22	16.8±2.9	126.3±32.5
F. rubrivestimenta	0.152±0.119	15.50±13.43	0	67.03±0.02	0.10±0.15	11.27±0.99	5.18±0.04	20.17±1.69	1.07±0.91	7.12±4.22	0.25±0.19	23.9±3.1	207.9±40.8
F. septica	0.042±0.040	31.26±0.00	1.61±0.00	20.12±4.16	0.87±0.28	0.90±1.27	4.97±7.02	17.75±0.81	1.98±0.30	1.70±2.88	0.06±0.09	16.6±2.8	104.1±5.1
F. subtrinervia	0.302±0.355	9.54±13.07	0	39.45±2.39	0.67±0.33	1.42±1.97	13.02±8.95	21.10±0.21	1.08±0.07	0.07±0.16	0.31±0.31	27.8±4.2	171.8±30.7
F. trachypison	0.028±0.020	29.20±0.51	0.14±0.31	14.03±0.72	0.54±0.26	0.36±0.80	0	17.61±0.66	2.50±0.39	176.4±47.54	0.15±0.12	17.1±1.1	142.4±43.1
F. variegata	0.015±0.011	24.93±2.52	0.50±0.48	19.91±2.29	0.67±0.20	1.50±1.37	2.79±5.92	19.05±0.69	1.65±0.47	86.24±115.67	0.11±0.09	17.4±1.6	274.9±82.6
F. virens	0.145±0.099	23.75±1.56	0.35±0.49	4.10±1.03	0.09±0.13	0	0	17.79±1.09	2.37±0.68	9.40±7.78	0.88±0.62	23.1±2.5	149.3±25.2
F. wassa	0.009±0.005	19.04±10.82	0.14±0.31	12.07±3.10	0.74±0.45	0.98±2.19	0	17.92±0.46	2.53±0.32	0.48±0.40	0.22±0.08	17.8±2.1	158.9±40.2

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

Martin Volf, Simon T Segar, Scott E Miller, Brus Isua, Mentap Sisol, Gibson Aubona, Petr Šimek, Martin Moos, Juuso Laitila, Jorma Kim, Jan Zima Jr, Jadranka Rota, George D Weiblen, Stewart Wossa, Juha-Pekka Salminen, Yves Basset and Vojtech Novotny

Table S5. Escalation of alkaloid diversity (Shannon) and oxidatively active phenolics (mg/g) as analysed by Adonis function. Table shows details on sums of squares (SSq) and p-values for individual *Ficus* species. Significant values are in bold. Species are ordered from the tip (*F. aurantiacafolia*) the root (*F. virens*) of the tree.

	Alkaloid diversity		**Oxidatively active phenolics**	
Species	**SSq**	**p**	**SSq**	**p**
F. aurantiacafolia	**0.01902**	**<0.001**	0.00006	0.854
F. hahliana	**0.01774**	**<0.001**	0.00004	0.874
F. hispidioides	**0.02032**	**<0.001**	0.00011	0.806
F. congesta	**0.02062**	**<0.001**	0.00009	0.828
F. pachyrrhachis	**0.02065**	**<0.001**	0.00012	0.801
F. botryocarpa	**0.01579**	**<0.001**	0.00004	0.855
F. septica	**0.01663**	**<0.001**	0.00002	0.886
F. pungens	**0.00228**	**0.043**	0	0.932
F. nodosa	0.00018	0.579	0.00063	0.297
F. variegata	0.00051	0.35	0.00012	0.649
F. dammaropsis	0.00008	0.723	0.0014	0.128
F. mollior	0.00019	0.634	**0.01107**	**<0.001**
F. rubrivestimenta	0.00023	0.601	**0.01243**	**<0.001**
F. conocepholia	0.00057	0.329	0.00033	0.457
F. copiosa	0.00463	0.053	0.00253	0.163
F. wassa	0.00463	0.053	0.00233	0.181
F. phaeosyce	**0.00478**	**0.047**	0.00249	0.162
F. gul	**0.00483**	**0.049**	0.00187	0.236
F. trachypison	0.00473	0.052	0.00202	0.217
F. subtrinervia	0.00056	0.33	0.00005	0.766
F. virens	0.00004	0.791	0.00035	0.449