

# Macroevolution of defense syndromes in *Ficus* (Moraceae)

by Zhao, J., Segar, S.T., McKey, D. and Chen, J.

**Copyright, publisher and additional information:** this is the author accepted manuscript. The final published version (version of record) is available online via Wiley. ***This article may be used for non-commercial purposes in accordance with Wiley Terms and Conditions for Self-Archiving.*** Please refer to any applicable terms of use of the publisher.

[DOI link to the version of record on the publisher's site](#)



**Harper Adams  
University**

1 Running head: Defense syndromes in *Ficus*

2 Title: Macroevolution of defense syndromes in *Ficus* (Moraceae)

3

4 Authors: Jin Zhao<sup>1</sup>, Simon T. Segar<sup>2</sup>, Doyle McKey<sup>3</sup>, Jin Chen<sup>1\*</sup>

5

6 <sup>1</sup> CAS Key Laboratory of Tropical Forest Ecology, Xishuangbanna Tropical Botanical Garden,

7 Chinese Academy of Sciences, Mengla, Yunnan 666303, People's Republic of China; email:

8 zhaojin@xtbg.org.cn

9

10 <sup>2</sup> Department of Crop and Environment Sciences, Harper Adams University, Newport, TF10

11 8NB, United Kingdom; email: simon.t.segar@gmail.com

12

13 <sup>3</sup>CEFE, UMR 5175, CNRS–University of Montpellier–University Paul–Valéry–EPHE–SupAgro

14 Montpellier–INRA–IRD, Montpellier, France; email: doyle.mckey@cefe.cnrs.fr

15

16 \* Author for correspondence: Jin Chen

17 Telephone: 86-691-8715457

18 Fax: 86-691-8715070

19 E-mail: cj@xtbg.org.cn

20

21 **Abstract (350 words limited)**

22 Recursive adaptations and counter-adaptations of plant-feeding insects are thought to have driven  
23 chemical and physical diversity in plant defenses. Among existing theories on defensive  
24 diversity, the syndromes hypothesis predicts that plants may evolve suites of covarying defense  
25 traits along evolutionary arms races with herbivores. We use the large, phenotypically diverse  
26 angiosperm genus *Ficus* (Moraceae) to test the major predictions of this hypothesis: i) plant  
27 species will form distinctive combinations of defensive traits; ii) these traits will be correlated  
28 within each syndrome. Trait combinations need not map well onto phylogenies because plant  
29 species can converge onto similar trait values, but strong phylogenetic signal driven by selection  
30 (as opposed to drift) suggests roles for escalation and co-evolution. Finally, *Ficus* species with  
31 complementary combinations of defenses will be less susceptible to insect damage and harbor  
32 distinct insect communities. We quantified susceptibility to insect herbivory and nine leaf traits  
33 related to resource acquisition and defense in 36 *Ficus* species growing in a common-garden  
34 setting, in dry and wet seasons over two years. We recovered a set of three syndromes defined by  
35 relatively small sets of trait combinations. Broadly speaking, these syndromes grouped fig  
36 species with different life forms. For example, epiphytic figs had nutrient-poor, tough, tannin-  
37 rich leaves, while free-standing trees tended to have leaves covered in trichomes and full of  
38 alkaloid-rich latex. When season and species identity were accounted for, the combination of two  
39 traits, higher C/N and higher latex tannin content, provided significantly stronger defense than  
40 did either trait taken singly. Several individual traits (C/N, latex tannin and trichome density)  
41 were significantly negatively correlated with herbivore damage, while alkaloid content was

42 positively correlated (perhaps as a result of feeding by adapted herbivores). Several defensive  
43 traits influenced insect herbivore community structure. Finally, traits followed different  
44 evolutionary trajectories. While latex tannin, C/N and leaf tannin fit a Brownian-motion model of  
45 evolution, the first two escalating across *Ficus* phylogeny, others appeared to have more limited  
46 phylogenetic signal or tended to de-escalate. Overall, the patterns we detected support the  
47 concept of coordinated defense syndromes, demonstrating that evolutionary arms races can drive  
48 combinations of traits in this genus.

49

50 **Keywords:** Defense syndrome, *Ficus*, Trade-off, Life form, Herbivory, Macroevolution,  
51 Phylogeny, Phylogenetic generalized linear mixed model

52

## 53 INTRODUCTION

54 Insects on plants are of great economic and ecological interest; they dominate terrestrial  
55 ecosystems in terms of species richness and abundance (Price 2002). While a large proportion of  
56 plants rely on insects for pollination, many are also under attack by insect herbivores (Ehrlich  
57 and Raven 1964). Multiple traits help to defend plants against leaf-chewing insects, including,  
58 but not limited to, i) limitations to nutritional quality (e.g., proteins and antiproteins) (Green and  
59 Ryan 1972, Ryan 1990), ii) physical defenses (e.g., spines, trichomes, and leaf toughness)  
60 (Wagner 1991, Lucas et al. 2000), iii) toxicity to insects (e.g., cyanogens and alkaloids) (Bennett  
61 and Wallsgrave 1994), iv) adaptive phenology (Feeny 1970, Takahashi and Yamauchi 2010), v)  
62 high regrowth capacity (i.e., tolerance) (Fornoni 2011), and vi) indirect defenses (e.g., volatile  
63 compounds that attract enemies of phytophagous insects) (Aljory and Chen 2016). Which trait,  
64 or suites of traits, contribute(s) to effective defense, and whether these traits are constrained by  
65 host phylogeny or ecological factors, are fundamental questions in plant defense (Agrawal and  
66 Fishbein 2006, Agrawal 2007). Although a range of different theories exist as to the origin and  
67 diversification of plant defenses, our understanding of the relative contribution of local  
68 ecological conditions vs. historical or physiological constraints in shaping the observed inter-  
69 specific variation in defenses is still limited (Agrawal 2007, 2011, Nuñez-Farfan et al. 2007, Züst  
70 and Agrawal 2017).

71 Plants have evolved different combinations of traits (Díaz et al. 2015, Wright et al. 2004),  
72 and typically employ a broad arsenal of defensive traits against herbivores (Duffey and Stout  
73 1996, Romeo et al. 1996). Several hypotheses envisaging syndromes of defensive traits have

74 been developed. Silvertown and Dodd (1996) showed that herbaceous and woody plants have  
75 distinct types of chemical defenses (alkaloids and tannins, respectively). Because growth and  
76 defense are interlinked (Kursar and Coley 2003, Züst and Agrawal 2017), defense syndromes  
77 also include growth-related traits. The repeated appearance of a particular defense syndrome  
78 might be driven by convergent adaptation of multiple traits in unrelated plant species growing  
79 under similar ecological conditions and herbivore pressures (Coley et al. 1985, Fine et al. 2006).  
80 Certain combinations of traits have been repeatedly selected across unrelated species for defense  
81 against herbivores in tropical forest. Kursar and Coley (2003) argue that in their production of  
82 young leaves, trees fall along an escape-defense continuum: young leaves of extreme “escape”  
83 species are predicted to have few chemical defenses, but rapid synchronous leaf expansion and  
84 low leaf nutritional quality during expansion; young leaves of extreme “defense” species have  
85 high chemical defense, low nutritional quality, and asynchronous leaf expansion. Ecologists also  
86 hypothesize that shared defensive traits or strategies could reflect a common ancestry; for  
87 example, Pearse and Hipp (2009) found that interactions between introduced oaks and their  
88 herbivores are driven independently by traits that track plant phylogeny.

89       When pairs of traits are considered separately from others, trade-offs between traits can be  
90 found (Steward and Keeler 1988, Herms and Mattson 1992). If defensive traits are costly, or to  
91 some extent redundant, then trade-offs will occur, especially when plants are nutrient-limited.  
92 Trade-offs can involve not only different types of chemical-defense compounds but also physical  
93 and chemical defenses (Steward and Keeler 1988, Eichenberg et al. 2015) and the ways in which  
94 defenses are deployed, e.g. between constitutive (always active) and inducible defenses

95 (activated through a previous consumer and conferring some degree of resistance to subsequent  
96 attacks) (Morris et al. 2006, Kempel et al. 2011). However, some studies have found limited  
97 evidence of defense syndromes and trade-offs. Mason et al. (2016) did not observe any defense  
98 syndrome based on trade-offs. Instead, they found leaf defenses to be strongly related to a plant's  
99 position on the leaf economic spectrum, with higher defenses being found in *Helianthus* species  
100 with more resource-conservative leaf economic traits supporting a slower-growth persistence  
101 strategy. However, there was little support for the evolution of higher defenses in low-resource  
102 habitats (but see Fine et al. 2006). The contrasting results of previous studies might suggest that  
103 coadaptation among defensive traits could be lineage-dependent (Agrawal 2011, Johnson et al.  
104 2014). More empirical studies of additional lineages are necessary to address whether and how  
105 defensive traits of a plant are co-adapted, while data on the specialization and identity of the  
106 insect herbivores provides much-needed context (Volf et al. 2019).

107 *Ficus* is a large, pantropical genus with about 800 species (Berg and Corner 2005),  
108 representing an important component of tropical floras. The genus is particularly species-rich in  
109 South-East Asia (Berg 1989, Stevens 2001). Figs are also ecologically diverse, with different life  
110 forms, including epiphytes, hemi-epiphytes (epiphytes that become free-standing trees as they  
111 grow), and free-standing trees. Fig plants are attacked by a wide range of different herbivores  
112 (Novotny and Basset 2005). Furthermore, figs are latex-bearing and latex is well known for its  
113 defensive role against herbivores (Agrawal and Konno 2009, Bauer and Speck 2012). Several  
114 important studies have explored the functional traits of *Ficus* in relation to defense against  
115 herbivores and their evolutionary implications (Volf et al. 2018, Villard et al. 2019). For

116 example, in a recent study, Volf et al. (2018) showed an evolutionary escalation of alkaloid  
117 diversity and of oxidative activity in fig species, which may have resulted from adaptation to  
118 generalist herbivorous insects. In contrast, they found significant divergence in the density of leaf  
119 trichomes between closely related fig species, which may have been driven by specialist  
120 herbivores. Other studies, conducted at our field site, have demonstrated that there is a trade-off  
121 between leaf water flux capacity and leaf drought tolerance-related traits such as LMA (leaf mass  
122 per area) across hemi-epiphytic and free-standing *Ficus* species (Hao et al. 2010). As the inverse  
123 of LMA, SLA (leaf area per mass) is considered to be related not only to plant palatability but  
124 also to plant growth (Schädler et al. 2003, Wright et al. 2004), implying that there is no strict  
125 boundary between traits evolved in response to biotic or to abiotic pressures. Some traits that  
126 affect leaf water exchange can also affect plants' resistance to herbivores or their ability to  
127 regrow after herbivory.

128         In this study, we combine comparative phylogenetics, detailed plant trait data, insect  
129 herbivore data and field observations to investigate the macroevolution of leaf defenses and  
130 resistance to herbivory in *Ficus* (Moraceae) in a common-garden setting. The use of common-  
131 garden experiments can minimize the contribution of plastic phenotypic adjustments to local site  
132 conditions, allowing the detection of genetically based species differences (Garland and Adolph  
133 1991). In this study, the individuals of the different *Ficus* spp. we studied are distributed in a  
134 relatively small area (about 1.3 ha), meaning that they are all exposed to a relatively uniform  
135 local community of herbivores.

136         Specifically, we addressed the following four questions: (1) Can defensive phenotypes of



137 *Ficus* spp. be classified into defense syndromes? (2) Are there correlations among defensive  
138 traits within each syndrome? (3) Are phylogenetic relationships among *Ficus* species congruent  
139 with patterns of defense trait similarity? (4) Which traits best predict observed herbivore damage  
140 and insect herbivore community structure, and do combinations of traits better predict resistance  
141 to attack than single traits?

142

## 143 **METHODS**

### 144 *Study system and study site*

145 The study was conducted in Xishuangbanna Tropical Botanical Garden (XTBG; 21°56'N,  
146 101°15'E, elevation 580 m), Chinese Academy of Sciences, Yunnan Province, southwestern  
147 China. The climate is dominated by the southwest monsoon, with a well-defined alternation of  
148 wet and dry seasons. Mean annual precipitation is about 1500 mm with 80% occurring during the  
149 May–October wet season. Mean annual air temperature is 22.7°C, with monthly means ranging  
150 from 14.8°C in January to 25.5°C in June. The garden is surrounded by a river and retains a ca.  
151 255-ha patch of relatively undisturbed tropical rain forest. The garden also adopts an organic  
152 management strategy, prohibiting the use of pesticides and chemical fertilizers, thus allowing  
153 many members of the natural herbivore community to colonize plants growing within the garden.  
154 Furthermore, the individual fig plants studied are distributed haphazardly in this common garden  
155 and are attacked by a wide range of different herbivores, including leaf beetles and larvae of  
156 butterflies and moths (Fig. 1). All fig plants studied were planted as seeds or seedlings  
157 introduced from the field at different years (Table S1) and were planted without a specific

158 arrangement in mind. Thirty-six species of *Ficus* belonging to five subgenera, including four  
159 epiphytes, 15 hemi-epiphytes and 17 free-standing tree species, were selected to address our  
160 questions. All of the *Ficus* species in our study are native to southwestern China. Our study  
161 focused on leaf defenses and susceptibility of figs to naturally occurring herbivores. All plants  
162 sampled were mature and growing under similar conditions at XTBG, providing a common  
163 environment for the analysis of evolutionary differences (Garland and Adolph 1991). All  
164 measurements were performed during March 10-25 (dry season) and August 10-25 (wet season)  
165 in both 2014 and 2015.

166

#### 167 *Measurement of leaf defenses*

168 Six traits of leaves and three traits of latex present in leaves were included in this study:  
169 specific leaf area (SLA,  $\text{cm}^2 \cdot \text{g}^{-1}$  dry mass), leaf water (%), C/N ratio, leaf tannin content (%),  
170 toughness (g), trichome density ( $\text{No.} \cdot \text{mm}^{-2}$ ), latex dry content ( $\text{g} \cdot \text{cm}^{-2}$ ), latex tannin content ( $\text{g} \cdot \text{L}^{-1}$ ), and latex alkaloid content ( $\text{g} \cdot \text{L}^{-1}$ ). SLA, leaf water, C/N, leaf tannin, and toughness are traits  
171 commonly correlated (positively or negatively) with leaf palatability and digestibility. Higher  
172 C/N ratio, toughness, and tannin content are often also correlated with reduced herbivory and  
173 slower herbivore growth rates (Choong et al. 1992, Hanley et al. 2007, Clissold et al. 2009,  
174 Kitajima et al. 2010, Kitajima et al. 2012, Prado et al. 2014). SLA and C/N are also considered as  
175 indices of resource capture ability and leaf quality, respectively. The latter might affect herbivore  
176 damage (Pérez-Harguindeguy et al. 2003), whereas the former affects the plant's ability to  
177 tolerate herbivory (e.g. by regrowth) because of its connection with carbon storage and  
178

179 photosynthetic capacity (Wright et al. 2004). Trichomes block or ensnare small herbivores such  
180 as insects or gastropods, interfering with feeding or oviposition, and may keep pathogen-  
181 containing water droplets away from the leaf surface (Hanley et al. 2007). Glandular trichomes  
182 are also known to secrete secondary compounds that may deter small herbivores and inhibit  
183 colonization and growth of pathogens (Hanley et al. 2007). Tannins are polyphenolic compounds  
184 classically recognized for their protein-precipitating capacity and more recently for their strong  
185 oxidative activity against herbivores (Salminen and Karonen 2011). Tannins are widespread in  
186 plants, and have been demonstrated to reduce herbivory by reducing leaf protein digestibility,  
187 damaging the digestive system, and generally interfering with metabolism and growth (Moles et  
188 al. 2011). Latex has been strongly implicated in defense against herbivorous insects not only  
189 because its sticky nature provides mechanical defense, but also owing to the bioactive defense-  
190 related compounds it contains, including (depending on the plant) tannins, alkaloids, cardiac  
191 glycosides, and digestive cysteine proteases (Agrawal et al. 2008, Agrawal and Konno 2009,  
192 Konno 2011). Together, the nine traits we studied reflect a wide variety of different classes and  
193 mechanisms of putative leaf defenses present in plants.

194 Three individuals per species were selected; six to ten leaves from each individual were  
195 collected for measuring leaf traits. Because latex immediately began flowing from the detached  
196 leaves, the first step was to collect the latex. We measured latex exudation on all plants by  
197 detaching the youngest fully expanded undamaged leaf from the plant and then cutting its tip off  
198 and collecting the latex in a pre-weighed microcentrifuge tube. We collected latex exuded from  
199 the apex of the leaf; the tip was repeatedly cut to keep the latex flowing until no more latex

200 exuded. These tubes were weighed by electronic balance in the laboratory nearby. The tube  
201 containing latex was oven-dried at 45°C for 72 h to measure its dry mass. The latex dry content  
202 was calculated as weight per leaf dry mass. Furthermore, 10 µl of latex (from the cut petiole  
203 when the leaf was detached) was collected from 1-10 additional leaves and dissolved with 90 µl  
204 purified water, and then diluted 10 times. The mixture was filtered through a 0.45 µm membrane.  
205 The extract was fully blended with 50 ml acetone for 40 min and the mixture was filtered  
206 through a medium-speed quantitative filter. Then 1 ml filtrate was mixed with 2.5 ml of sodium  
207 tungstate-phosphorus molybdenum acid and 5 ml sodium carbonate. The bromothymol blue  
208 colorimetric method (418 nm) was used to determine the latex alkaloid content (Shamsa et al.  
209 2008).

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

216 Following latex extraction, we used the same leaves to measure other traits. Leaf area was  
217 measured via a flatbed scanned image or digital photograph using ImageJ. Leaves were stored in  
218 plastic bags on ice in the field, then brought into the lab and weighed. Leaf toughness was  
219 measured using a leaf punch. We built a device consisting of a flat-ended cylindrical steel rod  
220 (punch, 2 mm diameter) mounted onto the moving head of the testing machine and a stationary

221 base with a sharp-edged hole with a 0-1 mm clearance (following Sanson et al. 2001). The punch  
222 was set to go through the hole without any friction. The punch moved downward at a constant  
223 speed of  $10 \text{ mm}\cdot\text{s}^{-1}$ . The leaves were positioned to avoid primary and secondary veins where  
224 possible. Trichome density was measured under a light stereoscope by counting the trichomes in  
225 a circle 5 mm in diameter on the abaxial side of the leaf. We then oven-dried leaves for 48 h  
226 ( $70^{\circ}\text{C}$ ) in the laboratory. Leaf water was estimated as the difference between the wet and dry leaf  
227 mass. Leaf area and dry mass were then used to calculate SLA. Additional leaves were collected  
228 for analysis (following exudation of all latex) of total C and N and leaf tannin, which required  
229 more material than the other analyses. Our measures of leaf dry mass, leaf water content, SLA  
230 and total C and N thus excluded latex from the calculations. Because latex accounted for only  
231 very small proportions ( $0.3 \sim 3.5\%$ ) of the total dry mass of leaves, its exclusion in these  
232 measures had little effect on our results. Owing to the fact that our measures of leaf tannin were  
233 conducted on leaves from which latex had been extracted, leaf tannin and latex tannin contents  
234 are two independent measures. Dry samples were then sent to the Biogeochemical Laboratory of  
235 the Kunming Division of Xishuangbanna Tropical Botanical Garden for chemical analyses. Total  
236 C and N were determined using a wet digestion procedure (Kalra and Maynard 1991).

237

### 238 *Susceptibility to naturally occurring herbivores*

239 The susceptibility of different *Ficus* spp. to herbivores was assessed in both dry and wet  
240 seasons for two years by measuring the proportion of leaf surface lost to chewing herbivores.

241 Similar-aged leaves were sampled in wet and dry seasons and in both years. We sampled at least

242 100 of the youngest fully expanded leaves, from 4-5 branches extending in different cardinal  
243 directions. These leaves were photographed, and the initial area of the intact leaf was  
244 reconstructed, with the percentage of lost leaf surface being calculated using ImageJ.

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

250

#### 251 *Phylogenetic reconstruction*

252 Phylogenetic relationships among the 36 *Ficus* species were inferred from genotypes at  
253 three loci: ITS, ETS and G3pdh sequences (Table S2). The phylogeny of *Ficus* was reconstructed  
254 using Bayesian inference as implemented in MrBayes. *Antiaropsis decipiens* and *Castilla*  
255 *elastica* were used as outgroups. The following substitution models were used for individual loci:  
256 ITS: GTR+I+G, ETS: HKY+G, G3pdh: GTR+G. Models were selected according to BIC using  
257 jModelTest 2.1.10. Sampling was carried out every 1000 generations for 10<sup>7</sup> generations, the  
258 first 20% of all generations were discarded as ‘burnin’ and the results were summarized with a  
259 maximum clade credibility tree. The phylogenetic tree of *Ficus* published by Cruaud et al. (2012)  
260 was used to confirm the accuracy of the placement of the 36 *Ficus* species in our study. For the  
261 species represented in both studies, topology of the tree we generated was congruent with that  
262 presented by Cruaud et al. (2012).

263

264 *Evolution of traits*

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

274 The effects of herbivore pressure on evolution of defense traits can vary, including  
275 divergence (predicted when pressure is exerted by specialists), escalation (predicted when  
276 generalists exert pressure) and even decline combined with increase in tolerance to herbivory  
277 (when defenses become ineffective) (Agrawal and Fishbein 2008, Agrawal et al. 2009c, Volf et  
278 al. 2018). Three evolutionary models were fitted for each trait in this study: Brownian motion  
279 (the correlation structure among trait values is proportional to the extent of shared ancestry  
280 between species); white noise, a non-phylogenetic null model; and Pagel’s lambda, allowing a  
281 more complex model of evolution with strong ( $\lambda = 1$ ) to weak ( $\lambda = 0$ ) phylogenetic covariation.  
282 The ‘fitContinuous’ function in the R package ‘Geiger’ was used for fitting these models  
283 (Harmon et al. 2008). AICc weights of each model were compared to evaluate the best-fitting

284 one. We also used the values of trait disparity through time (DTT) from the root to tips using the  
285 function ‘*dt*’ in the R package ‘*Geiger*’ to further examine the evolution of individual traits  
286 through time (Harmon et al. 2008). Furthermore, we used the average square distance metric to  
287 calculate trait disparity, and created a null distribution of DTT with 95% confidence intervals  
288 using 999 simulations under Brownian motion. We also used Permutational Multivariate  
289 Analysis of Variance (PERMANOVA) as implemented in the function ‘*adonis*’ in the R package  
290 ‘*vegan*’ (Oksanen et al. 2017) to test the correlations between leaf traits and phylogenetic  
291 distance among figs. To test the general directional changes in trait values from the root of the  
292 tree, the correlation between Abouheif’s distance (distance from the root) and trait values was  
293 determined as calculated in the R package ‘*adephylo*’ (Jombart et al. 2010). Because  
294 evolutionary trends may not be linear over time (e.g., in late-diverging vs. early-diverging  
295 lineages), we also examined whether polynomial regressions gave better fits than linear  
296 correlations.

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

299

### 300 *Testing correlation between traits by the convex hull method*

301 We used convex hull methods to explore combinations of traits. Convex hull methods, a  
302 construct from computational geometry, provide an n-dimensional measure of the volume of trait  
303 space occupied by species, and reflect shared ecological tolerances (Cornwell et al. 2006).  
304 Convex hull methods have been applied successfully to a wide range of datasets, including those



305 used to examine the global spectrum of plant form and function (Díaz et al. 2015). In our study,  
306 we computed a nine-dimensional convex hull volume ( $H_{ob}$ ) on the basis of the observed values  
307 of latex dry, latex tannin, and latex alkaloid content, leaf water content, SLA, C/N, toughness,  
308 trichome density and leaf tannin content, and compared it to four null model volumes ( $H_n$ :  $H_1 \sim$   
309  $H_4$ ) constructed under four different sets of assumptions (Díaz et al. 2015). Observed data were  
310 lg- or square root-transformed and standardized to zero mean and unit variance (z-  
311 transformation).

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

326 observed dataset.  $H_4$  assumes that there are fewer than nine independent axes of specialization  
327 because of the correlation among these traits and that extreme values are selected against. The  
328 hypervolume of  $H_4$  is a hyperellipsoid.

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

333

#### 334 *Covariation among traits*

335 Fig trait data were lg- or square root-transformed to fit a normal distribution, and we  
336 examined covariation among traits using two methods. First, we estimated pairwise correlation  
337 coefficients (Pearson's  $r$ ) among all traits while accounting for species' mean values across four  
338 sampling times ( $n = 36$ ). When the residual errors of the correlation among all traits displayed  
339 phylogenetic signal, we also tested the effect of shared evolutionary history in a maximum  
340 likelihood phylogenetic generalized least squares (PGLS) framework using Pagel's Continuous,  
341 implemented in BayesTraits (Pagel 1999), also using mean values for each species across four  
342 sampling times ( $n = 36$ ). When the raw and the PGLS analyses gave the same result, only the  
343 latter is presented; otherwise, both analyses are presented. We further analyzed correlations  
344 among all traits, using mean values for each fig species in each of the four sampling times ( $n =$   
345 144). Secondly, because many traits were correlated, we simplified this covariation using  
346 principal components analysis (PCA) using mean values for each *Ficus* species across four

347 sampling times ( $n = 36$ ). PCA was appropriate because our traits varied continuously and any  
348 correlation among variables was generally linear, which we assessed visually by examining all  
349 pairwise biplots.

350

### 351 *Ficus traits correlated with herbivore damage*

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

359 In addition, we used LM to examine correlations between herbivore damage (response  
360 variable) and defensive traits (fixed explanatory variables) for each sampling time considered  
361 separately ( $n = 144$ ). Generalized Linear Mixed-Effects Models (GLMM) were constructed in  
362 the R package ‘phyr’ to test the influence of fixed effects (*Ficus* traits) and random effects  
363 (species, year and season) on herbivore damage. Models were fitted using restricted maximum  
364 likelihood. We used the mean value for herbivore damage of each sampling time from three  
365 individuals of each fig species. These analyses included a correlation matrix derived from a fully  
366 unresolved species level phylogenetic tree for *Ficus* as a random effect. Using a star phylogeny  
367 effectively excluded phylogeny from the analysis, but provided a modelling framework directly

368 in line with subsequent phylogenetic generalized linear mixed models. Following non-  
369 phylogenetic analyses, we conducted a second set of models controlling for phylogenetic  
370 relationships. Phylogenetic Generalized Linear Mixed Models (PGLMMs) were used to analyze  
371 the response of herbivores (response variable) to *Ficus* traits (fixed explanatory variables), while  
372 including a correlation matrix derived from *Ficus* inter-species phylogenetic relationships as a  
373 random effect alongside year and season. The only difference between GLMM and PGLMM was  
374 that PGLMM included phylogenetic relationships of *Ficus* species. We also tested for  
375 phylogenetic signal in the residuals of each analysis. Year and season were also included in the  
376 PGLMMs as random effects in order to assess the power of both phylogeny and its covariance  
377 with traits to explain the residual variance not captured by our traits (n = 144). By including a  
378 phylogenetic term, we were able to assess i) the need to control for phylogeny and ii) the power  
379 of phylogenetic relationships in predicting herbivore damage. All the figures were visualized by  
380 using the package 'ggplot2' in R 3.5.1.

381

### 382 *Ficus species and herbivores interaction network*

383 We explored the identity of key herbivores and the overlap in community structure between  
384 figs by collecting herbivorous insects between July and October 2019. Three to ten individuals of  
385 each *Ficus* species were sampled (we checked each sampled plant three times per week), and the  
386 total area of foliage sampled over multiple sampling times ranged from 50 to 150 m<sup>2</sup> per species.  
387 For externally feeding adult insects such as Orthoptera, we hand collected insects from foliage  
388 and provided them with fresh leaves of the plant on which they were found until they fed or died.

389 Only those insects that fed on the leaves of their host fig were included in our analysis (Novotny  
390 et al. 2002).

391 All externally chewing larvae were collected by hand from the foliage, and taken to the  
392 laboratory where they were fed with their host plant until pupation and emergence. Leaf miners  
393 and piercing-sucking insects (leaf feeders only) were also hand sampled from foliage. Relatively  
394 few sucking species were collected. All insects were assigned to morphospecies using external  
395 morphological characteristics and identified to the lowest possible taxonomic level with  
396 identification keys. We classified insects as specialists or generalists; a third category ‘other’ was  
397 used to place species for which no published evidence was available. Specialist insects included  
398 those whose diet was largely composed of fig species, while generalists consumed leaves of figs  
399 alongside those of a wide range of plants. Classifications were built with the assistance of R.G.S.  
400 Tharanga Aluthwattha at XTBG and with reference to the published literature. Insect-plant  
401 associations were analyzed using three separate analyses: one univariate analysis considering  
402 abundance and two multivariate analyses considering distance measures. First, a chi-square test  
403 was used to compare the abundance of specialists and generalists across *Ficus* life forms.  
404 Second, we created quantitative adjacency matrices using the leaf-chewer data, and quantified  
405 interactions using the number of insect individuals recorded (standardized to leaf area sampled  
406 and with singleton species removed). We then used this interaction matrix as the biological  
407 matrix in canonical correlation analysis (CCA) to understand how herbivore insect occurrence  
408 and abundance (community structure) relates to the defensive traits of figs. Ordination of our  
409 interaction matrix was constrained by the set of nine defensive (environmental) trait variables

410 measured. Finally, we used non-metric multi-dimensional scaling (NMDS) to group *Ficus*  
411 species based on the similarity of their insect herbivore communities. Significance of differences  
412 in insect herbivore community between *Ficus* life forms was tested using PERMANOVA using  
413 999 random permutations and Euclidean distance. The response variable was the distance matrix  
414 derived from the *Ficus* – herbivore interaction matrix, and the explanatory variable was the  
415 categorical variable life form.

416

#### 417 *Comparing the defensive traits of damaged vs. intact leaves*

418 Because our leaf trait measurements were taken from intact leaves, not herbivore-damaged  
419 ones, it was important to explore whether the defensive traits we measured can be induced by  
420 herbivory. We carried out measurements of leaf defenses using both intact and artificially  
421 damaged leaves of *Ficus* species in August, 2019. Three individuals per species were selected;  
422 six to ten leaves from each individual were collected for measuring leaf traits. During a period of  
423 field observations, we designed a collection regime that allowed us to standardize leaf age  
424 sampled. Immediately following bud appearance, we marked the developing leaves with labels.  
425 During leaf expansion, we left half of the leaves of each species intact and injured the others  
426 using a hole punch (5 mm diameter) once every 4 h for three times within one day and ensured  
427 that the size of the removed area matched with the mean value of natural herbivory observed  
428 over wet and dry seasons for each fig species. Following complete expansion, we collected  
429 leaves of the intact and damaged groups for measurement of defensive traits using the methods  
430 described earlier. We examined the correlation between defenses of intact and damaged leaves

431 using Linear Models (LM) (n = 36). Our approach allowed us to establish i) if relative levels of  
432 defense traits in different species are similar whether leaves are damaged or not, and ii) for each  
433 species, if damaged leaves have levels of defense similar to those measured in intact leaves.

434

## 435 **RESULTS**

### 436 *Susceptibility to herbivores*

437 Season and life form significantly affected herbivory (Fig. S1, Table S3). Feeding intensity  
438 on epiphytic figs was significantly lower than on hemi-epiphytic and free-standing figs, while  
439 there was no significant difference between the latter two groups. Leaf area lost was greater on  
440 leaves collected in the dry season than on those collected in the wet season. However, results of  
441 PGLMM (Table 1) showed that sampling time did not contribute significantly to explaining  
442 relationships between herbivory and leaf traits. As such, seasonal variation will not be discussed  
443 further. We also measured leaf area loss in natural populations in the relatively undisturbed rain  
444 forest near XTBG in the wet season. For *F. subulata*, the leaf area lost in natural populations was  
445 0.5 - 27.3% (mean 8.1%), while this figure was 0.2 - 15.5% (mean 5.8%) for *F. auriculata*. In the  
446 wet season in our common garden *F. subulata* suffered leaf area losses of 18.1 - 21.1% (mean  
447 19.6%), while *F. auriculata* lost 13.4 ~ 32.8% (mean 23.1%) of its leaf area. Levels of herbivory  
448 of natural populations were lower than plants on the common-garden setting. Furthermore, the  
449 percentage of leaf area lost was larger than has been reported from other studies of wild *Ficus*  
450 populations in the Australasian region, e.g. Sam et al. (2020) report a maximum value of 6.1%.

451

453 In analyses of each trait, strong phylogenetic signal of individual traits was recovered (Table  
454 2). Latex tannin, SLA, C/N and leaf tannin all displayed strong phylogenetic signals. Life form  
455 also showed strong phylogenetic signal (Epiphyte,  $D = -0.99$ ,  $P = 0.01$  when compared to  
456 Phylogenetic randomness,  $P = 0.84$  when compared to Brownian threshold model; Hemi-  
457 epiphyte,  $D = -0.57$ ,  $P = 0.00$  when compared to Phylogenetic randomness,  $P = 0.90$  when  
458 compared to Brownian threshold model; Free-standing,  $D = -0.22$ ,  $P = 0.00$  when compared to  
459 Phylogenetic randomness,  $P = 0.68$  when compared to Brownian threshold model). Strong  
460 phylogenetic signal was also detected for PC1, PC2 and herbivore damage (Table 2).

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



473 detected among late-divergent lineages (i.e. closely related fig species) (Fig. 3e-i).

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

477

#### 478 *Covariation among traits*

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

484 We detected four positive and three negative pairwise correlations among traits of these 36  
485 *Ficus* species with average values across four sampling times (Table 3). Latex dry content was  
486 positively correlated with latex alkaloid content, but only when phylogenetic non-independence  
487 was accounted for (Pearson's  $r = -0.01$ ,  $P = 0.97$ , PGLS  $r = 0.35$ , LR = 4.60,  $P = 0.03$ ). Latex  
488 tannin content was also positively correlated with latex alkaloid content, but again only when  
489 phylogenetic non-independence was accounted for (Pearson's  $r = 0.31$ ,  $P = 0.06$ , PGLS  $r = 0.36$ ,  
490 LR = 4.93,  $P = 0.03$ ). C/N was positively correlated with both toughness (PGLS  $r = 0.52$ , LR =  
491 11.58,  $P = 0.001$ ) and leaf tannin (PGLS  $r = 0.41$ , LR = 6.58,  $P = 0.01$ ). Three negative  
492 correlations were also found, between latex dry content and SLA (PGLS  $r = -0.33$ , LR = 4.18,  $P$   
493 = 0.04), latex dry content and leaf tannin content (but not when phylogenetic non-independence

494 was accounted for [Pearson's  $r = -0.22$ ,  $P = 0.19$ , PGLS  $r = -0.43$ ,  $LR = 7.43$ ,  $P = 0.01$ ] and C/N  
495 and trichome density (PGLS  $r = -0.46$ ,  $LR = 8.39$ ,  $P = 0.003$ ). When we considered values from  
496 each of the four sample times separately ( $n = 144$ ), five positive and ten negative pairwise  
497 correlations among traits of these 36 *Ficus* species were observed (Table S4).

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

502

### 503 *Plant traits that predict resistance to herbivores*

504 We first analyzed the relationship between leaf traits and herbivore damage using mean  
505 values for each fig species for each of the four sampling times. Analysis of model residuals  
506 demonstrated a lack of phylogenetic signal (Table S5), the results using the linear models were in  
507 broad agreement with those derived from GLMMs and PGLMMs, with strong negative  
508 relationships between herbivore damage and latex tannin and between damage and C/N  
509 remaining once temporal and phylogenetic effects had been filtered out (Table 4). Life form and  
510 leaf toughness also remained as significant explanatory variables in GLMMs. In addition, suites  
511 of traits predicted herbivore damage better than single traits, in both linear models and mixed  
512 effects analyses (Table 5). Leaf traits explained a significant proportion of the variation in  
513 herbivore damage ( $R^2 = 0.38$ ,  $P < 0.001$ ), followed by variation among species not explained by  
514 the traits we measured ( $R^2 = 0.16$ ,  $P < 0.001$ ). Neither phylogenetic history of figs nor sampling

515 times contributed significantly to explaining variation measured in herbivore damage (Table 1).  
516 Phylogenetic signal in model residuals was minimal; as such, the GLMM without a phylogenetic  
517 term remains the most powerful analysis.

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

529

### 530 *Ficus - herbivore interaction network*

531 The *Ficus* - herbivore network (Fig. 1) was composed of 1285 insects from 65  
532 morphospecies, with a total of 342 distinct interactions. Among insects, 1207 (94%) individuals  
533 were leaf-chewers and 78 (6%) were leaf-suckers. Leaf-chewers (including 38 Lepidoptera, 20  
534 Coleoptera and one Orthoptera species) represented the principal herbivorous insects of *Ficus*  
535 species and inflicted most of the damage. In the communities, the majority of caterpillars were

536 Erebidae (85% of individuals), followed by Noctuidae (10% of individuals) and Crambidae (3%  
537 of individuals). Furthermore, 50% of Erebidae individuals were from the genus *Asota*, which is  
538 believed to be able to sequester or detoxify alkaloids of *Ficus* species (Sourakov and Emmel  
539 2001). The relatively polyphagous group Crambidae (Novotny et al. 2002) represented 4% of all  
540 polyphagous individuals, Erebidae represented 52% of all polyphagous individuals (Erebidae  
541 included 49% polyphagous individuals, 50% *Asota* and 1% others); the other generalists  
542 included Coleoptera and Noctuidae. Among all of the individual phytophagous insects, 54% (698  
543 individuals, 48 species) fed on free-standing figs, 44% (564 individuals, 39 species) interacted  
544 with hemi-epiphytic figs and only 2% (23 individuals, 11 species) fed on epiphytic figs. The  
545 abundance of specialist and generalist herbivores differed significantly among fig life forms (Fig.  
546 S4). Of the individual insects feeding on epiphytic figs, 45% were specialists, which accounted  
547 for 30% and 34% of individuals feeding on hemi-epiphytic and free-standing figs, respectively.  
548 Several defensive traits were correlated with herbivore community structure (Fig. 6), including  
549 trichome density ( $F = 2.00$ ,  $p = 0.005$ ), C/N ( $F = 2.08$ ,  $p = 0.01$ ), leaf toughness ( $F = 2.29$ ,  $p =$   
550  $0.01$ ), leaf tannin content ( $F = 2.09$ ,  $p = 0.02$ ) and latex alkaloid content ( $F = 1.94$ ,  $p = 0.005$ ).  
551 Life form was not a significant predictor of insect herbivore community structure ( $F_{2,33} = 0.73$ ,  $P$   
552  $= 0.91$ ,  $R^2 = 0.04$ ).

553

#### 554 *Comparing the defensive traits of damaged vs. intact leaves*

555 Levels of all nine defense traits of intact *Ficus* leaves were significantly and positively  
556 correlated with those in damaged leaves (Fig. S3), with all traits showing very similar levels of

557 defense in intact and damaged leaves. The order of species along defense levels was also the  
558 same whether leaves are damaged or intact. In addition, the slope of the relationship between  
559 defensive traits of intact and damaged leaves ranged from 0.75 to 1.01 (median value was 0.94)  
560 while intercepts ranged from -0.93 to 0.17 (median value was 0.06). Coefficients of  
561 determination ( $R^2$ ) of the relationships ranged from 0.74 to 0.99, except for latex dry content ( $R^2$   
562 = 0.58). Thus, there was neither large upregulation of defenses in damaged leaves nor differences  
563 in ‘inducibility’ among species.

564

## 565 **DISCUSSION**

566 Although the concept of ‘defense syndromes’ has been proposed as an evolutionary  
567 outcome of arms races between plants and their herbivores for decades (Agrawal and Fishbein  
568 2006), empirical evidence supporting the concept is rare. Using convex hull methods, we showed  
569 that only a small subset of all possible trait combinations occurred or were frequent, indicating  
570 strong inherent correlations among traits in *Ficus*. These syndromes grouped together fig species  
571 with different life forms. Furthermore, these traits combined predicted resistance better than did  
572 each trait individually in a common-garden setting where all plant individuals were freely  
573 accessible to the herbivore community. We also found strong phylogenetic signal in combination  
574 with escalation in defense-related traits, including latex tannin and C/N, suggestive of selection  
575 rather than drift. Other traits, in contrast, appeared to have more limited phylogenetic signal or  
576 tended to de-escalate. The study therefore suggests that an evolutionary arms race can drive the  
577 evolution of defensive syndromes in this tropical genus.

578

579 *Evolution of Ficus traits*

580 In our study, C/N, latex tannin content, leaf tannin content and SLA displayed strong  
581 phylogenetic signal among *Ficus* species. Previous studies have suggested the occurrence of  
582 macroevolutionary escalation (Farrell et al. 1991, Agrawal and Fishbein 2008, Becerra et al.  
583 2009, Pearse and Hipp 2012) or divergence (Becerra 2007, Kursar et al. 2009, Salazar et al.  
584 2016) of defensive traits. Whether these leaf traits displayed phylogenetic signal depended on the  
585 plant lineage studied (Agrawal et al. 2009b, Rasmann and Agrawal 2011, Nakadai et al. 2014,  
586 Eichenberg et al. 2015, Liu et al. 2015, Volf et al. 2018). The carbon/nitrogen ratio (C/N) is one  
587 of the most important indices of leaf quality for herbivores (Agrawal and Fishbein 2006, Mason  
588 and Donovan 2015). Our study reports C/N to undergo strong and significant escalation,  
589 suggesting that this trait could be particularly important in the evolutionary response of plants to  
590 selective pressures exerted by herbivores. A high C/N ratio in leaf tissue slows the development  
591 of insects that feed on them (Bryant et al. 1983). Leaf N has been reported to influence insect  
592 herbivory and low leaf N contents might negatively affect the preference and performance of  
593 insect herbivores (Minkenbergh and Ottenheim 1990, Stamp and Casey 1993, Anderson et al.  
594 2004, Kagata and Ohgushi 2012), particularly when combined with carbon-rich fiber and  
595 tannins. The importance of leaf N content to insect preference and performance is further  
596 indicated by the fact that in some plants, damage to leaves results in reallocation of nutrients,  
597 leading to a decrease in N content (Wold and Marquis 1997, Cornelissen and Fernandes 2001).

598 Secondary metabolites have been shown to have strong phylogenetic signal in some studies

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

612

### 613 *Covariation of traits*

614       Considering analyses of pairwise covariation of individual traits, we encountered four  
615 positive and three negative correlations when we considered species' mean values across the four  
616 sampling times ( $n = 36$ ). These are fewer than we expected (and fewer than we found when all  
617 values from the four sample times were used [ $n = 144$ ]), but these low numbers are in line with  
618 results of some other studies. For example, in a global investigation of relationships among four  
619 chemical and six physical defenses of 261 plant species, only five of the 45 pairwise correlations

620 between defense traits were significant and three of these correlations were negative, indicating  
621 tradeoffs (Moles et al. 2013). In another study, which examined potential defense syndromes in  
622 24 species of milkweeds (*Asclepias* spp.) in a field experiment, analysis employing  
623 phylogenetically independent contrasts found few correlations between seven defensive traits.  
624 No bivariate trade-offs were detected, while two positive correlations were found, between  
625 trichome density and latex production and between C/N ratio and leaf toughness (Agrawal and  
626 Fishbein 2006). That relatively few correlations and tradeoffs were demonstrated might be  
627 explained by two main reasons. First, theory that predicts tradeoffs between defenses is based on  
628 allocation of resources, but different traits may place demands on different resources, and  
629 ‘exchange rates’ between different resources are not clear. Traits that place demands on the same  
630 resource (e.g. different N-based defenses, or different C-based defenses) may be more likely to  
631 show negative correlations than traits using different resources (e.g. N-based vs. C-based  
632 defenses). Second, leaf traits that play roles in plant resistance against insect herbivores may  
633 have multiple additional functions. For example, trichomes confer protection against herbivores,  
634 but also contribute to drought resistance (Ohri et al. 2007). Defenses may coexist because each  
635 has different additional functions. Similarly, defenses may coexist because they have different  
636 defensive functions, for example, deterring different types of herbivores or pathogens. Finally,  
637 defenses can act synergistically to reduce damage (Agrawal, 2007). This could explain the  
638 occurrence of positive associations. Conversely, negative associations might be explained not  
639 only by resource-allocation tradeoffs but also by antagonism (the opposite of synergy) between  
640 two kinds of defenses. Several interspecific comparative studies have reported negative



641 correlations between distributions of tannins and alkaloids (Gartlan et al. 1980, Janzen and  
642 Waterman 1984, Silvertown and Dodd 1996, Mali and Borges 2003). These might be explained  
643 by the formation, when both are present, of insoluble alkaloid tannates that reduce the  
644 effectiveness of both kinds of defenses (Janzen and Waterman 1984, Mali and Borges 2003).  
645 However, we detected no negative associations between alkaloids and tannins in our study.

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

654

#### 655 *Defense syndromes as predictors of resistance against herbivores*

656       The defense-syndrome hypothesis predicts that trait combinations should provide better  
657 protection against herbivores than any single trait alone. However, relatively few studies have  
658 tested this prediction (Agrawal 2011). Although some studies have detected defense syndromes  
659 (Agrawal and Fishbein 2006, Travers-Martin and Mueller 2008, Pearse 2011, Moreira et al.  
660 2016, Raffa et al. 2017), few of them have tested the relationship between these syndromes and  
661 resistance to herbivores (Agrawal and Fishbein 2006, Travers-Martin and Mueller 2008, Pearse

2011). In this study, suites of traits combined did indeed predict level of herbivory better than individual traits, suggesting that these suites of traits provide better protection than individual traits (Table 5). We identify two important considerations that should be taken into account in studies of defense syndromes. First, studies must be capable of detecting resistance conferred by suites of defensive traits not only against a particular herbivore, but against all herbivores. The function of defense is to reduce herbivory, regardless of its source. Many studies have focused on particular herbivores, sometimes specialists (Travers-Martin and Muller 2008, Haak et al. 2014), sometimes generalists (Mason et al. 2016). Second, defense syndromes are unlikely to exist in the form originally outlined because herbivores are just one selective pressure among many in nature that act on leaf traits. Defense syndromes are likely to reflect adaptation to the great diversity of environmental challenges, both abiotic and biotic, that plants face (Janzen 1980, Strauss et al. 2005). As demonstrated by our GLMM and PGLMM results, variation in the defense traits we studied explains only a part of interspecific variation in plant resistance to herbivores ( $R^2 = 0.38$  for level of herbivory). There must exist other important traits affecting resistance that were not included in our study. Additional unmeasured defenses might include protease activity, oxidative activity and alkaloid diversity, which have been confirmed to be correlated with the community structure of herbivorous insects (Volf et al. 2018). In the present study, latex alkaloid content was positively correlated with herbivore damage (Table S5), implying that latex alkaloid might be an ineffective trait. Intriguingly, alkaloid-rich *Ficus* species can host distinct insect communities (Volf et al. 2018), and the abundance across *Ficus* species of one *Asota* species studied here was positively correlated with latex alkaloid content.

683           Interestingly, there was variation among the three life forms of figs both in herbivore  
684 damage to their leaves (Fig. S1, Table S3) and in the combinations of defensive traits of their  
685 leaves. Epiphytic figs suffered lower herbivore damage compared to hemi-epiphytic and free-  
686 standing *Ficus* species. In our study, leaves of epiphytic species possessed significantly higher  
687 C/N and leaf tannin content and were tougher than leaves of other figs (Fig. 5). Free-standing fig  
688 species displayed high trichome density, along with high concentrations of tannin and alkaloids  
689 in their latex, whereas hemi-epiphytic figs had higher leaf water content and SLA. Similar results  
690 were found for these two life forms in *Ficus* spp. in Papua New Guinea (Volf et al. 2018). These  
691 results imply that different life forms of *Ficus* have evolved different defensive strategies against  
692 the multiple selective pressures imposed by the biotic and abiotic environment (also see Agrawal  
693 and Fishbein 2006). Epiphytic figs, with no access to nutrients and water in soil, face limited  
694 resource availability compared to hemi-epiphytic and free-standing figs and appear to have the  
695 most effective (and probably costliest) defenses against herbivores, consistent with resource-  
696 availability theory (Coley et al. 1985, Fine et al. 2006). By comparison, hemi-epiphytic and free-  
697 standing figs appear to have less effective defenses and suffer more damage from herbivorous  
698 insects. Tolerance to herbivory (not measured here) might be an available strategy for these figs,  
699 congruent with the compensatory regrowth hypothesis (Strauss and Agrawal 1999, Stowe et al.  
700 2000). We also detected linear and tight positive correlations, with slopes differing little from 1  
701 and intercepts differing little from zero, between the defenses of intact and damaged leaves. This  
702 is highly suggestive of only small induced responses in our focal traits for the *Ficus* species we  
703 studied (we note that volatile organic compounds were not collected during our study). We

704 recorded higher levels of herbivory within our common-garden setting than in natural  
705 populations (especially for *F. auriculata*), perhaps driven by higher densities of available hosts or  
706 a simpler landscape. While the magnitude of the effects measured may thus have been larger than  
707 in natural populations, it is doubtful as to whether this increased rate of herbivory influenced the  
708 general conclusions of our study.

709

#### 710 *Ficus - herbivore interaction network*

711 Our study demonstrated that epiphytic figs interacted with fewer herbivorous insects than  
712 hemi-epiphytic and free-standing figs (Fig. S4), and that herbivores of epiphytic figs tended to  
713 be more specialized. The diverse set of pressures imposed by species-rich communities of insect  
714 herbivores is believed to select for variability in plant defenses (Ehrlich and Raven 1964;  
715 Agrawal and Fishbein 2006). Correspondingly, the community structure of insect herbivores is  
716 driven by plant defenses (Volf et al. 2018). In our study, epiphytic figs had nutrient-poor leaves  
717 that were tough and rich in tannins while free-standing trees tended to have leaves covered in  
718 trichomes and full of alkaloid-rich latex. Our results imply that generalists might be deterred by  
719 the high tannin levels (or by other, unmeasured, defensive traits) of epiphytic figs, resulting in  
720 lower damage levels compared to hemi-epiphytic and free-standing figs. However, while levels  
721 of resistance varied, there was no overall difference in insect community structure between each  
722 life form. We surveyed herbivore communities for only one season. Longer-term investigation  
723 of the structure of the herbivore community might improve our understanding of the roles of  
724 different evolutionary processes in generating the interaction network between *Ficus* and

725 insects. Our modest data set was, however, sufficient to reveal that traits with distinct  
726 evolutionary trajectories shaped insect herbivore communities. For example, both escalating  
727 (C/N) and divergent (leaf tannin) traits explained significant proportions of variance in insect  
728 community structure. In agreement with Volf et al. (2018), we also found a role for latex  
729 alkaloids and trichome density in predicting herbivore community composition.

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

741 ACKNOWLEDGMENTS

742 We thank Chunfen Xiao, Junwei Liu and Hongyun Zhou for their assistance with  
743 the field work and the Public Technology Service Center of XTBG, CAS, for support  
744 with chemical analyses. We thank R.G.S. Tharanga Aluthwattha for his assistance  
745 with the herbivore insect identification. We thank Kyle Tomlinson for analytical  
746 advice. In addition, we thank Anthony R. Ives for his help with PGLMM analysis.  
747 Comments of two anonymous reviewers greatly improved the manuscript. This study  
748 was funded by the National Natural Science Foundation of China (U1402264 and  
749 31300369), the West Light Foundation of the Chinese Academy of Sciences and the  
750 CAS 135 program (No. 2017XTBG-T01). STS acknowledges departmental support  
751 from Harper Adams University. DM acknowledges support from the Center for  
752 Functional and Evolutionary Ecology (CEFE).

753 LITERATURE CITED

- 754 Agrawal, A. A. 2007. Macroevolution of plant defense strategies. *Trends in Ecology*  
755 *& Evolution* **22**:103-109.
- 756 Agrawal, A. A. 2011. Current trends in the evolutionary ecology of plant defence.  
757 *Functional Ecology* **25**:420-432.
- 758 Agrawal, A. A., and M. Fishbein. 2006. Plant defense syndromes. *Ecology* **87**:S132-  
759 S149.
- 760 Agrawal, A. A., and M. Fishbein. 2008. Phylogenetic escalation and decline of plant  
761 defense strategies. *Proceedings of the National Academy of Sciences of the*  
762 *United States of America* **105**:10057-10060.
- 763 Agrawal, A. A., M. Fishbein, R. Halitschke, A. P. Hastings, D. L. Rabosky, and S.  
764 Rasmann. 2009a. Evidence for adaptive radiation from a phylogenetic study of  
765 plant defenses. *Proceedings of the National Academy of Sciences of the*  
766 *United States of America* **106**:18067-18072.
- 767 Agrawal, A. A., M. Fishbein, R. Jetter, J. P. Salminen, J. B. Goldstein, A. E. Freitag,  
768 and J. P. Sparks. 2009b. Phylogenetic ecology of leaf surface traits in the  
769 milkweeds (*Asclepias* spp.): chemistry, ecophysiology, and insect behavior.  
770 *New Phytologist* **183**:848-867.
- 771 Agrawal, A. A., and K. Konno. 2009. Latex: a model for understanding mechanisms,  
772 ecology, and evolution of plant defense against herbivory. *Annual Review of*  
773 *Ecology, Evolution and Systematics* **40**:311-331.
- 774 Agrawal, A. A., M. J. Lajeunesse, and M. Fishbein. 2008. Evolution of latex and its  
775 constituent defensive chemistry in milkweeds (*Asclepias*): a phylogenetic test  
776 of plant defense escalation. *Entomologia Experimentalis Et Applicata*  
777 **128**:126-138.

778 Agrawal, A. A., J. P. Salminen, and M. Fishbein. 2009c. Phylogenetic trends in  
779 phenolic metabolism of milkweeds (*Asclepias*): evidence for escalation.  
780 *Evolution* **63**:663-673.

781 Aljibory, Z., and M. S. Chen. 2016. Indirect plant defense against insect herbivores: a  
782 review. *Insect Science* **25**: 2-23.

783 Anderson, T. R., M. Boersma, and D. Raubenheimer. 2004. Stoichiometry: linking  
784 elements to biochemicals. *Ecology* **85**:1193-1202.

785 Appel, H. M. 1993. Phenolics in ecological interactions: the importance of oxidation.  
786 *Journal of Chemical Ecology* **19**:1521-1552.

787 Bajaj, K. L., and A. K. Devsharma. 1977. A colorimetric method for the determination  
788 of tannins in tea. *Microchimica Acta* **68**:249-253.

789 Bauer, G., and T. Speck. 2012. Restoration of tensile strength in bark samples of *Ficus*  
790 *benjamina* due to coagulation of latex during fast self-healing of fissures.  
791 *Annals of Botany* **109**:807-811.

792 Becerra, J. X. 2007. The impact of herbivore-plant coevolution on plant community  
793 structure. *Proceedings of the National Academy of Sciences of the United*  
794 *States of America* **104**:7483-7488.

795 Becerra, J. X., K. Noge, and D. L. Venable. 2009. Macroevolutionary chemical  
796 escalation in an ancient plant-herbivore arms race. *Proceedings of the National*  
797 *Academy of Sciences of the United States of America* **106**:18062-18066.

798 Bennett, R. N., and R. M. Wallsgrove. 1994. Secondary metabolites in plant defense –  
799 mechanisms. *New Phytologist* **127**: 617-633.

800 Berg, C. 1989. Classification and distribution of *Ficus*. *Experientia* **45**:605-611.

801 Berg, C. C., and E. J. H. Corner. 2005. *Moraceae-Ficus*. *Flora Malesiana Series I*  
802 (Seed Plants) Volume 17/Part 2. National Herbarium of the Netherlands,



803 Leiden.

804 Bryant, J., F. Chapin, and D. Klein. 1983. Carbon/nutrient balance of boreal plants in  
805 relation to vertebrate herbivory. *Oikos* **40**: 357-368.

806 Cárdenas, R. E., R. Valencia, N. J. B. Kraft, A. Argoti, and O. Dangles. 2014. Plant  
807 traits predict inter- and intraspecific variation in susceptibility to herbivory in a  
808 hyperdiverse Neotropical rain forest tree community. *Journal of Ecology* **102**:  
809 939-952.

810 Choong, M. F., P. W. Lucas, J. S. Y. Ong, B. Pereira, H. T. W. Tan, and I. M. Turner.  
811 1992. Leaf fracture-toughness and sclerophylly - their correlations and  
812 ecological implications. *New Phytologist* **121**:597-610.

813 Clissold, F. J., G. D. Sanson, J. Read, and S. J. Simpson. 2009. Gross vs. net income:  
814 How plant toughness affects performance of an insect herbivore. *Ecology*  
815 **90**:3393-3405.

816 Coley, P. D., J. P. Bryant, and F. S. Chapin. 1985. Resource availability and plant  
817 antiherbivore defense. *Science* **230**:895-899.

818 Cornelissen, T.G., and G.W. Fernandes. 2001. Defense, growth and nutrient allocation  
819 in the tropical shrub *Bauhinia brevipes* (Leguminosae). *Austral Ecology* **26**:  
820 246-253.

821 Cornwell, W. K., L. D. Schwilk, and D. D. Ackerly. 2006. A trait-based test for habitat  
822 filtering: convex hull volume. *Ecology* **87**: 1465-1471.

823 Cruaud, A., N. Rønsted, B. Chantarasuwan, L. S. Chou, W. L. Clement, A. Couloux ,  
824 B. Cousins,, G. Genson, R.D. Harrison and P. E. Hanson. 2012. An extreme  
825 case of plant–insect codiversification: figs and fig-pollinating wasps.  
826 *Systematic Biology* **61**: 1029-1047.

827 Díaz, S., J. Kattge, J. H. C. Cornelissen, I. J. Wright, S. Lavorel, S. Dray, B. Reu, M.

828 Kleyer, C. Wirth, I. C. Prentice, E. Garnier, G. Bönisch, M. Westoby, H.  
829 Poorter, P. B. Reich, A. T. Moles, J. Dickie, A. N. Gillison, A. E. Zanne, J.  
830 Chave, S. J. Wright, S. N. Sheremet'ev, H. Jactel, C. Baraloto, B. Cerabolini,  
831 S. Pierce, B. Shipley, D. Kirkup, F. Casanoves, J. S. Joswig, A. Günther, V.  
832 Falczuk, N. Rüger, M. D. Mahecha, and L. D. Gorné. 2015. The global  
833 spectrum of plant form and function. *Nature* **529**: 7585.

834 Duffey, S. S., and M. J. Stout. 1996. Antinutritive and toxic components of plant  
835 defense against insects. *Archives of Insect Biochemistry and Physiology* **32**:3-  
836 37.

837 Ehrlich, P. R., and P. H. Raven. 1964. Butterflies and plants – a study in coevolution.  
838 *Evolution* **18**:586-608.

839 Eichenberg, D., O. Purschke, C. Ristok, L. Wessjohann, and H. Bruelheide. 2015.  
840 Trade-offs between physical and chemical carbon-based leaf defence: of  
841 intraspecific variation and trait evolution. *Journal of Ecology* **103**:1667-1679.

842 Farrell, B. D., D. E. Dussourd, and C. Mitter. 1991. Escalation of plant defense – do  
843 latex and resin canals spur plant diversification? *American Naturalist* **138**:881-  
844 900.

845 Feeny, P. 1970. Seasonal changes in oak leaf tannins and nutrients as a cause of spring  
846 feeding by winter moth caterpillars. *Ecology* **51**:565-581.

847 Fine, P. V. A., Z. J. Miller, I. Mesones, S. Irazuzta, H. M. Appel, M. H. H. Stevens, I.  
848 Saaksjarvi, L. C. Schultz, and P. D. Coley. 2006. The growth-defense trade-off  
849 and habitat specialization by plants in Amazonian forests. *Ecology* **87**:S150-  
850 S162.

851 Fornoni, J. 2011. Ecological and evolutionary implications of plant tolerance to  
852 herbivory. *Functional Ecology* **25**:399-407.

853 Fritz, S. A., and A. Purvis. 2010. Selectivity in mammalian extinction risk and threat  
854 types: a new measure of phylogenetic signal strength in binary traits.  
855 Conservation Biology **24**:1042-1051.

856 Garland, T. Jr., and S. C. Adolph. 1991. Physiological differentiation of vertebrate  
857 populations. Annual Review of Ecology and Systematics **22**:193-228.

858 Gartlan, J. S., D. B. McKey, P. G. Waterman, and T. T. Struhsaker. 1980. A  
859 comparative study of the phytochemistry of two African rain forests.  
860 Biochemical Systematics and Ecology **8**:401-422.

861 Green, T. R., and C. A. Ryan. 1972. Wound-induced proteinase inhibitor in plant  
862 leaves: a possible defense mechanism against insects. Science **175**:776-77.

863 Haak, D. C., B. A. Ballenger, and L. C. Moyle. 2014. No evidence for phylogenetic  
864 constraint on natural defense evolution among wild tomatoes. Ecology  
865 **95**:1633-1641.

866 Hanley, M. E., B. B. Lamont, M. M. Fairbanks, and C. M. Rafferty. 2007. Plant  
867 structural traits and their role in anti-herbivore defence. Perspectives in Plant  
868 Ecology Evolution and Systematics **8**:157-178.

869 Harmon, L. J., J. T. Weir, C.D. Brock, R.E. Glor, and W. Challenger. 2008. GEIGER:  
870 investigating evolutionary radiations. Bioinformatics **24**:129-131.

871 Hao, G. Y., L. Sack, A. Y. Wang, K. F. Cao, and G. Goldstein. 2010. Differentiation of  
872 leaf water flux and drought tolerance traits in hemiepiphytic and non-  
873 hemiepiphytic *Ficus* tree species. Functional Ecology **24**:731-740.

874 Herms, D. A., and W. J. Mattson. 1992. The dilemma of plants—to grow or defend.  
875 Quarterly Review of Biology **67**:283-335.

876 Janzen, D. H. 1980. When is coevolution? Evolution **34**:611-612.

877 Janzen, D. H., and P. G. Waterman. 1984. A seasonal census of phenolics, fibre and

878 alkaloids in foliage of forest trees in Costa Rica: some factors influencing their  
879 distribution and relation to host selection by Sphingidae and Saturniidae.  
880 *Biological Journal of the Linnean Society* **21**:439-454.

881 Jombart, T., F. Balloux, and S. Dray. 2010. Adephylo: new tools for investigating the  
882 phylogenetic signal in biological traits. *Bioinformatics* **26**:1907-1909.

883 Johnson, M. T. J., A. R. Ives, J. Ahern, and J. P. Salminen. 2014. Macroevolution of  
884 plant defenses against herbivores in the evening primroses. *New Phytologist*  
885 **203**:267-279.

886 Kagata, H., and T. Ohgushi. 2012. Carbon to nitrogen excretion ratio in lepidopteran  
887 larvae: relative importance of ecological stoichiometry and metabolic scaling  
888 *Oikos* **121**:1869-1877.

889 Kalra, Y. P., and D.G. Maynard. 1991. *Methods manual for forest soil and plant*  
890 *analysis*. Alberta, Canada: Forestry Canada, Northwest Region, Northern  
891 Forestry Center.

892 Kembel, S. W., P. D. Cowan, M. R. Helmus, W. K. Cornwell, H. Morlon, D. D.  
893 Ackerly, and C. O. Webb. 2010. Picante: R tools for integrating phylogenies  
894 and ecology. *Bioinformatics* **26**:1463-1464.

895 Kempel, A., Schadler, M., Chrobock, T., Fischer, M., and M. van Kleunen. 2011.  
896 Tradeoffs associated with constitutive and induced plant resistance against  
897 herbivory. *Proceedings of the National Academy of Sciences of the United*  
898 *States of America* **108**:5685-5689.

899 Kitajima, S., K. Kamei, S. Taketani, M. Yamaguchi, F. Kawai, A. Komatsu, and Y.  
900 Inukai. 2010. Two chitinase-like proteins abundantly accumulated in latex of  
901 mulberry show insecticidal activity. *BMC Biochemistry* **11**:6.

902 Kitajima, S., T. Taira, K. Oda, K. T. Yamato, Y. Inukai, and Y. Hori. 2012.

903 Comparative study of gene expression and major proteins' function of  
904 laticifers in lignified and unlignified organs of mulberry. *Planta* **235**:589-601.  
905 Konno, K. 2011. Plant latex and other exudates as plant defense systems: Roles of  
906 various defense chemicals and proteins contained therein. *Phytochemistry*  
907 **72**:1510-1530.  
908 Kursar, T. A., and P. D. Coley. 2003. Convergence in defense syndromes of young  
909 leaves in tropical rainforests. *Biochemical Systematics and Ecology* **31**:929-  
910 949.  
911 Kursar, T. A., K. G. Dexter, J. Lokvam, R. T. Pennington, J. E. Richardson, M. G.  
912 Weber, E. T. Murakami, C. Drake, R. McGregor, and P. D. Coley. 2009. The  
913 evolution of antiherbivore defenses and their contribution to species  
914 coexistence in the tropical tree genus *Inga*. *Proceedings of the National*  
915 *Academy of Sciences of the United States of America* **106**:18073-18078.  
916 Lewinsohn, T. M., V. Novotny, and Y. Basset. 2005. Insects on plants: Diversity of  
917 herbivore assemblages revisited. *Annual Review of Ecology, Evolution and*  
918 *Systematics* **36**: 597-620.  
919 Liu, H., Q. Y. Xu, P. C. He, L. S. Santiago, K. M. Yang, and Q. Ye. 2015. Strong  
920 phylogenetic signals and phylogenetic niche conservatism in ecophysiological  
921 traits across divergent lineages of Magnoliaceae. *Scientific Reports* **5**: 12246.  
922 Lucas, P. W., Turner, I. M., Dominy, N. J., and N. Yamashita. 2000. Mechanical  
923 defences to herbivory. *Annals of Botany* **86**:913-920.  
924 Mali, S. and R. M. Borges. 2003. Phenolics, fibre, alkaloids, saponins, and  
925 cyanogenic glycosides in a seasonal cloud forest in India. *Biochemical*  
926 *Systematics and Ecology* **31**:1221-1246.  
927 Mason, C.M., and L. A. Donovan. 2015. Evolution of the leaf economics spectrum in

928 herbs: evidence from environmental divergences in leaf physiology across  
929 *Helianthus* (Asteraceae). *Evolution* **69**:2705-2720.

930 Mason, C. M., A. W. Bowsher, B. L. Crowell, R. M. Celoy, C. J. Tsai, and L. A.  
931 Donovan. 2016. Macroevolution of leaf defenses and secondary metabolites  
932 across the genus *Helianthus*. *New Phytologist* **209**:1720-1733.

933 Mesa, J. M., D. R. Scholes, J. A. Juvik, and K. N. Paige. 2017. Molecular constraints  
934 on resistance-tolerance trade-offs. *Ecology* **98**:2528-2537.

935 Minkenberg, O. P. J. M., and J. J. G. W. Ottenheim. 1990. Effect of leaf nitrogen  
936 content of tomato plants on preference and performance of a leafmining fly.  
937 *Oecologia* **83**: 291-298.

938 Moles, A. T., B. Peco, I. R. Wallis, W. J. Foley, A. G. B. Poore, E. W. Seabloom, P. A.  
939 Vesk, A. J. Bisigato, L. Cella-Pizarro, C. J. Clark, P. S. Cohen, W. K.  
940 Cornwell, W. Edwards, R. Ejrnaes, T. Gonzales-Ojeda, B. J. Graae, G. Hay, F.  
941 C. Lumbwe, B. Magana-Rodriguez, B. D. Moore, P. L. Peri, J. R. Poulsen, J.  
942 C. Stegen, R. Veldtman, H. Zeipel, N. R. Andrew, S. L. Boulter, E. T. Borer, J.  
943 H. C. Cornelissen, A. G. Farji-Brener, J. L. DeGabriel, E. Jurado, L. A. Kyhn,  
944 B. Low, C. P. H. Mulder, K. Reardon-Smith, J. Rodriguez-Velazquez, A. De  
945 Fortier, Z. Zheng, P. G. Blendinger, B. J. Enquist, J. M. Facelli, T. Knight, J.  
946 D. Majer, M. Martinez-Ramos, P. McQuillan, and F. K. C. Hui. 2013.  
947 Correlations between physical and chemical defences in plants: tradeoffs,  
948 syndromes, or just many different ways to skin a herbivorous cat? *New*  
949 *Phytologist* **198**:252-263.

950 Moles, A. T., I. R. Wallis, W. J. Foley, D. I. Warton, J. C. Stegen, A. J. Bisigato, L.  
951 Cella-Pizarro, C. J. Clark, P. S. Cohen, W. K. Cornwell, W. Edwards, R.  
952 Ejrnaes, T. Gonzales-Ojeda, B. J. Graae, G. Hay, F. C. Lumbwe, B. Magana-

953 Rodriguez, B. D. Moore, P. L. Peri, J. R. Poulsen, R. Veldtman, H. von Zeipel,  
954 N. R. Andrew, S. L. Boulter, E. T. Borer, F. F. Campon, M. Coll, A. G. Farji-  
955 Brener, J. De Gabriel, E. Jurado, L. A. Kyhn, B. Low, C. P. H. Mulder, K.  
956 Reardon-Smith, J. Rodriguez-Velazquez, E. W. Seabloom, P. A. Vesk, A. van  
957 Cauter, M. S. Waldram, Z. Zheng, P. G. Blendinger, B. J. Enquist, J. M.  
958 Facelli, T. Knight, J. D. Majer, M. Martinez-Ramos, P. McQuillan, and L. D.  
959 Prior. 2011. Putting plant resistance traits on the map: a test of the idea that  
960 plants are better defended at lower latitudes. *New Phytologist* **191**:777-788.

961 Moreira, L. F., N. C. Teixeira, N. A. Santos, J. O. S. Valim, R. M. Mauricio, R. N. C.  
962 Guedes, M. G. A. Oliveira, and W. G. Campos. 2016. Diamondback moth  
963 performance and preference for leaves of *Brassica oleracea* of different ages  
964 and strata. *Journal of Applied Entomology* **140**:627-635.

965 Morris, W. F., Traw, M. B., and J. Bergelson. 2006. On testing for a tradeoff between  
966 constitutive and induced resistance. *Oikos* **112**:102-110.

967 Nakadai, R., M. Murakami, and T. Hirao. 2014. Effects of phylogeny, leaf traits, and  
968 the altitudinal distribution of host plants on herbivore assemblages on  
969 congeneric *Acer* species. *Oecologia* **175**:1237-1245.

970 Novotny, V., and Y. Basset. 2005. Review - Host specificity of insect herbivores in  
971 tropical forests. *Proceedings of the Royal Society B-Biological Sciences* **272**:  
972 1083-1090.

973 Novotny, V., Y. Basset, S.E. Miller, P. Drozd, and L. Cizek. 2002. Host specialisation  
974 of leaf chewing insects in a New Guinea rainforest. *Journal of Animal Ecology*  
975 **71**: 400-412.

976 Nuñez-Farfán, J., J. Fornoni, and P. Luis Valverde. 2007. The evolution of resistance  
977 and tolerance to herbivores. *Annual Review of Ecology, Evolution and*

978 Systematics **38**: 541-566.

979 Ohrui, H. T., H. Nobira, Y. Sakata, T. Taji, C. Yamamoto, K. Nishida, T. Yamakawa, Y.  
980 Sasuga, Y. Yaguchi and H. Takenaga. 2007. Foliar trichome- and aquaporin-  
981 aided water uptake in a drought-resistant epiphyte *Tillandsia ionantha*  
982 Planchon. *Planta* **227**:47-56.

983 Oksanen, J., F.G. Blanchet, R. Kindt, P. Legendre, P.R. Minchin, R.B. O'hara, G.L.  
984 Simpson, P. Solymos, M.H.H. Stenvens, E. Szoecs and H. Wagner. 2017.  
985 vegan: Community Ecology Package. R package version 2.4-3. Available at:  
986 <https://CRAN.R-project.org/package=vegan>.

987 Pagel, M. 1999. Inferring the historical patterns of biological evolution. *Nature* **401**:  
988 877-884.

989 Pearse, I. S. 2011. The role of leaf defensive traits in oaks on the preference and  
990 performance of a polyphagous herbivore, *Orgyia vetusta*. *Ecological*  
991 *Entomology* **36**:635-642.

992 Pearse, I. S., and A. L. Hipp. 2009. Phylogenetic and trait similarity to a native species  
993 predict herbivory on non-native oaks. *Proceedings of the National Academy of*  
994 *Sciences of the United States of America* **106**:18097-18102.

995 Pearse, I. S., and A. L. Hipp. 2012. Global patterns of leaf defenses in oak species.  
996 *Evolution* **66**:2272-2286.

997 Pérez-Harguindeguy, N., Díaz, S., Vendramini, F., Cornelissen, J.H.C., Gurvich, D.E.,  
998 and M. Cabido. 2003. Leaf traits and herbivore selection in the field and in  
999 cafeteria experiments. *Austral Ecology* **28**: 642-650.

1000 Prado, A., A. Sierra, D. Windsor, and J. C. Bede. 2014. Leaf traits and herbivory  
1001 levels in a tropical gymnosperm, *Zamia stevensonii* (Zamiaceae). *American*  
1002 *Journal of Botany* **101**:437-447.



- 1003 Price, P. W. 2002. Resource-driven terrestrial interaction webs. *Ecological Research*  
1004 **17**: 241-247.
- 1005 Raffa, K. F., C. J. Mason, P. Bonello, S. Cook, N. Erbilgin, K. Keefover-Ring, J. G.  
1006 Klutsch, C. Villari, and P. A. Townsend. 2017. Defence syndromes in  
1007 lodgepole - whitebark pine ecosystems relate to degree of historical exposure  
1008 to mountain pine beetles. *Plant Cell and Environment* **40**:1791-1806.
- 1009 Rasmann, S., and A. A. Agrawal. 2011. Evolution of specialization: A phylogenetic  
1010 study of host range in the Red Milkweed Beetle (*Tetraopes tetraophthalmus*).  
1011 *American Naturalist* **177**:728-737.
- 1012 Romeo, J. T., J. A. Saunders, and P. Barbosa, editors. 1996. Phytochemical diversity  
1013 and tedundancy in ecological interactions. Plenum Press, New York, New  
1014 York, USA.
- 1015 Ryan, C. A. 1990. Protease inhibitors in plants: Genes for improving defenses against  
1016 insects and pathogens. *Annual Review of Phytopathology* **28**:425-449.
- 1017 Salazar, D., A. Jaramillo, and R. J. Marquis. 2016. The impact of plant chemical  
1018 diversity on plant-herbivore interactions at the community level. *Oecologia*  
1019 **181**:1199-1208.
- 1020 Salminen, J. P., and M. Karonen. 2011. Chemical ecology of tannins and other  
1021 phenolics: we need a change in approach. *Functional Ecology* **25**:325-338.
- 1022 Sam, K., B. Koane, L. Sam, A. Mrazova, S. Segar, M. Volf, M. Moos, P. Simek, M.  
1023 Sisol, and V. Novotny. 2020. Insect herbivory and herbivores of *Ficus* species  
1024 along a rain forest elevational gradient in Papua New Guinea. *Biotropica* **52**:  
1025 263-276.
- 1026 Sanson, G., J. Read, N. Aranwela, F. Clissold, and P. Peeters. 2001. Measurement of  
1027 leaf biomechanical properties in studies of herbivory: Opportunities, problems

- 1028 and procedures. *Austral Ecology* **26**:535-546.
- 1029 Schädler, M., G. Jung, H. Auge, and R. Brandl. 2003. Palatability, decomposition and  
1030 insect herbivory: patterns in a successional old-field plant community. *Oikos*  
1031 **103**:121–132.
- 1032 Schofield, P., D. M. Mbugua, and A. N. Pell. 2001. Analysis of condensed tannins: a  
1033 review. *Animal Feed Science and Technology* **91**:21-40.
- 1034 Shamsa, F., H. Monsef, R. Ghamooshi, and M. Verdianrizi. 2008. Spectrophotometric  
1035 determination of total alkaloids in some Iranian medicinal plants. *Journal of*  
1036 *Applied Horticulture* **32**:17-20.
- 1037 Silvertown, J. and M. Dodd. 1996. Comparing plants and connecting traits.  
1038 *Philosophical Transactions of the Royal Society London Series B, Biological*  
1039 *Sciences* **351**:1233-1239.
- 1040 Sourakov, A. and T. C. Emmel. 2001. On the toxic diet of day-flying moths in the  
1041 Solomon Islands (Lepidoptera: Arctiidae). *Tropical Lepidoptera Research*  
1042 **12**:5-6.
- 1043 Stamp, N.E. and T.M. Casey (Eds.). 1993. *Caterpillars: Ecological and Evolutionary*  
1044 *Constraints on Foraging*, New York, Chapman and Hall pp **587**.
- 1045 Stevens, P. 2001. Angiosperm phylogeny website. Version 12, July 2012. See  
1046 <http://www.mobot.org/MOBOT/research/APweb/>.
- 1047 Steward, J. L., and K. H. Keeler. 1988. Are there trade-offs among antiherbivore  
1048 defenses in *Ipomoea* (Convolvulaceae)? *Oikos* **53**:79-86.
- 1049 Stowe, K. A., R. J. Marquis, C. G. Hochwender, and E. L. Simms. 2000. The  
1050 evolutionary ecology of tolerance to consumer damage. *Annual Review of*  
1051 *Ecology, Evolution, and Systematics* **31**:565-595.
- 1052 Strauss, S. Y., and A. A. Agrawal. 1999. The ecology and evolution of plant tolerance

1053 to herbivory. *Trends in Ecology and Evolution* **14**:179-185.

1054 Strauss, S. Y., H. Sahli, and J. K. Conner. 2005. Toward a more trait-centered  
1055 approach to diffuse (co)evolution. *New Phytologist* **165**:81-89.

1056 Takahashi, D., and A. Yamauchi. 2010. Optimal defense schedule of annual plants  
1057 against seasonal herbivores. *American Naturalist* **175**:538-550.

1058 Travers-Martin, N., and C. Mueller. 2008. Matching plant defence syndromes with  
1059 performance and preference of a specialist herbivore. *Functional Ecology*  
1060 **22**:1033-1043.

1061 Villard, C., R. Larbat, R. Munakata, and A. Hehn. 2019. Defence mechanisms of  
1062 *Ficus*: pyramiding strategies to cope with pests and pathogens. *Planta* **249**:  
1063 617-633.

1064 Volf, M., J. P. Salminen, and S. T. Segar, 2019. Evolution of defences in large tropical  
1065 plant genera: perspectives for exploring insect diversity in a tri-trophic  
1066 context. *Current Opinion in Insect Science* **32**: 91-97.

1067 Volf, M., S. T. Segar, S. E. Miller, B. Isua, M. Sisol, G. Aubona, P. Simek, M. Moos,  
1068 J. Laitila, J. Kim, J. Zima, J. Rota, G. D. Weiblen, S. Wossa, J. P. Salminen, Y.  
1069 Basset, and V. Novotny. 2018. Community structure of insect herbivores is  
1070 driven by conservatism, escalation and divergence of defensive traits in *Ficus*.  
1071 *Ecology Letters* **21**:83-92.

1072 Wagner, G. J. 1991. Secreting glandular trichomes – more than just hairs. *Plant*  
1073 *Physiology* **96**: 675-679.

1074 Wold, E.N., and R.J. Marquis. 1997. Induced defenses in white oak: effects on  
1075 herbivores and consequences for the plant. *Ecology* **78**: 1356-1369.

1076 Wright, I.J., P. B. Reich, M. Westoby, D. D. Ackerly, Z. Baruch, F. Bongers, J.  
1077 Cavender-Bares, T. Chapin, J. H. C. Cornelisson, M. Diemer, J. Flexas, E.

1078 Garnier, P. K. Groom, J. Gulias, K. Hikosaka, B. B. Lamont, T. Lee, W. Lee,  
1079 C. Lusk, J. J. Midgley, M. Navas, U. Niinemets, J. Oleksyn, N. Osada, H.  
1080 Poorter, P. Poot, L. Prior, V. I. Pyankov, C. Roumet, S. C. Thomas, M. G.  
1081 Tjoelker, E. J. Veneklaas, R. Villar. 2004. The worldwide leaf economics  
1082 spectrum. *Nature* **428**:821-827.

1083 Zhao, J., and J. Chen. 2012. Interspecific variation in compensatory regrowth to  
1084 herbivory associated with soil nutrients in three *Ficus* (Moraceae) Saplings.  
1085 *PLoS One* **7**:e45092.

1086 Zust, T., and A. A. Agrawal. 2017. Trade-offs between plant growth and defense  
1087 against insect herbivory: An emerging mechanistic synthesis. *Annual Review*  
1088 *of Plant Biology* **68**:513-534.

1089

1090 Table 1 Contributions of leaf traits and other variables to explaining variation among  
1091 species in herbivore damage to leaves, using PGLMM analyses (n = 144; \* :  $P < 0.05$ ,  
1092 \*\* :  $P < 0.01$ , \*\*\* :  $P < 0.001$ ).

Variables	R <sup>2</sup>	<i>P</i>
Leaf traits	0.38	< 0.001
Variation among species	0.16	< 0.001
Sampling times	0.03	0.14
Phylogeny	0.005	0.69

1093

1094 Table 2 Selected models of evolution and phylogenetic signal for individual *Ficus*  
1095 traits measured by Blomberg's K and PIC (n = 36, mean values across four sampling  
1096 times; \* :  $P < 0.05$ , \*\* :  $P < 0.01$ , \*\*\* :  $P < 0.001$ ). BM: Brownian motion, i.e. the  
1097 correlation structure among trait values is proportional to the extent of shared ancestry  
1098 between species; White: white noise, a non-phylogenetic null model; Lambda:  
1099 Lambda model, a more complex model of evolution with strong ( $\lambda = 1$ ) to weak ( $\lambda =$   
1100 0) phylogenetic covariation.

Trait	Model (AICc)	K	PIC		P
			observed mean	randomized mean	
Lg (Latex dry, g·cm <sup>-2</sup> )	White	0.23	11.78	8.92	0.91
Sqrt (Latex tannin, g·L <sup>-1</sup> )	White	0.49	92.54	146.88	<b>0.02*</b>
Lg (Latex alkaloid, g·L <sup>-1</sup> )	White	0.51	81.48	114.93	0.13
Sqrt (Leaf water, %)	White	0.28	8.78	7.97	0.73
Lg (SLA, cm <sup>2</sup> ·g <sup>-1</sup> )	Lambda ( $\lambda = 0.46$ )	0.52	9.10	15.02	<b>0.02*</b>
Lg (C/N)	BM	0.74	3.73	8.79	<b>0.001***</b>
Lg (Toughness, g)	White	0.37	15.96	19.23	0.29
Lg (Trichome density, No.·mm <sup>-2</sup> )	White	0.39	76.16	97.22	0.23
Lg (Leaf tannin, %)	BM	0.63	33.25	63.50	<b>0.01**</b>
Sqrt (Herbivore damage, %)	Lambda ( $\lambda = 0.41$ )	0.47	122.97	187.40	<b>0.04*</b>
PC1	BM	0.84	109.69	279.41	<b>0.001***</b>
PC2	BM	0.89	70.67	205.80	<b>0.001***</b>

1101

1102 Table 3 Correlations among leaf traits of 36 *Ficus* species using mean values across four sampling times (two seasons in each of two years) as  
 1103 measured in a common garden. Shown are Spearman correlation coefficient (above the diagonal), phylogenetic generalized least squares (PGLS)  
 1104 coefficient (below the diagonal) and likelihood ratios (LR, also below the diagonal, in parentheses), calculated as twice the difference in  
 1105 likelihood ratio of the estimated model, and a model where trait covariance is set to zero (n = 36, \* :  $P < 0.05$ , \*\* :  $P < 0.01$ , \*\*\* :  $P < 0.001$ ).

Traits	Lg (Latex dry, g·cm <sup>-2</sup> )	Sqrt (Latex tannin, g·L <sup>-1</sup> )	Lg (Latex alkaloid, g·L <sup>-1</sup> )	Sqrt (Leaf water, %)	Lg (SLA, cm <sup>2</sup> ·g <sup>-1</sup> )	Lg (C/N)	Lg (Toughness, g)	Lg (Trichome, No.·mm <sup>-2</sup> )	Lg (Leaf tannin, %)
Lg (Latex dry, g·cm <sup>-2</sup> )		0.02	-0.01	-0.01	<b>-0.43**</b>	0.06	-0.00	0.13	-0.22
Sqrt (Latex tannin, g·L <sup>-1</sup> )	0.12(0.52)		0.31	-0.28	-0.12	-0.21	0.02	<b>0.35*</b>	-0.11
Lg (Latex alkaloid, g·L <sup>-1</sup> )	<b>0.35(4.60)*</b>	<b>0.36(4.93)*</b>		-0.03	-0.14	-0.19	0.07	0.12	-0.14
Sqrt (Leaf water, %)	0.13(0.58)	-0.10(0.38)	-0.01(0.38)		0.04	-0.01	0.02	-0.17	-0.30
Lg (SLA, cm <sup>2</sup> ·g <sup>-1</sup> )	<b>-0.33(4.18)*</b>	-0.05(0.14)	-0.15(0.86)	0.08(0.22)		<b>-0.36*</b>	-0.19	-0.03	-0.27
Lg (C/N)	-0.29(3.14)	-0.31(3.69)	-0.31(3.69)	0.13(0.60)	-0.02(0.01)		<b>0.36*</b>	<b>-0.48**</b>	<b>0.62***</b>
Lg (Toughness, g)	-0.13(0.66)	-0.09 (0.28)	-0.09(0.28)	0.23(2.01)	-0.04(0.06)	<b>0.52(11.60) ***</b>		0.01	-0.01
Lg (Trichome, No.·mm <sup>-2</sup> )	0.01(0.001)	0.20(1.50)	0.20(1.50)	-0.22(1.87)	0.08(0.26)	<b>-0.46(8.39)**</b>	-0.14(0.68)		-0.31
Lg (Leaf tannin, %)	<b>-0.43(7.43)**</b>	0.12(0.01)	-0.02(0.01)	-0.21(1.69)	-0.14(0.67)	<b>0.41(6.75)**</b>	-0.05(0.10)	-0.26(2.61)	

1106

1107 Table 4 Results of LM (first five lines of data), GLMM (three lines in the middle) and  
 1108 PGLMM (last two lines) analyses of the effects of traits on herbivore damage giving  
 1109 model coefficients and significance with fixed effects listed, and random effects being  
 1110 *Ficus* species, season and year for PGLMM (n = 144, \* :  $P < 0.05$ , \*\* :  $P < 0.01$ , \*\*\* :  
 1111  $P < 0.001$ ). Only traits for which results of the analysis were significant are shown.  
 1112 (LM, Linear Models; LME, Linear Mixed-Effects Models; PGLMM, Phylogenetic  
 1113 Linear Mixed Models.)

Fixed effect	Estimate	Standard error	t-value	P-value
Lg (C/N)	-2.24	0.47	-4.82	< 0.001***
Sqrt (Latex tannin, g·L <sup>-1</sup> )	-0.39	0.10	-3.94	< 0.001***
Lg (Trichome density, No.·mm <sup>-2</sup> )	-0.36	0.16	-2.31	0.02*
Free-standing	1.33	0.43	3.09	0.01**
Hemi-epiphyte	1.06	0.45	2.38	0.02**

Fixed effect	Estimate	Standard error	z-value	P-value
Lg (C/N)	-1.57	0.53	-2.97	< 0.003***
Sqrt (Latex tannin, g·L <sup>-1</sup> )	-0.32	0.12	-2.60	0.009**
Lg (Toughness, g)	-0.77	0.38	-2.03	0.04*
Free-standing	1.41	0.64	2.21	0.03*

Fixed effect	Estimate	Standard error	z-value	P-value
Lg (C/N)	-1.48	0.53	-2.78	0.01**
Sqrt (Latex tannin, g·L <sup>-1</sup> )	-0.31	0.12	-2.55	0.01**



1115 Table 5 Results of LM, GLMM and PGLMM analyses giving model coefficients and  
 1116 significance with fixed effects listed, and random effects being *Ficus* species, season  
 1117 and year for PGLMM (n = 144; \* :  $P < 0.05$ , \*\* :  $P < 0.01$ , \*\*\* :  $P < 0.001$ ).

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

1118 For PGLMMs the additional random effect of phylogenetic covariance was included.

1119 Only traits for which results were significant are shown.

1120

1121 Fig. 1 Interaction networks between *Ficus* and herbivorous insects, constructed from  
1122 observations in the wet season of 2019. The bars above represent insect  
1123 morphospecies (purple bars represent herbivore species that are specialists on *Ficus*,  
1124 green bars represent generalists), the black bars below represent *Ficus* species. Bar  
1125 width corresponds to the proportion of interactions contributed by each species to the  
1126 network. Links between species are indicated by lines that are proportional to  
1127 interaction strength. Red circles represent epiphytic, yellow circle represent hemi-  
1128 epiphytic, and blue circle represent free-standing *Ficus* species respectively.

1129

1130 Fig. 2 Distribution of *Ficus* defenses across the phylogeny. Traits following  
1131 Brownian-motion (purple), Lambda model (green) and white-noise (white) models are  
1132 differentiated by background color. *Ficus* traits include latex dry ( $\text{g}\cdot\text{cm}^{-2}$ ), latex tannin  
1133 ( $\text{g}\cdot\text{L}^{-1}$ ), latex alkaloid ( $\text{g}\cdot\text{L}^{-1}$ ), leaf water (%), specific leaf area (SLA,  $\text{cm}^2\cdot\text{g}^{-1}$ ), C/N,  
1134 toughness (g), trichome density ( $\text{No.}\cdot\text{mm}^{-2}$ ) and leaf tannin (%) ( $n = 36$ , data are the  
1135 mean values for four sampling times (two seasons in each of two years)).

1136

1137 Fig. 3 Mean disparity through time (DTT) for traits with significant effects on  
1138 herbivore damage (solid line). The dashed line indicates the median DTT based on  
1139 999 simulations of character evolution on the phylogeny of the studied *Ficus* species  
1140 under Brownian motion. The grey shaded area indicates the 95% confidence interval  
1141 for the simulated data ( $n = 36$ , data are the mean values across four sampling times  
1142 (two seasons in each of two years)).

1143

1144 Fig. 4 The volume in trait space occupied by *Ficus* species is constrained compared to  
1145 theoretical null models. A volume of  $H_{0b}$  is less than volume of  $H_1$  (a),  $H_2$  (b) and  $H_3$

1146 (c), but higher than H<sub>4</sub> (d). The hypervolumes are constructed on the basis of lg- or  
1147 square root-transformed observed values of latex dry, latex tannin, latex alkaloid  
1148 content, leaf water content, SLA, C/N, toughness, trichome density and leaf tannin  
1149 content (observed hypervolume = H<sub>ob</sub>, indicated by solid diamond), or on the basis of  
1150 four different null models of multivariate variation of those traits (H<sub>1</sub> to H<sub>4</sub>). VD  
1151 (volume difference) means the difference between H<sub>ob</sub> and H<sub>1</sub> to H<sub>4</sub> (see Methods).

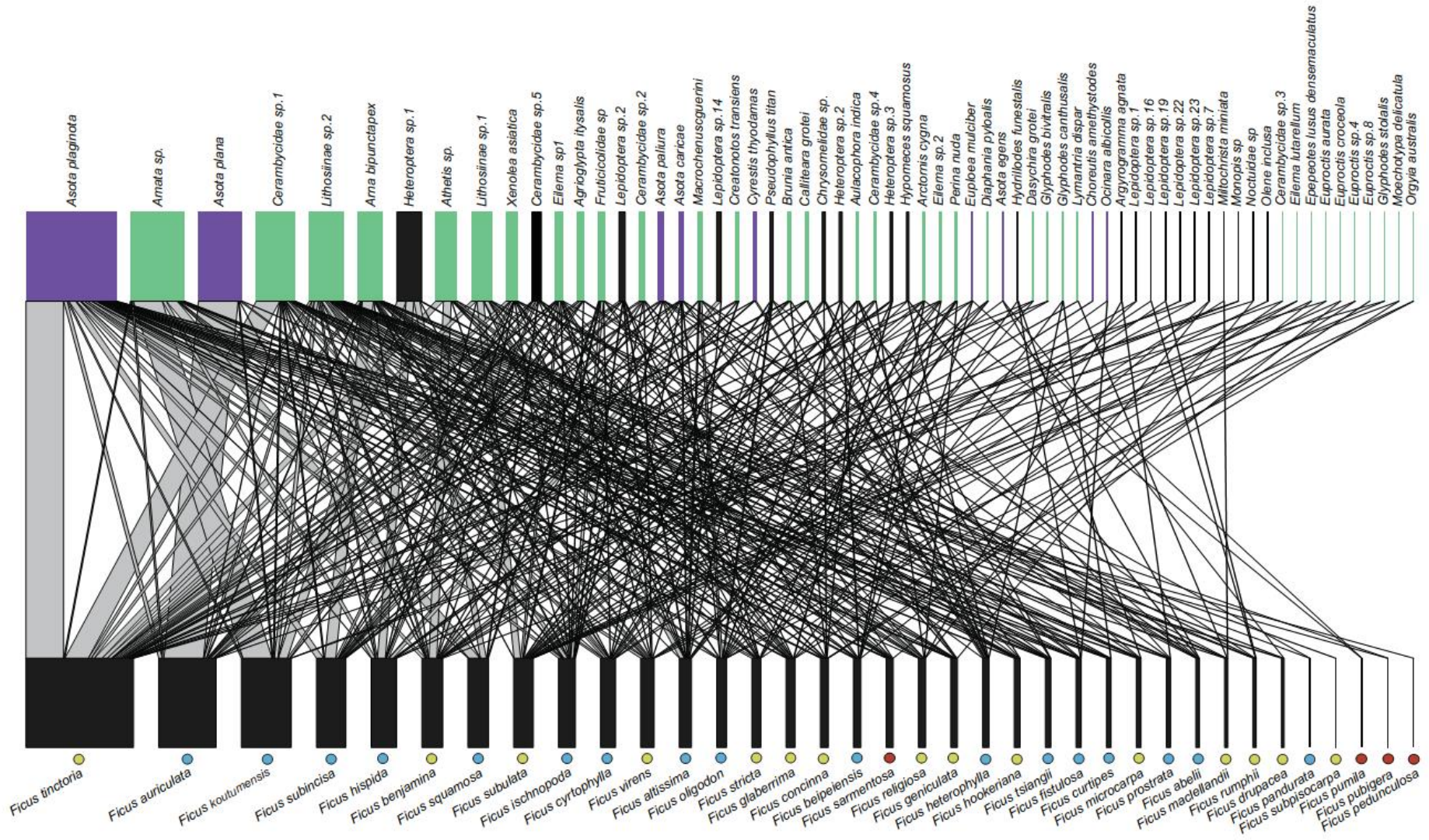
1152

1153 Fig. 5 Correlations between *Ficus* traits, as visualized by the principal components  
1154 analysis biplot (a) and regressions showing how PC1 predicts resistance to herbivores  
1155 (b). The individual points show the species' raw means across four sampling times  
1156 while the line shows the regression slope from the linear mixed model, with the  
1157 corresponding statistics shown (each point depicts the mean for a *Ficus* species across  
1158 four sampling times, n = 36).

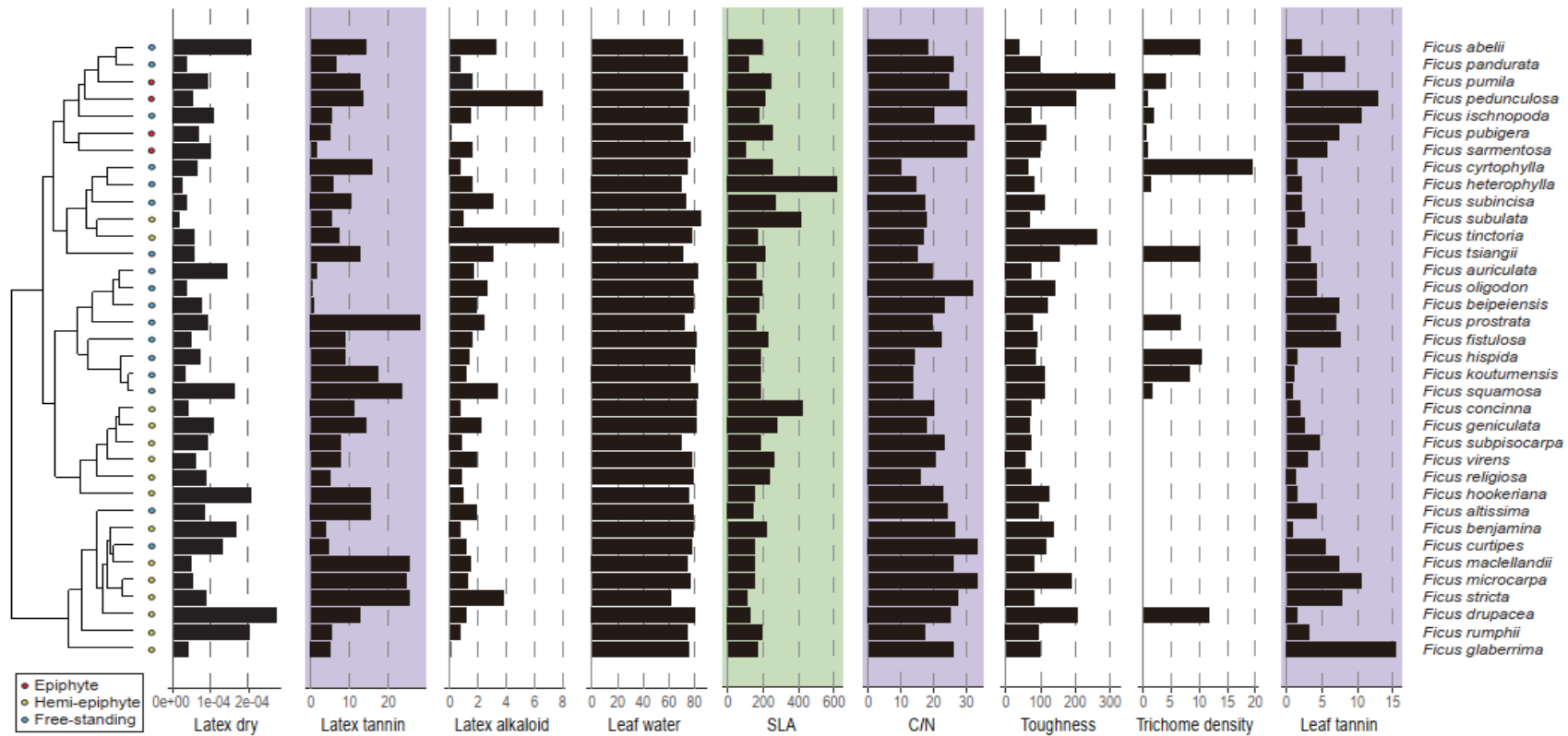
1159

1160 Fig. 6 Correlations between herbivore insect community structure and *Ficus* traits  
1161 visualized by the Canonical Correlation Analysis biplot (point depicts herbivore insect  
1162 species and lines represent direction and strength of correlations).

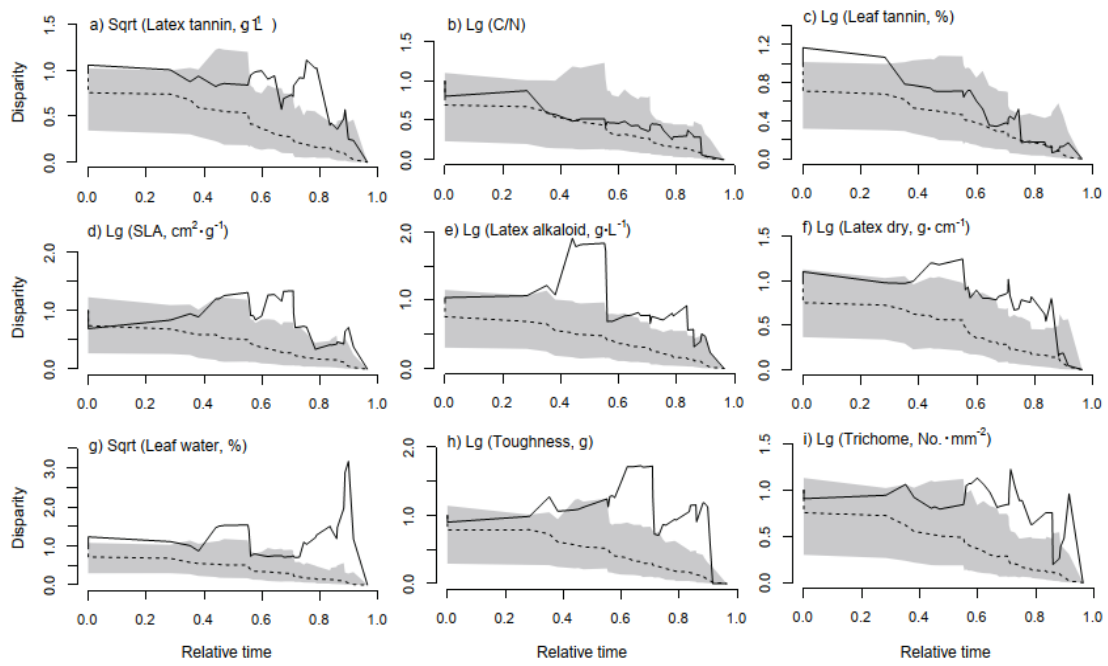
1163 Fig. 1 Interaction networks between *Ficus* and herbivorous insects, constructed from  
1164 observations in the wet season of 2019. The bars above represent insect  
1165 morphospecies (purple bars represent herbivore species that are specialists on *Ficus*,  
1166 green bars represent generalists), the black bars below represent *Ficus* species. Bar  
1167 width corresponds to the proportion of interactions contributed by each species to the  
1168 network. Links between species are indicated by lines that are proportional to  
1169 interaction strength. Red circles represent epiphytic, yellow circle represent hemi-  
1170 epiphytic, and blue circle represent free-standing *Ficus* species respectively.  
1171



1173 Fig. 2 Distribution of *Ficus* defenses across the phylogeny. Traits following Brownian-motion (purple), Lambda model (green) and white-noise  
 1174 (white) models are differentiated by background color. *Ficus* traits include latex dry ( $\text{g}\cdot\text{cm}^{-2}$ ), latex tannin ( $\text{g}\cdot\text{L}^{-1}$ ), latex alkaloid ( $\text{g}\cdot\text{L}^{-1}$ ), leaf  
 1175 water (%), specific leaf area (SLA,  $\text{cm}^2\cdot\text{g}^{-1}$ ), C/N, toughness (g), trichome density ( $\text{No.}\cdot\text{mm}^{-2}$ ) and leaf tannin (%) (n = 36, data are the mean  
 1176 values for four sampling times (two seasons in each of two years)).

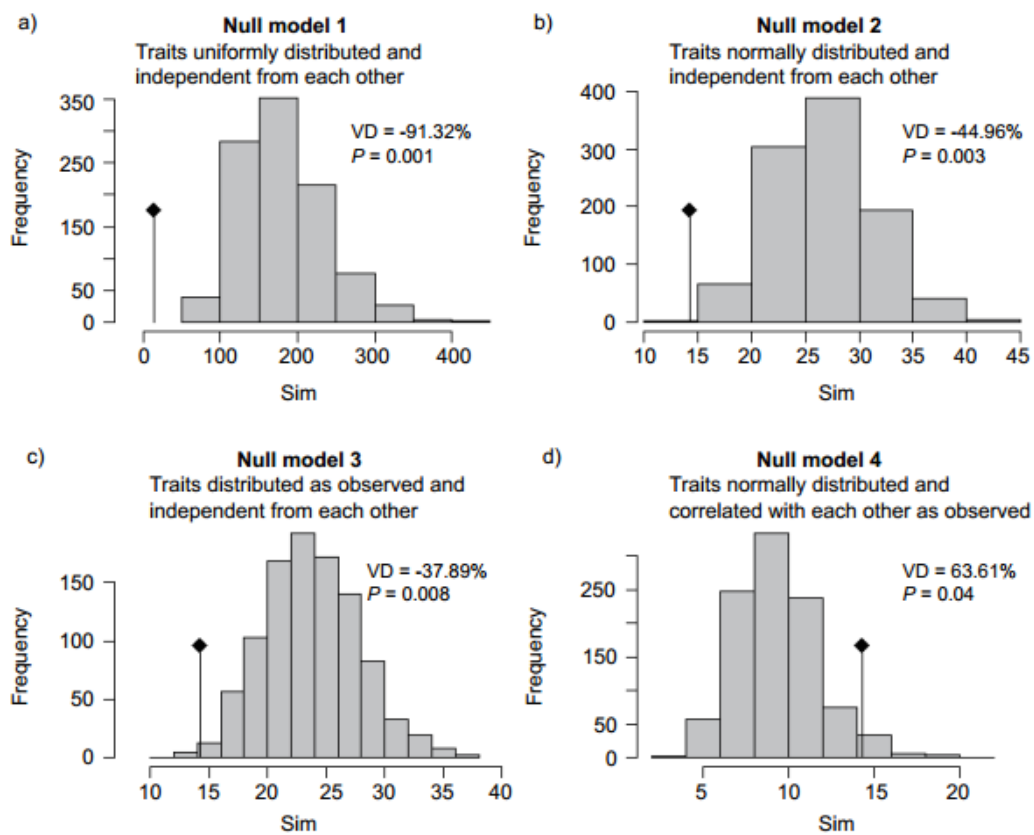


1178 Fig. 3 Mean disparity through time (DTT) for traits with significant effects on  
 1179 herbivore damage (solid line). The dashed line indicates the median DTT based on  
 1180 999 simulations of character evolution on the phylogeny of the studied *Ficus* species  
 1181 under Brownian motion. The grey shaded area indicates the 95% confidence interval  
 1182 for the simulated data (n = 36, data are the mean values across four sampling times  
 1183 (two seasons in each of two years)).



1184

1185 Fig. 4 The volume in trait space occupied by *Ficus* species is constrained compared to  
 1186 theoretical null models. A volume of  $H_{ob}$  is less than volume of  $H_1$  (a),  $H_2$  (b) and  $H_3$   
 1187 (c), but higher than  $H_4$  (d). The hypervolumes are constructed on the basis of lg- or  
 1188 square root-transformed observed values of latex dry, latex tannin, latex alkaloid  
 1189 content, leaf water content, SLA, C/N, toughness, trichome density and leaf tannin  
 1190 content (observed hypervolume =  $H_{ob}$ , indicated by solid diamond), or on the basis of  
 1191 four different null models of multivariate variation of those traits ( $H_1$  to  $H_4$ ). VD  
 1192 (volume difference) means the difference between  $H_{ob}$  and  $H_1$  to  $H_4$  (see Methods).

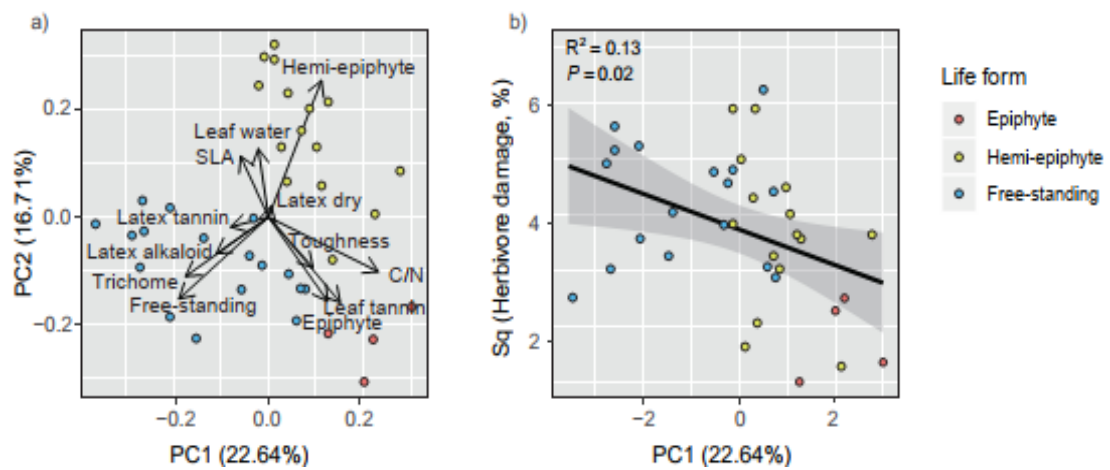


1193

1194

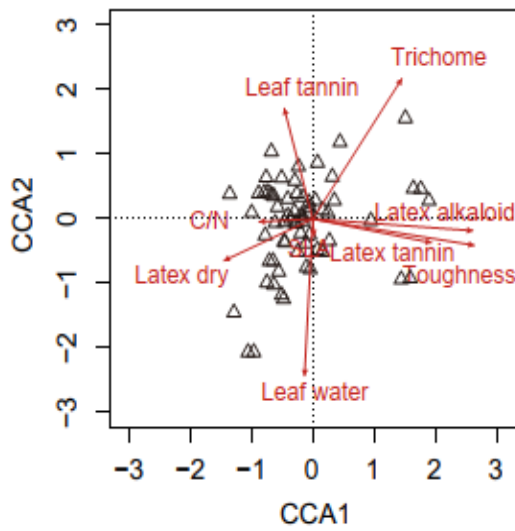


1195 Fig. 5 Correlations between *Ficus* traits, as visualized by the principal components  
 1196 analysis biplot (a) and regressions showing how PC1 predicts resistance to herbivores  
 1197 (b). The individual points show the species' raw means across four sampling times  
 1198 while the line shows the regression slope from the linear mixed model, with the  
 1199 corresponding statistics shown (each point depicts the mean for a *Ficus* species across  
 1200 four sampling times, n = 36).



1201

1202 Fig. 6 Correlations between herbivore insect community structure and *Ficus* traits  
1203 visualized by the Canonical Correlation Analysis biplot (point depicts herbivore insect  
1204 species and lines represent direction and strength of correlations).



1205