

Macroevolution of defense syndromes in *Ficus* (Moraceae)

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**Harper Adams
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1 Running head: Defense syndromes in *Ficus*

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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 ($\text{g} \cdot \text{L}^{-1}$),
171 and latex alkaloid content ($\text{g} \cdot \text{L}^{-1}$). SLA, leaf water, C/N, leaf tannin, and toughness are traits
172 commonly correlated (positively or negatively) with leaf palatability and digestibility. Higher
173 C/N ratio, toughness, and tannin content are often also correlated with reduced herbivory and
174 slower herbivore growth rates (Choong et al. 1992, Hanley et al. 2007, Clissold et al. 2009,
175 Kitajima et al. 2010, Kitajima et al. 2012, Prado et al. 2014). SLA and C/N are also considered as
176 indices of resource capture ability and leaf quality, respectively. The latter might affect herbivore
177 damage (Pérez-Harguindeguy et al. 2003), whereas the former affects the plant's ability to
178 tolerate herbivory (e.g. by regrowth) because of its connection with carbon storage and

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°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 10⁷ 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 m² 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).

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

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

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 ⁻¹)	-0.39	0.10	-3.94	< 0.001***
Lg (Trichome density, No.·mm ⁻²)	-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 ⁻¹)	-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 ⁻¹)	-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 ²	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_4 (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_{ob} , indicated by solid diamond), or on the basis of
1150 four different null models of multivariate variation of those traits (H_1 to H_4). VD
1151 (volume difference) means the difference between H_{ob} and H_1 to H_4 (see Methods).

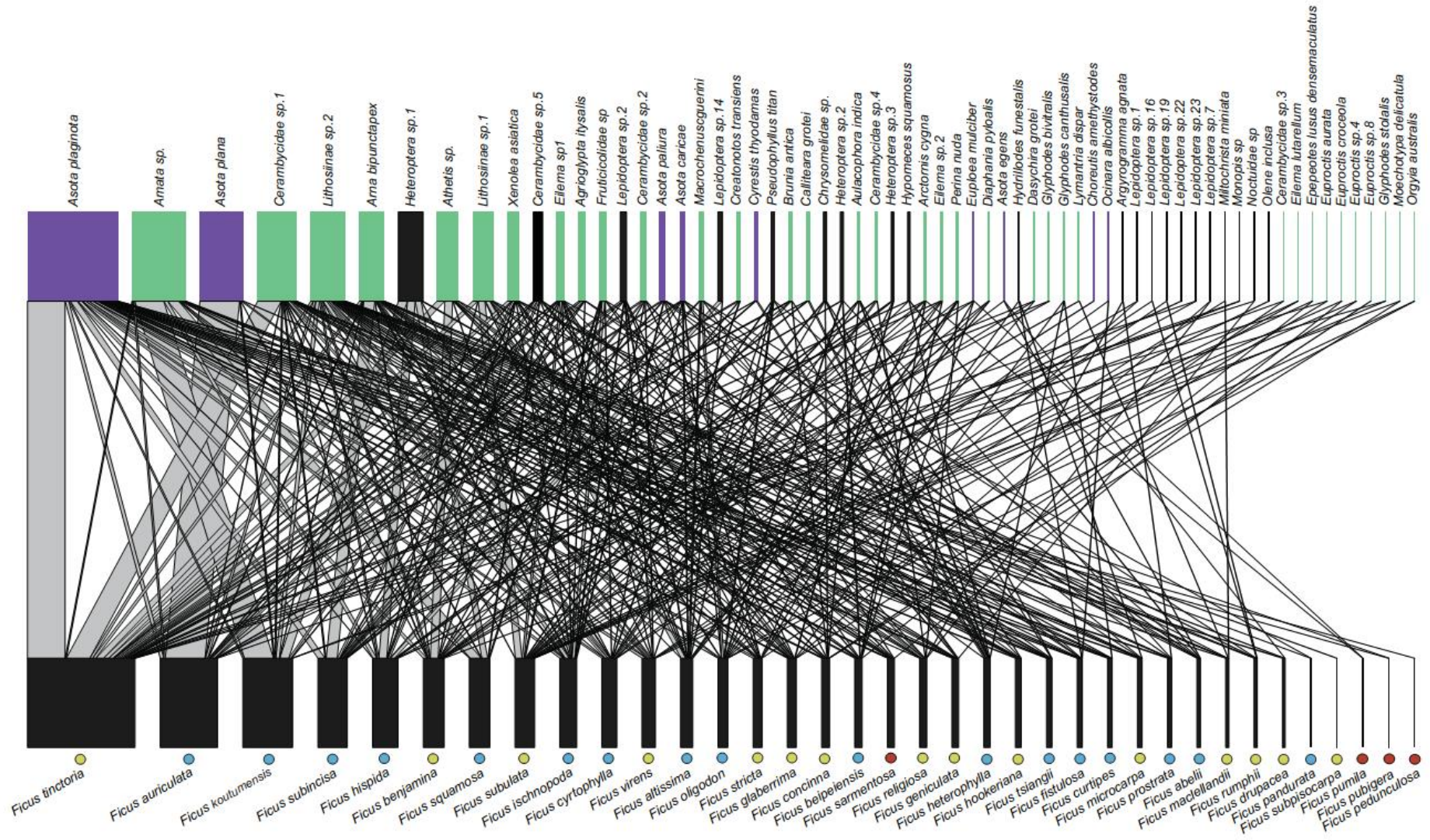
1152

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, $n = 36$).

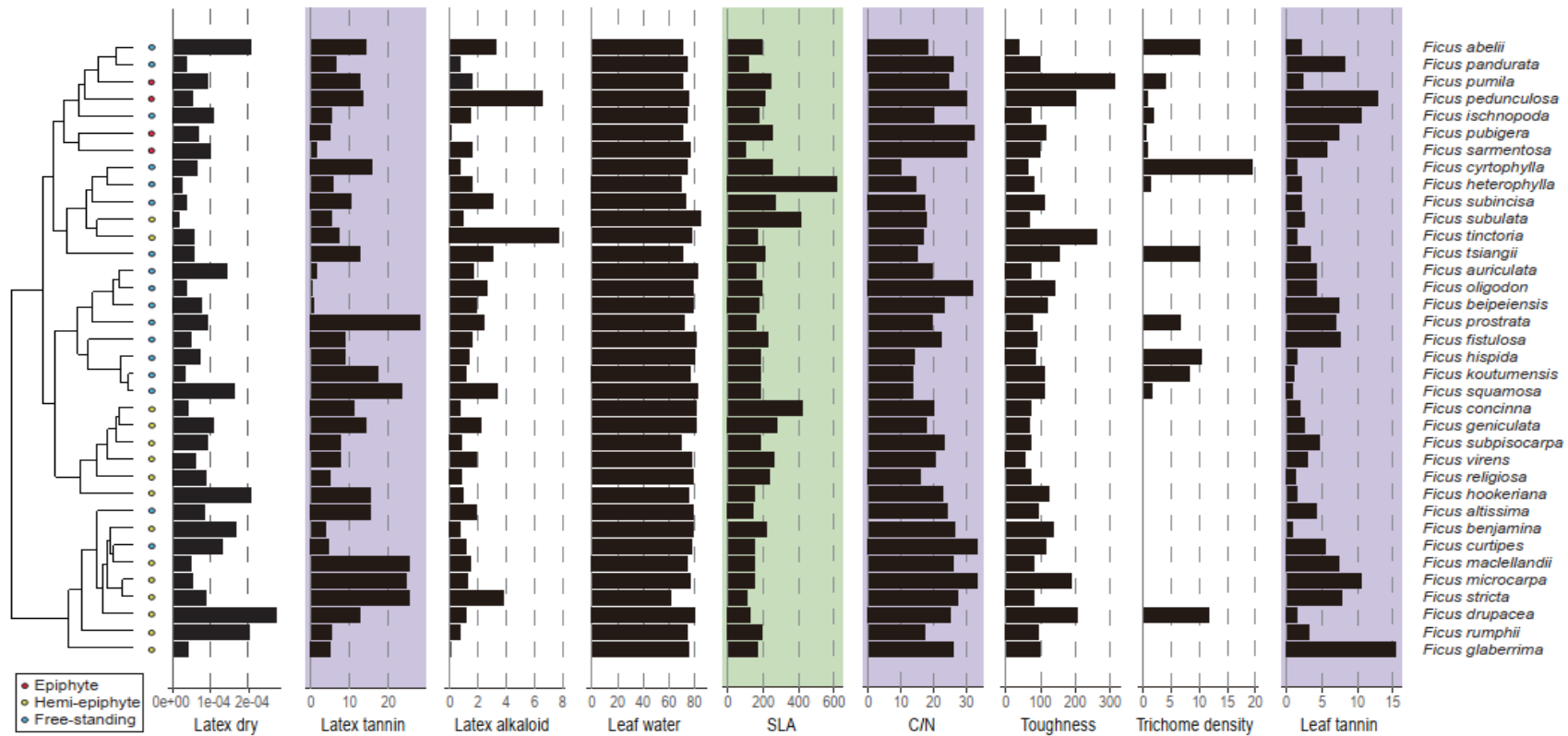
1159

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).

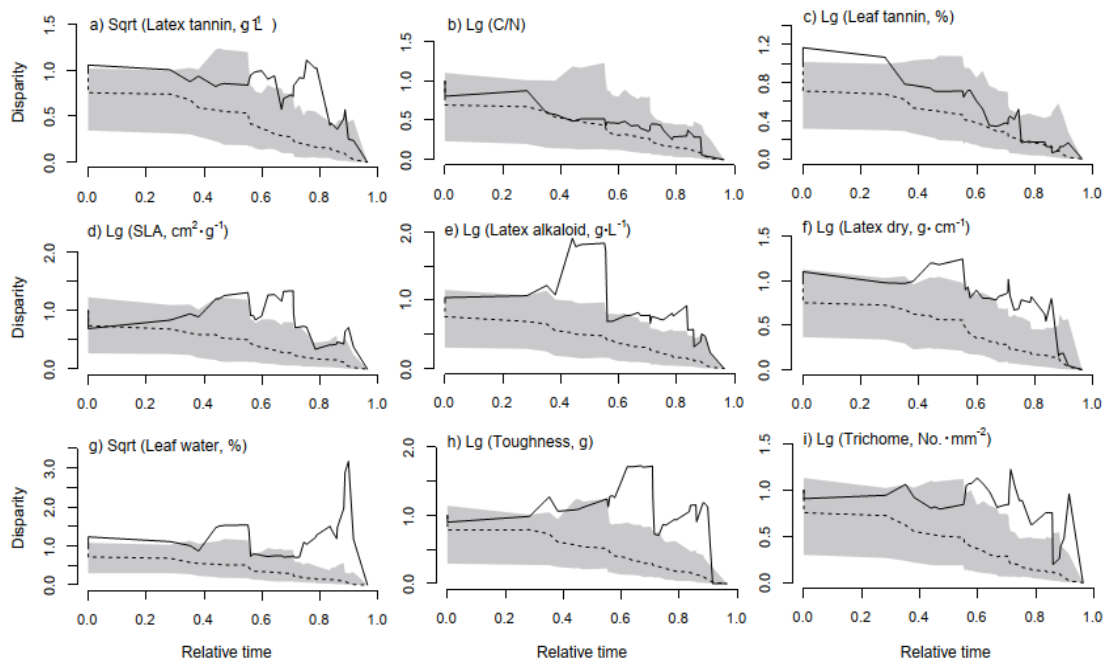
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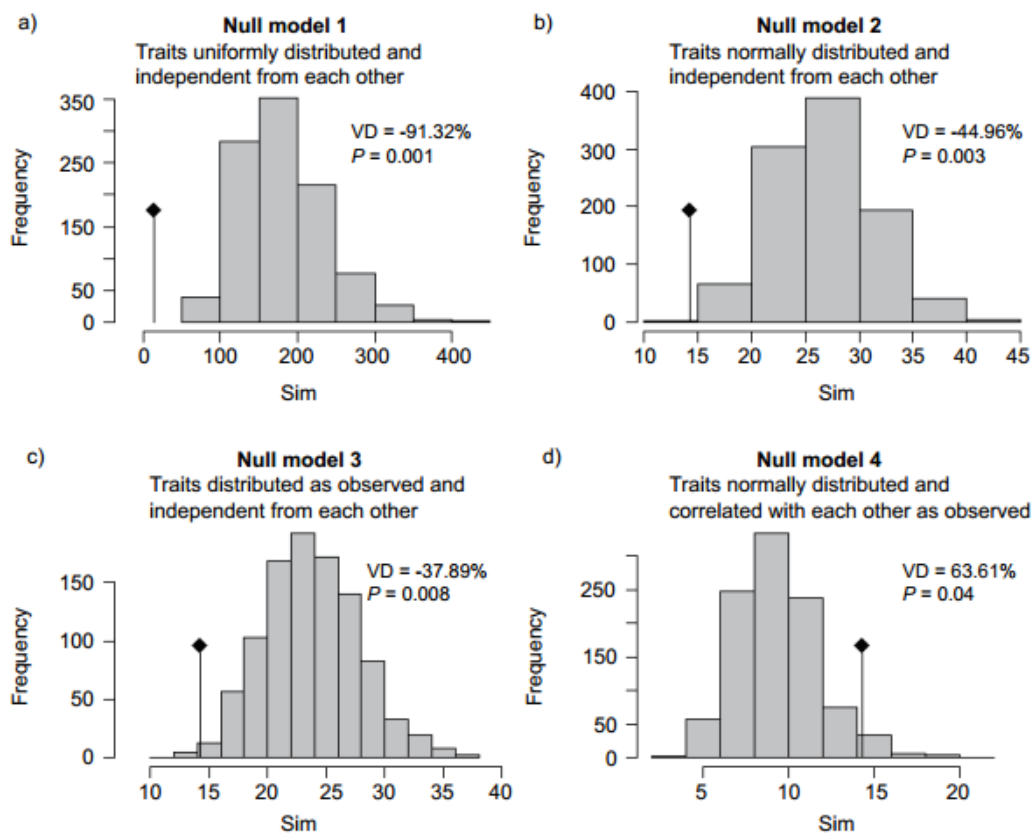


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1184

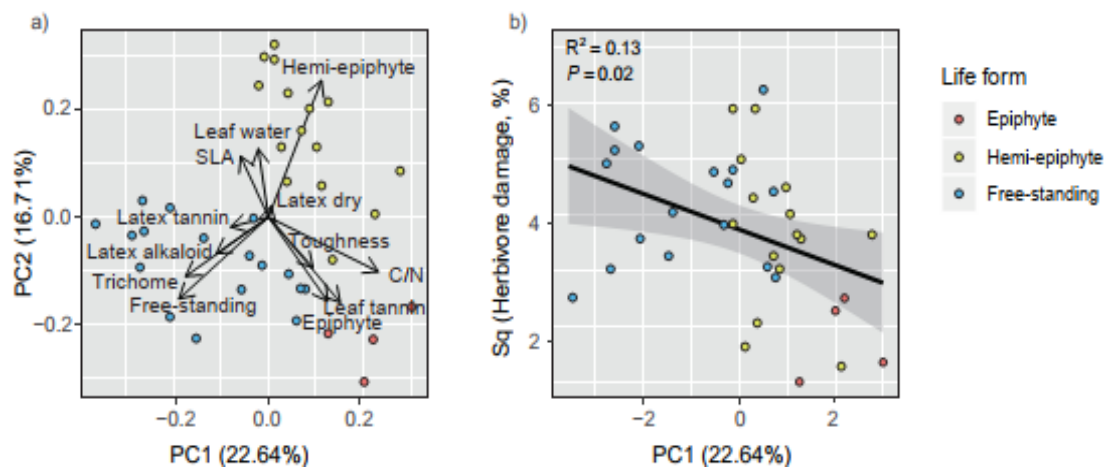
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1193

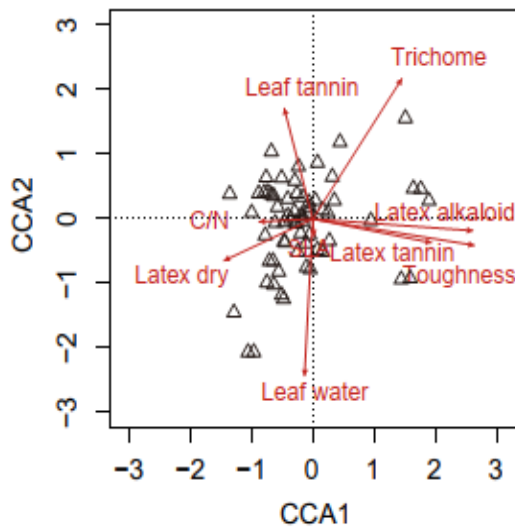
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1205