Journal of Chemical Ecology (2020) 46:442–454
https://doi.org/10.1007/s10886-020-01173-7

Compound Specific Trends of Chemical Defences in *Ficus* Along an Elevational Gradient Reflect a Complex Selective Landscape

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Received: 9 January 2020 / Revised: 25 February 2020 / Accepted: 16 March 2020 / Published online: 21 April 2020

Abstract

Elevational gradients affect the production of plant secondary metabolites through changes in both biotic and abiotic conditions. Previous studies have suggested both elevational increases and decreases in host-plant chemical defences. We analysed the correlation of alkaloids and polyphenols with elevation in a community of nine *Ficus* species along a continuously forested elevational gradient in Papua New Guinea. We sampled 204 insect species feeding on the leaves of these hosts and correlated their community structure to the focal compounds. Additionally, we explored species richness of folivorous mammals along the gradient. When we accounted for *Ficus* species identity, we found a general elevational increase in flavonoids and alkaloids. Elevational trends in non-flavonol polyphenols were less pronounced or showed non-linear correlations with elevation. Polyphenols responded more strongly to changes in temperature and humidity than alkaloids. The abundance of insect herbivores decreased with elevation, while the species richness of folivorous mammals showed an elevational increase. Insect community structure was affected mainly by alkaloid concentration and diversity. Although our results show an elevational increase in several groups of metabolites, the drivers behind these trends likely differ. Flavonoids may provide figs with protection against abiotic stressors. In contrast, alkaloids affect insect herbivores and may provide protection against mammalian herbivores and pathogens. Concurrent analysis of multiple compound groups alongside ecological data is an important approach for understanding the selective landscape that shapes plant defences.

Keywords Coleoptera · Folivorous mammals · Herbivory · Lepidoptera · New Guinea · Phenanthroindolizidine alkaloids · Polyphenols · Possum · Tannins

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s10886-020-01173-7) contains supplementary material, which is available to authorized users.

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Introduction

Elevational gradients lead to local adaptations and differential selection on traits, rapid turnover in community composition, and changing interaction networks (Segar et al. 2017b; Toussaint et al. 2013). As a result, long wet elevational gradients in the tropics are often among the most diverse places on earth in terms of both species richness and functional diversity (Perrigo et al. 2020). In plants, elevational gradients can drive significant changes in the production of secondary metabolites in response to changes in both biotic and abiotic conditions (Defossez et al. 2018; Moreira et al. 2018). These changes in plant chemistry have cascading effects on the associated organisms, as plant secondary chemistry underpins patterns of diversity across multiple trophic levels (Richards et al. 2015; Volf et al. 2019).

Plants might be expected to invest progressively less into chemical defences with increasing elevation because insect abundance and herbivory generally decrease towards higher elevations (Garibaldi et al. 2011; Pellissier et al. 2014; Sam et al. 2020). However, the costs of compensating for biomass lost to herbivores show a strong elevational increase too. This may favour a higher investment into defences at the expense of growth by plants at higher elevations (Defossez et al. 2018; Givnish 1999; Salgado et al. 2016). Elevational trends in anti-herbivore defences can be further modified by changes in herbivore communities that normally show a strong turnover with elevation (Novotny et al. 2005). As different herbivores respond to different plant defences (Volf et al. 2015, 2018), such changes in insect community composition can modify the relative importance of individual defensive traits along elevational gradients. Furthermore, while studies have typically focused on elevational trends in insect herbivory, the abundance of plant pathogens and other groups of herbivores, such as folivorous mammals, also show pronounced elevational trends (Brown and Vellend 2014; Geml et al. 2014; Tallowin et al. 2017). Thus, the plant chemotype observed is a result of multiple biotic drivers operating over both ecological and evolutionary scales.

While herbivores and pathogens are important drivers of secondary metabolite diversity, abiotic factors also play an important role. Temperature, and in most cases resources, decrease with elevation and this can impair some of the metabolic pathways responsible for producing secondary metabolites. This is largely true in the alpine zone, above the tree line, where plants are exposed to extreme abiotic conditions (Pellissier et al. 2014). On the other hand, secondary metabolites involved in protection against low temperatures and UV irradiation, such as various flavonoids, should increase in concentration with elevation (Rasmann et al. 2014). This increase in specific metabolite groups stimulated by abiotic conditions can secondarily affect insect herbivores that also respond to the changing environmental conditions themselves (Escobar-Bravo et al. 2017).

Indeed, it is the interaction between biotic and abiotic factors that drives elevational trends in host plant defences (Defossez et al. 2018). Given the complexity of these interactions, elevational gradients do not generate a simple directional change in the overall intensity of chemical defences. Instead they act to modify the relative importance of individual groups of secondary metabolites and forms of plant defence (Defossez et al. 2018; Moreira et al. 2018; Rasmann et al. 2014). Quantification of herbivore or pathogen communities and environmental variables is necessary for the correct interpretation of trends in host-plant defences (Moreira et al. 2018).

Here we focus on the compound specific leaf chemistry of figs (*Ficus*; Moraceae) along one of the world's most diverse elevational gradients, the New Guinean Central Range. *Ficus* has a pantropical distribution and is an extraordinarily species rich genus of woody plants, containing over 800 species, of which ca. 150 occur in Papua New Guinea (PNG) (Berg and Corner 2005; Cruaud et al. 2012). *Ficus* is a keystone plant genus. It supports diverse communities of herbivorous insects and several groups of frugivorous and herbivorous birds and mammals (Kanowski et al. 2003; Novotny et al. 2005; Shanahan et al. 2001). The insect herbivores associated with the genus can typically feed on multiple con-generics which is thought to have contributed to the chemical divergence among *Ficus* species (Volf et al. 2018, 2019). The majority of the mammalian herbivores feeding on *Ficus* in the New Guinean region are possums, cuscuses or tree mice (Flannery 1995). *Ficus* is over-represented amongst plant species with wide elevational ranges (Novotny et al. 2005) and in PNG, elevational gradients have probably played an important role in the speciation within the genus. Parapatric speciation has likely generated distinctive lowland/highland populations, sister species, and communities (Segar et al. 2017b; Souto-Vilarós et al. 2019).

Fig leaves contain a variety of secondary metabolites, including alkaloids, polyphenols, and terpenoids (Volf et al. 2018). Phenanthroindolizidine alkaloids are among the most important alkaloid groups in *Ficus*. They have a rather restricted distribution among plants and are typically produced by species of Moraceae, Apocynaceae, and Caricaceae (Damu et al. 2005; Han et al. 2013; Konno et al. 2004). Phenanthroindolizidine alkaloids exhibit a pronounced cytotoxicity and inhibit the enzymes involved in the synthesis of DNA (Stærk et al. 2000). They are strong antifeedants for generalist herbivores (Miller and Feeny 1983). In contrast, some specialized and highly adapted insect herbivores feeding on *Ficus*, such as moths from the genus *Asota*, are probably able to sequester these metabolites (Sourakov and Emmel 2001). Some phenanthroindolizidine alkaloids, such as antofine, also show anti-pathogen activities, being effective inhibitors of bacteria and fungi (Mogg et al. 2008). Polyphenols are a diverse group of secondary metabolites with a broad variety of functions. Their anti-herbivore

function against insects results from at least three factors: (1) oxidative activation mediated by the high pH of the insect gut, or by plant polyphenol oxidases release by cell lysis, (2) binding and precipitation of nutritive proteins at the low to neutral pH present at the oral cavity or in the gut of some insect species, and (3) activity resulting from degradation/hydrolysis products of polyphenols that may be accelerated by high pH or microbe action (Salminen 2014; Salminen and Karonen 2011). Importantly, the high pH found especially in the gut of lepidopteran larvae favours the oxidation of polyphenols and inhibits their protein precipitation functions (Salminen and Karonen 2011). In addition, flavonols are often involved in abiotic protection, such as against UV irradiation (Escobar-Bravo et al. 2017; Harborne and Williams 2000).

Our aim was to document elevational trends in the concentration, diversity, and composition of *Ficus* alkaloids and polyphenols. We analysed trends in chemical data in the context of caterpillar and leaf-chewing beetle communities. Furthermore, we reported patterns in the elevational species richness of mammalian herbivores because these may represent an important factor driving investment in defence. We expected a general elevational increase in *Ficus* defences as the plants growing at high elevations need to protect their biomass against both biotic and abiotic factors more intensely.

Methods and Materials

Study Sites and Field Sampling We carried out a detailed survey at six study sites along an elevational gradient (200, 700, 1200, 1700, 2200, and 2700 m a.s.l.) on Mt. Wilhelm in Papua New Guinea from June 2013 to February 2014 (Figure S1, Table S1). Our study transect has been subject to intensive study and is home to 51% species of New Guinea mainland birds, 27% of PNG butterflies and 15% of PNG frogs (Novotny and Toko 2015). There are 157 *Ficus* species known from New Guinea (Whitfeld and Weiblen 2010), including 73 species documented along the Mt Wilhelm transect. The majority of species surveyed at our study site are widespread in Papua New Guinea and frequently recorded in large scale floristic surveys (Berg and Corner 2005). We focused on nine *Ficus* species common along the gradient: *F. arfakensis* King, *F. copiosa* Steud., *F. pungens* Reinw. ex Blume, *F. erythrosperma* Miq., *F. hahliana** Diels, *F. hombroniana** Corner, *F. itoana* Diels, Diels, *F. microdictya* and *F. umbrae* Weiblen. The last three species are part of a monophyletic complex, with *F. umbrae* Weiblen being a newly described species recently split from *F. itoana* (Ezedin and Weiblen 2019; Souto-Vilarós et al. 2018). We treated the *F. itoana* species complex as a single species for the purpose of statistical analyses. Species marked with an asterisk may comprise further genetically distinct entities above the population level. Highland individuals of *F. hombroniana* resemble the closely related *F. ihuensis* and populations of *F. hahliana* at 1700 m a.s.l. and above are genetically and morphologically distinct from lowland populations, although they form a monophyletic clade within the current sampling context (Segar et al. 2017b).

At each elevation, we set up ten 10 × 500 m transects and marked all focal *Ficus* species with a DBH (diameter at breast height) greater than 1 cm that were growing within the transect. We identified each tree and gave it a unique identifier number (Segar et al. 2017b). Our selection of individual trees for sampling chemistry was guided largely by the range of sizes used to sample insects (see below), although in both cases we aimed to avoid extremely young individuals (i.e. saplings with a DBH < 1.0 cm). We sampled 142 trees for chemical data and recorded DBH data for 132 of these individuals. The mean diameter at breast height (DBH) for each species was as follows (standard error in parentheses): *Ficus afarkensis* 5.0 cm (± 0.9), *Ficus copiosa* 7.5 cm (± 2.2), *Ficus erythrosperma* 6.8 cm (± 0.9), *Ficus hahliana* 5.8 cm (± 0.8), *Ficus hombroniana* 2.5 cm (± 0.4), *Ficus itoana* complex 7.8 (± 0.9) and *Ficus pungens* 11.6 (± 1.6). We collected forty leaf discs from up to six individuals per species per elevation using a cork borer 2.4 cm in diameter (avoiding the midrib) from fully expanded mature leaves. We avoided sampling from plants heavily damaged by herbivores or pathogens. We stored half of the leaf discs in HPLC grade acetone in order to prevent enzymatic degradation and oxidization of the studied metabolites in the field and transferred them to a dark − 20ºC freezer on return to the New Guinea Binatang Research Centre. Later, we used these discs for secondary metabolite analysis. We weighed the other half of leaf discs fresh and dry in order to estimate both the percentage of water per leaf disc and the dry weight contained in each tube of acetone (Volf et al. 2018).

We sampled all *Ficus* individuals for Lepidoptera leaf-chewing larvae (caterpillars) and adult leaf chewing beetles. Trained collectors walked the same ten transects per elevation as described above and systematically (leaf to leaf) searched all accessible (≤ 3 m height) foliage for herbivores on *Ficus* trees. Collection was exhaustive across the accessible foliage such that the number of leaves surveyed varied from tree to tree. We repeated this sampling ten times, in approximately ten-day intervals over a 3.5 month period, for each transect and across all study sites. A total of 300 km across sites was walked across surveys and months. We tested all herbivores for feeding on the plant species from which they were collected in 24-hour no-choice experiments to confirm host associations. Where possible we reared the larvae to adults and photographed both stages. We morphotyped individuals by cross-referencing them to collections at the New Guinea Binatang Research Center. We shipped the adult Lepidoptera to the National Museum of Natural History, Smithsonian Institution for further identification. Legs of representative

samples were shipped to Institute of Entomology, Biology Centre, Czech Academy of Sciences. We sampled dry legs from 486 Lepidoptera individuals to obtain COI barcode sequences (Wilson 2012). Following this we either shipped the samples directly for sequencing with standard Sanger protocols at the Biodiversity Institute of Ontario or sent them as extracted and amplified DNA for sequencing at Macrogen Korea. We uploaded the sequences to BOLD and assigned them to Barcoding Index Numbers (BINs) which we used as corroborating evidence, alongside photographs and taxonomic examination by SEM, to further improve our field-based identifications (Appendix 1). Our approach allowed us to place the barcoded specimens within a wider sampling context (of 25,000 New Guinean Lepidoptera sequences) and to connect and refine species concepts across tens of years of sampling. We have released data for 408 sequences representing 198 barcode clusters (putative species) on GenBank (MT256509 - MT256916) including the standard fields for the BARCODE data standard and more data, including images and host plants, are available on BOLD (www.boldsystems.org; Ratnasingham and Hebert 2007, 2013), in a dataset accessible using a DOI (https://doi.org/10.5883/DS-WILFC).

We used the leaf area sampled for herbivores to standardize insect abundance across sites and *Ficus* species (Table 1). Specifically, we counted the number of leaves sampled for herbivores on each tree. We then haphazardly sampled one leaf per tree and photographed it. We randomly selected at least ten individuals per *Ficus* species and elevation (if available), measured the leaf area from photographs and used these data to generate mean area of one leaf per *Ficus* species per elevation. The final estimates of the leaf area sampled for herbivores were calculated by multiplying the number of leaves sampled for a given *Ficus* species and elevation by the corresponding mean area per leaf.

Non-volant mammals were surveyed at every elevation during the dry season of 2019 (June-September). We sampled every site for ten consecutive nights using between 177 and 266 traps per night. We used the following trap types: rat-type snap traps, medium Sherman box live traps, Elliott box live traps, roofed Tomahawk cage live traps (cat size and squirrel size), and roofed pitfall live traps (provided with hay or moss in higher altitudes). We positioned trapping lines to start at least 50 m from each camp. The terrestrial traps were in 4–6 lines, at ~ 7 m intervals and placed in diverse habitats (primary and secondary forest, creeks and food gardens). The pitfalls were set 10 m apart along a 50 mm high barrier from a black plastic foil. Additionally, we set a mean of 39 arboreal traps per site in accessible trees between a height of seven to 15 m at the altitudes of 700, 1700, and 2700 m a.s.l., using a combination of snap traps, Sherman box live traps, and roofed Tomahawk cage live traps. We checked our traps at least twice per 24-hour sampling period (dusk and sunrise). We baited all traps except for the pitfalls before dawn, mostly with a mixture of peanut butter, tinned fish, and rolled oats or with sweet potatoes. Arboreal traps were occasionally baited with banana. We also conducted spotlighting and night walks with local hunters to find and capture mammals. We inspected hunted animals, including older bones and skins, provided by local hunters (a total of 142 bones and 18 skins and other remains). Finally, we conducted opportunistic interviews with local inhabitants and recorded their mammal sightings for each site. The methods, including sampling protocol, were approved by the PNG National Research Institute as a basis for the issue of a Special Exemption Research Visa no. 99902702887. All animals were handled in accordance with ethical guidelines approved by the State of Papua New Guinea.

Finally, we measured average temperature and humidity at each elevation as surrogates for climatic changes along the gradient as described in detail in Sam et al. (2019). Temperature and humidity at each site were recorded every hour by R3120 dataloggers (Comet Systems, Rožnov pod Radhoštěm) placed in the understory (1 m above ground). The temperature and humidity were monitored for 12 months in 2010 and six months in 2013. Only at 700 and 1200 m,

Table 1 Number of individuals of *Ficus* species sampled for chemical traits and the leaf area of conspecific individuals searched for herbivores (in brackets; m^2) across elevations. Species and elevations with low leaf area sampled for herbivores are marked with NAs and were excluded from the analyses using herbivore data. Species codes used in Fig. 3 are given in the brackets following the scientific names

Species	200 m	700 m	1200 m	1700 m	2200 m	2700 m	Total
F. arfakensis (ARF)	5 (138.08)	5 (64.42)	5 (39.20)	3 (395.41)			17 (637.11)
F. copiosa (COP)	6 (47.41)	5 (165.96)	4 (18.13)	5 (116.67)			20 (348.17)
F. erythrosperma (ERY)		5 (46.63)	4 (114.73)	5 (120.34)			14 (281.7)
F. hahliana (HAH)	5 (148.30)	5 (246.15)	5 (274.08)	5 (96.82)	3 (661.90)	2 (1664.84)	25 (2497.05)
F. hombroniana (HOM)	3 (22.88)	5 (23.63)	5 (4.38)	5 (421.77)	5 (667.71)		23 (1140.37)
F. itoana complex (IXM)	5 (11.94)	4 (147.48)		5 (241.67)	5 (14.96)	5 (NA)	24 (416.05)
F. pungens (PUN)	5 (NA)	5 (NA)	4 (NA)	5 (NA)			19 (NA)
Total	29 (368.61)	34 (694.27)	27 (450.52)	33 (1392.27)	13 (1344.57)	7 (1664.84)	142 (5320.45)

where the original dataloggers were stolen, the data represent six months of measurements in 2011 and six months of measurements in 2013. The values obtained were used for calculating mean temperature and humidity at each elevation.

Chemical Analysis We stored the leaf discs collected for alkaloid and polyphenol analysis (ca. 0.5 g of dry leaf tissue in total for each individual) in 40 ml of HLPC grade acetone. In the laboratory, we transferred this first acetone extract into a 50 ml falcon tube. We added 5 ml of ultrapure water and concentrated the solution to water phase under a flow of nitrogen at room temperature. We cut the leaf discs into smaller blades and transferred them into grinding tubes (DT-50, IKA-Werke GmbH & Co. KG, Germany) containing 35 ml acetone/water (80:20, v/v). We extracted the remaining alkaloids and polyphenols from the leaves by grinding them for 30 min using tube dispensers at room temperature (Ultra-Turrax Tube Drive, IKA-Werke GmbH & Co. KG, Germany). Then we removed the leaf material and combined the extract with the water phase obtained from the first acetone extraction above. We diluted the combined extract with acetone to a uniform volume of 50 ml. We split this volume of extract, with 10 ml being taken for polyphenol analysis and the remaining 40 ml being freeze-dried and used for alkaloid analysis.

For the analysis of alkaloids, we suspended the dried extract in 10 ml of 5% aq. HCl, vortexed it and transferred it into a 15 ml Falcon tube and centrifuged it (9000 rpm, 10 min) before transferring it to a 10 ml clear vial. Subsequently, we took 8 ml of the sample and adjusted its pH to 10 with 25% NH_3. We extracted the alkaline solution in a 50 ml extraction funnel with an equal volume of $CHCl_3$. We dried the chloroform solution under nitrogen and dissolved it into ethanol, filtered it with a 0.2 μm PTFE filter and analysed it by UPLC-DAD-HESI-Orbitrap-MS in the positive ion mode as described in Volf et al. (2018). The Acquity UPLC systems consisted of a binary solvent manager, a sample manager, a column oven and a diode array detector (Waters Corporation, Milford, MA, USA). We used an Acquity UPLC BEH phenyl column (30 mm × 2.1 mm i.d., 1.7 μm; Waters Corporation). The UPLC system was attached to a Q Exactive Orbitrap mass spectrometer with a heated electrospray ion source (HESI II; Thermo Fisher Scientific GmbH, Bremen, Germany). The flow rate of the eluent was 0.650 mL/min and 0.1% HCOOH (A) and acetonitrile (B) were used in the gradient elution. The gradient profile was as follows: 0–0.1 min: 97% A and 3% B (isocratic); 0.1–3.0 min: 97–55% A and 3–45% B (linear gradient); 3.0–5.0 min: 55%–10% A and 45–90% B (linear gradient); 5.0–7.0 min: 10% A and 90% B (isocratic); 7.0–7.1 min: 10–97% A and 90–3% B (linear gradient); 7.1–7.2 min: 97% A and 3% B (isocratic). The injection volume was 5 μL by full loop injection. The resolution of the mass spectrometer was set to 70 000, automatic gain control (AGC) was 3×10^6, maximum injection time was 200 ms and the scan range was 150–1200 *m/z*. The HESI conditions were as follows: spray voltage + 4.0 kV, capillary temperature 380 °C, sheath gas (N_2) flow rate 60 units, auxiliary gas (N_2) flow rate 20 units and Slens RF level 60. The mass spectrometer was calibrated with Pierce LTQ Velos ESI Positive Ion Calibration Solution (Thermo Fischer Scientific, Rockford, IL, USA). We processed the data with Thermo Xcalibur Qual Browser and Thermo Xcalibur Quan Browser software packages (Thermo Fischer Scientific). To identify the alkaloids in the samples, we took a portion of each alkaloid extract and pooled them together by plant species. We then identified the alkaloids from each plant species by analysing the pooled samples with UPLC-DAD-HESI-Orbitrap-MS/MS. We identified the compounds mainly by their molecular formulas, which we constructed from the high-resolution mass spectrometric data and then compared them to literature (e.g.Damu et al. 2005; Khan et al. 1993; Lee et al. 2011). Additionally, we used UV spectra and MS^2 data for the compound identification (Baumgartner et al. 1990; Bruneton et al. 1983; Cui et al. 2004; Xiang et al. 2002). We assigned the individual compounds to following structural sub-groups: phenanthroindolizidines, *seco*-phenanthroindolizidines, dehydro-*seco*-phenanthroindolizidines, tetrahydrobenzylisoquinolines, and ficuseptamines. Subsequently, we semi-quantified the alkaloids from the extracts with extracted ion chromatograms (EIC) as area of peak/mg (dry weight) of plant material. To control for the possible fluctuations in the performance of the MS system, we analysed a *Ficus septica* extract periodically and monitored the area of ficuseptine with an EIC. We normalized all initial peak areas of the EICs of the analytes taking into account the possible changes in the ficuseptine peak areas.

In the case of polyphenols, we ran two separate sets of assays. First, we quantified concentrations of the main polyphenol sub-groups (in mg/g dry weight) by UPLC-QqQ-MS/MS with the methods of Engström et al.. (Engström et al. 2014, 2015) as described in e.g. Malisch et al. (2016). The measured polyphenol sub-groups included (1) hydrolysable tannins that we divided into galloyl derivatives and hexahydroxydiphenoyl derivatives (HDDP, ellagitannins), (2) proanthocyanidins that we divided into procyanidin and prodelphinidin subunits, (3) flavonol glycosides that we divided into kaempferol, quercetin and myricetin derivatives, and (4) quinic acid derivatives. Second, from each species we chose all individual polyphenols we were able to characterize on the basis of their UV and MS spectra (e.g. Moilanen et al. 2013). For the quantification of the selected compounds from the negative ion full scan trace of the UPLC-QqQ-MS/MS analyses, we used the *m/z* value of each compound that corresponded to its deprotonated molecule. We quantified these compounds against calibration curves obtained with our own standards (chlorogenic acid, epicatechin, quercetin galactoside, kaempferol glucoside).

In addition, we ran two activity assays to quantify two major functions of polyphenols in anti-herbivore protection – oxidative activity and protein precipitation capacity. We measured polyphenol oxidative activity following Salminen

and Karonen (2011) using gallic acid as the standard. We measured protein precipitation capacity following Hagerman's radial diffusion assay (Hagerman and Butler 1978) using pentagalloylglucose as the standard. Both assays gave activities in mg/g dry weight.

Finally, we calculated the Shannon diversity index for alkaloids and polyphenols based on the concentration (in area of peak/mg dry weight and in mg/g dry weight, respectively) of main structural sub-groups listed above to account for structural diversity rather than for the number of compounds in a sample.

Statistical Analysis First, we explored overall elevational trends in the concentration and diversity of main alkaloid and polyphenol structural sub-groups, and in the two measured activities. We performed a *Redundancy Analysis* (RDA) with chemical data as the response variables to analyse what percentage of variability in *Ficus* chemical profiles is explained by the elevation. We used elevation as the explanatory variable and *Ficus* species identity as a covariable defining permutation blocks. All chemical and activity data were log-transformed prior to the analyses. We used *Ficus* species from individual elevations as samples. We identified the relative effects of elevation and species identity on alkaloid and polyphenol profiles using 9999 permutations and adjusted the explained variability following Ter Braak and Smilauer (2012). In addition, in the next step we added average temperature and humidity as surrogates for climatic variation along the gradient in the RDA and compared their effects with the effect of elevation by variance partitioning. We conducted all multivariate analyses conducted in CANOCO 5 (Ter Braak and Smilauer 2012).

Second, we used compound level data to test for specific elevational trends within focal metabolite sub-groups as individual compounds can exhibit differential responses to elevation. We modelled the overall correlation between the major classes of individual compounds (alkaloids, non-flavonoid polyphenols, flavonoids (flavonols and flavones)) and elevation with a separate linear mixed model for each polyphenol group using the R package 'nlme' (Pinheiro et al. 2019) and a generalised linear mixed model for alkaloids as implemented in the R package 'lme4' (Bates et al. 2015). Such an approach is informative when both correlations and opposing trends are expected between explanatory variables. In each model, we used the concentration of each individual compound present in at least 50% of all species and samples as the response variables. For analytical purposes we arranged the data so that the only unique row value was concentration, each individual tree was coded as an observation (repeating 1-142) while species (seven levels), elevation and compound identity were also included to group the rows of concentration values. The fixed explanatory variables were elevation and compound. We used *Ficus* species as the random effect. We also included a constant variance function for the term 'compound' that allowed a different standard error for each level (e.g. each compound) along with a general correlation structure between observations from the same individual grouped within species. Finally, we ran mixed models for each individual compound, with the random effect being species. Values in the alkaloid data set were typically high or zero, due to a lack of universal compound presence, as such we converted alkaloid concentration to binary values (presence or absence) and modelled this variable as having a binomial distribution of errors (e.g. we used a generalised linear mixed model with a logit link).

Third, we analysed the elevational trends in insect abundance and the number of herbivores shared between the studied *Ficus* species. To assess the elevational trends in leaf-chewer abundance, we analysed the correlation between the elevation and log-transformed insect abundance standardized by leaf area using linear mixed effect models. We used *Ficus* species identity as a random factor. To assess the elevational trends in leaf-chewer specialization, we calculated the dissimilarity of leaf-chewer communities between pairs of studied *Ficus* species at individual elevations using Bray-Curtis abundance-based index and correlated it to elevation. We used quasibinomial generalised linear models with the response variable Bray-Curtis dissimilarity and the explanatory variable elevation, with and without a second order polynomial fit. We chose a quasibinomial error structure because the response variable was bounded by 0 and 1 and the model showed overdispersion. We compared the two models using ANOVA with an F test and selected the more complex model if it explained significantly more of the deviance.

To analyse the effects of the studied compounds on the leaf-chewer community structure, we analysed the effects of alkaloids and polyphenols on leaf-chewer communities by hierarchical *Canonical Correspondence Analysis* (CCA). Firstly, we ran an analysis of the effects of total concentrations of alkaloids and polyphenols, their diversities, concentrations of their sub-groups, and the two types of activities. Secondly, we ran an analysis of the effects of individual compounds. We standardized insect data by leaf area, log-transformed them, and down-weighted rare insect species (Ter Braak and Smilauer 2012). We used *Ficus* species trait means at individual elevations as explanatory variables. We used *Ficus* species identity and elevation as covariables and defined the permutation blocks by species identity. We identified the chemical traits with significant effects using 9999 permutations and forward selection. We conducted all multivariate analyses in CANOCO 5 (Ter Braak and Smilauer 2012).

We removed singleton herbivore species from all analyses. We also excluded *F. pungens*, which had only a small leaf area sampled for herbivores, and the *F. itoana* complex from 2700 m, for which only one singleton herbivore was sampled, from all analyses using the insect data.

Results

In total, we analysed 142 trees for polyphenols and alkaloids. We characterized a total of 29 alkaloids belonging to five alkaloid sub-groups and 49 polyphenols belonging to five polyphenol sub-groups (Table S2 and S3). See Appendix 2 for details on their distribution among the studied *Ficus* species.

Both polyphenol and alkaloid total and sub-group concentrations, their diversities, and activities changed along the elevational gradient (Fig. 1). Diversities of both alkaloids and polyphenols showed an increasing trend along the gradient (Figure S2). There was an increase in alkaloid concentration towards 2200 m while they decreased at 2700 m when not accounting for *Ficus* species identity. This was caused by differential responses of individual alkaloid sub-groups to elevation – phenanthroindolizidines, *seco*-phenanthroindolizidines showed an almost linear increase towards higher elevations while dehydro-*seco*-phenanthroindolizidines and tetrahydrobenzylisokinolines decreased towards higher elevations but more slowly, with a plateau at mid elevations (ca. 1700–2200 m a.s.l.). Ficuseptamines were not present at low elevations and were found only in the *F. hahliana* population at 2700 m a.s.l.

Importantly, when analysed by the RDA accounting for species identity, most alkaloid structural sub-groups, alkaloid concentration, and their diversity showed significant positive

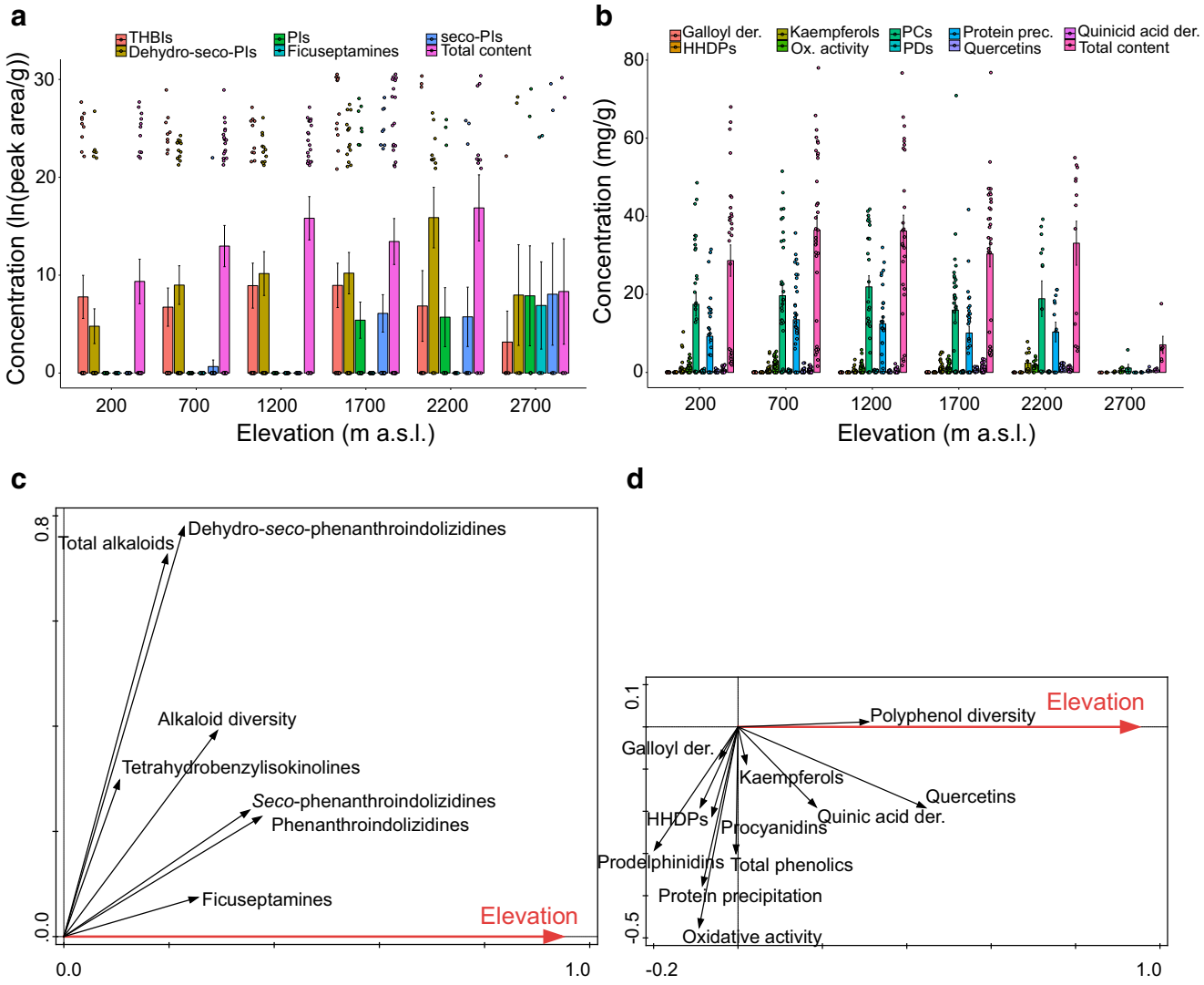

Fig. 1 Elevational trends in individual alkaloid (**a**) and polyphenol (**b**) structural sub-groups and effects of elevation on alkaloid (**c**) and polyphenol (**d**) composition in the studied *Ficus* species. The bars show means ± sd. The concentrations are given per g of dry leaf material. The overall effects of elevation on *Ficus* alkaloids, polyphenols, and their main structural groups were summarized by RDA. Elevation explained 7.4% of the adjusted variability in alkaloids (pseudo-F = 11.8, $p < 0.001$,) and 4.3% of the adjusted variability in polyphenols (pseudo-F = 8.0, $p < 0.001$). The RDA diagrams show the first two canonical axes. The thick arrow standing for elevation points in the direction of its increase. The thin arrows point in the direction of the increase of the studied chemical traits, while the angle between arrows indicates the correlation between them. The correlation is positive when the angle is sharp and negative when the angle is larger than 90 degrees

correlation with elevation (Table S4). Elevation explained 7.4% of the adjusted variability in alkaloids (pseudo-F = 11.8, $p < 0.001$, Fig. 1). When combined with average temperature and humidity, all three variables together explained 8.1% of the adjusted variability in alkaloids (pseudo-F = 5.0, $p = 0.001$). The unique effect of elevation was significant, explaining 1.9% of the variability in alkaloids (pseudo-F = 3.5, $p = 0.026$). In contrast, the unique effect of average temperature and humidity was not significant, explaining 0.8% of the variability in alkaloids (pseudo-F = 1.5, $p = 0.211$). The positive correlation in the concentration of several alkaloid groups with elevation was also supported by generalised linear mixed effect models analysing the elevational trends in individual compounds ($t_{1826} = 9.76$ $p < 0.001$). Ten out of 13 compounds showed a significant positive trend with elevation (Table S5).

The concentration of total phenolics showed a hump-shaped distribution with the maximum at mid elevations. The trend in total phenolics was driven by procyanidins, which were present in the highest concentration. The overall trend in procyanidins was mirrored by the protein precipitation capacity. When analysed by RDA analysis accounting for species identity, polyphenols generally responded to elevation but showed various elevational trends (4.3% of adjusted variability explained, pseudo-F = 8.0, $p < 0.001$). Polyphenol diversity, quercetins, and quinic acid derivatives showed the strongest positive correlation with elevation whereas prodelphinidins showed the strongest negative correlation with elevation. The response of other polyphenols was much weaker. Galloyl and HHDP derivatives (hydrolysable tannins) were present in very low levels (< 0.2 mg/g) in only a few of the samples and did not show any reliable patterns (Table S4). When combined with the average temperature and humidity, all three variables together explained 8.4% of the adjusted variability in polyphenols (pseudo-F = 5.1, $p = 0.001$). Both the unique effect of average temperature and humidity and the unique effect of elevation were significant, explaining 4.3% (pseudo-F = 4.0, $p = 0.003$) and 3.2% (pseudo-F = 5.3, $p = 0.003$) of the variability in polyphenols, respectively. The results from linear mixed effect models analysing the elevational trends in individual polyphenol compounds broadly supported the multivariate results outlined above. While flavonoids showed generally a positive correlation with elevation ($t = 6.086_{,1262}$, $p < 0.001$), non-flavonoid polyphenols did not show a significant trend ($t = -1.141_{,980}$, $p = 0.254$; Table S5). Specifically, the concentrations of three out of four flavonoid compounds correlated to elevation showed a positive elevational trend while only epicatechin was negatively correlated ($t = -3.865_{,134}$, $p < 0.001$). On the contrary, the five non-flavonoid compounds significantly correlated with elevation showing contrasting elevational trends. For example, concentration of PCPC dimer 1 was negatively correlated ($t = -2.364_{,134}$, $p < 0.001$) while chlorogenic acid was positively correlated ($t = 4.272_{,134}$, $p < 0.001$).

We sampled 56 Lepidoptera species (387 individuals) and 148 Coleoptera species (839 individuals) during the survey of insect herbivore communities associated with our *Ficus* species (Table S6, Appendix 1). Insect abundance decreased with elevation ($\chi^2(4) = 9.5$, $p = 0.002$). The dissimilarity in leaf-chewer communities between coexisting pairs of *Ficus* species measured by the Bray-Curtis index showed a hump-shaped distribution with the minimum dissimilarity at mid elevations (Fig. 2). The model including a second order polynomial relationship between Bray-Curtis dissimilarity and elevation explained significantly more deviance than the model with a first order relationship ($\Delta DF = 1$, $\Delta Deviance = 0.487$, $F = 4.736$, $p = 0.034$). There was a significant curvilinear relationship between elevation and Bray-Curtis dissimilarity ($F_{50,2} = 6.671$, $p = 0.044$).

CCA with forward selection identified ficuseptamines (pseudo-F = 2.0, $p = 0.009$) and alkaloid diversity (pseudo-F = 1.5, $p = 0.023$) as the chemical traits with significant effects on communities, together explaining 7.9% of the adjusted variability in leaf-chewer composition ($p = 0.002$ for the whole model including both traits). In the analysis of the effect of individual compounds, ficuseptamine (A or B) or pentamethoxy-phenanthroindolizidine (the presence of these compounds was collinear and their effects were identical; pseudo-F = 2.1, $p = 0.002$), dihydroxy-dimethoxy-dehydro-*seco*-phenanthroindolizidine (pseudo-F = 1.7, $p = 0.010$), kaempferol glucoside/galactoside (pseudo-F = 1.7, $p = 0.046$), hydroxy-trimethoxy-phenanthroindolizidine (pseudo-F = 1.5, $p = 0.042$), 5-caffeoylquinic acid (chlorogenic acid, pseudo-F = 1.3, $p = 0.033$), and epicatechin (pseudo-F = 1.5, $p = 0.030$) were selected as the variables that best explained herbivore community structure, together explaining 20.4% of the adjusted variability in leaf-chewer composition ($p < 0.001$ for the whole model including all six traits) (Fig. 3).

We recorded 21 species of folivorous mammalian herbivores along the gradient (Table S7). Their species richness increased towards higher elevations, with the maximum number of species (15) recorded at 2700 m a.s.l. (Fig. 2).

Discussion

We quantified alkaloid and polyphenol-based defences in a community of fig species along a forested elevational gradient in Papua New Guinea. At the community level, we found a hump-shaped trend in the concentration of both alkaloids and phenolics. However, when we accounted for *Ficus* species identity, we found an elevational increase in almost all studied groups of alkaloids that likely serve as potent and phylogenetically restricted anti-herbivore and anti-pathogen defences.

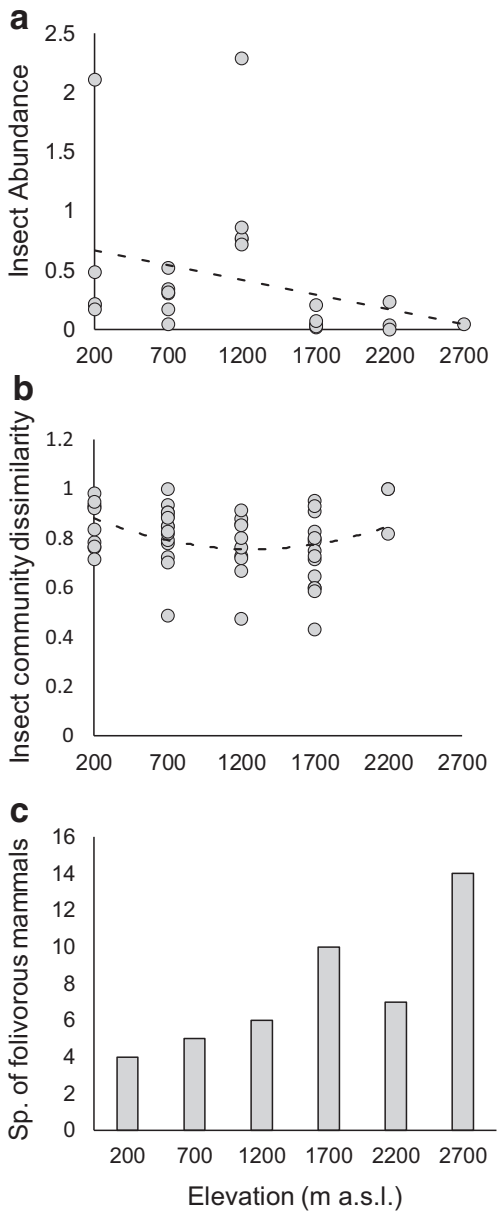

Fig. 2 Elevational trends in insect abundance (**a**), pairwise insect community dissimilarity between the studied *Ficus* species (**b**), and species richness of folivorous mammals along the studied gradient (**c**). The insect abundance decreased with elevation ($\chi^2(4) = 9.5$, $p = 0.0020$). The dissimilarity in leaf-chewer communities between coexisting pairs of *Ficus* species measured by the Bray-Curtiss index showed a hump-shaped distribution with the minimum at mid elevations ($F_{50,2}=6.671$, $p = 0.044$). *F. pungens*, which had only a small leaf area sampled for herbivores, and *F. itoana* complex from 2700 m, from which only one singleton herbivore was sampled, were removed from the analyses. This left *F. hahliana* as the only *Ficus* species with insect data at 2700 m a.s.l. and made bipartite comparisons of community dissimilarity impossible at this elevation. The comparisons of dissimilarity in insect communities thus span only up to 2200 m a.s.l. Mammal species were counted based on records from an active search, identified bone remains, and by questionnaire survey among the local villagers

The elevational trends in polyphenols were more diverse. We suggest that the elevational trends in individual metabolites and their groups depend on their ecological function.

Elevational increase in plant defences is generally stimulated by unfavourable conditions at higher elevations that cause higher levels of environmental stress and render compensation for lost biomass more costly (Givnish 1999; Salgado et al. 2016). The unfavourable conditions in tropical montane forests involve negative effects of lower temperature and higher rainfall that reduce rates of N mineralization and increase nutrient leaching (Givnish 1999). Here the changes in temperature and humidity explained a larger share of variation in polyphenol composition than the changes in elevation itself. This suggests that these two variables may play important roles in the elevational trends in some groups of polyphenols we studied. Additionally, highland plants are also exposed to higher UV-irradiation. We observed a general correlation between individual flavonoids and elevation while the direct response to elevation was weaker or non-linear in the case of non-flavonoid polyphenols. We did not test the activity of these particular metabolites. But flavonols, such as rutin, or kaempferol derivatives are known for their strong role in anti-UV protection (Harborne and Williams 2000). As they did not show a particularly strong correlation to insect communities, we suggest that their elevational increase in *Ficus* could be most likely attributed the role they play in protecting plants against detrimental environmental effects.

We found an elevational increase in almost all sub-groups of phenanthroidolizidine alkaloids. This group of alkaloids represents a specialized defence in *Ficus* species, having a relatively limited distribution among plants and strong effects on insect herbivores (Damu et al. 2005; Han et al. 2013; Konno et al. 2004; Volf et al. 2018). The herbivore communities studied here were most affected by ficuseptamines or pentamethoxy-phenanthroindolizidine, which were unique to *F. hahliana* at the highest elevation. Alkaloid diversity also played a significant role. This highlights the importance of rare or species-specific compounds for structuring insect herbivore communities. Such defences may be especially important in the genus *Ficus*, which harbours many herbivores able to potentially use multiple *Ficus* species as their hosts (Novotny et al. 2010; Volf et al. 2018). Indeed, insect herbivore communities associated with lowland *Ficus* populations are significantly structured by phenanthroidolizidine alkaloid diversity. These alkaloids limit the sharing of certain herbivores between closely related *Ficus* hosts (Volf et al. 2018) and may explain the turnover of specialist caterpillars across populations of the same hosts at different elevations (Novotny et al. 2005). Unlike in the case of polyphenols, their composition was not explained by the unique effects of climatic variables we measured. This is suggestive of their defensive role against insect herbivores in this system, although laboratory experiments with leaf extracts would be needed to confirm this.

The increased alkaloid concentration in high elevation figs may also serve to protect against mammals and pathogens. We

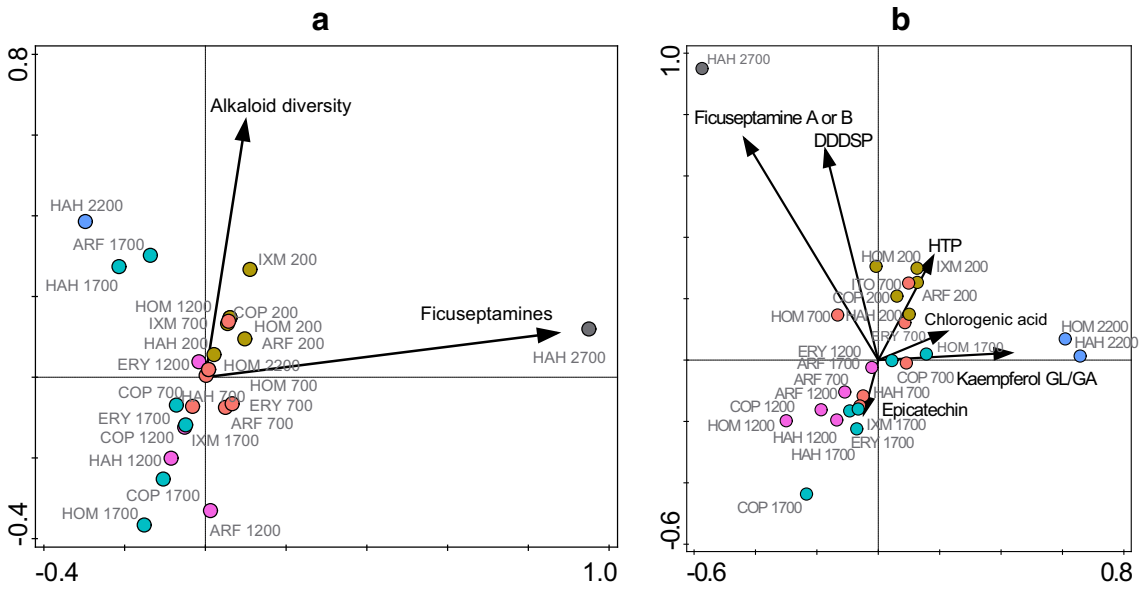

Fig. 3 Effects of *Ficus* chemical traits on the associated herbivore communities analysed with CCA with forward selection identified ficuseptamines (pseudo-F = 1.92.0, p = 0.009) and alkaloid diversity (pseudo-F = 1.65, p = 0.023) as the chemical traits with significant effects on communities, together explaining 7.9% of the adjusted variability in leaf-chewer composition (p = 0.002 for the whole model including both traits) (**a**). In the analysis of the effect of individual compounds, ficuseptamine A or B (pseudo-F = 2.1, p = 0.002), dihydroxy-dimethoxy-dehydro-*seco*-phenanthroindolizidine (DDDSP, pseudo-F = 1.7, p = 0.010), kaempferol glucoside/galactosidequercetin glycoside (kaempferol GL/GA, pseudo-F = 1.7, p = 0.046), hydroxy-trimethoxy-phenanthroindolizidine (HTP, pseudo-F = 1.5, p = 0.042), 5-caffeoylquinic acid (chlorogenic acid, pseudo-F = 1.3, p = 0.033), and epicatechin (pseudo-F = 1.5, p = 0.030) were selected as the variables that best explained herbivore community structure, together explaining 20.4% of the adjusted variability in leaf-chewer composition (p < 0.001 for the whole model including all six traits) (**b**). *F. pungens* (all elevations) and *F. itoana* complex (2700 m) had low leaf area sampled for herbivores and were excluded from the analysis. The presence of ficuseptamine (A or B) and pentamethoxy-phenanthroindolizidine were collinear and their effects were identical. Pentamethoxy-phenanthroindolizidine is not shown in the figure. Elevations are colour coded. See Table 1 for the species codes. The CCA diagrams show the first two canonical axes and the thick black arrows standing for chemical traits with significant effects on herbivore community structure point in the direction of their increase. The circles represent *Ficus* species and their insect communities from individual elevations. The distance between the circles approximates their insect community dissimilarity as measured by chi-square distances. Perpendicular projections of the circles onto the line overlaying the arrows of chemical traits can be used to approximate the trait values in individual samples

observed an elevational increase in species richness of folivorous mammals. Although we cannot present abundance-based data, our findings are in line with the observations of previous studies that report an elevational increase in abundance and diversity of folivorous mammals, such as various possums or cuscuses, in the Australo-Papuan region (Flannery 1995; Tallowin et al. 2017). Several possum species have been shown to be important consumers of *Ficus* leaves (Kanowski et al. 2003). Their dietary preferences are known to be affected by leaf secondary metabolites (Moore et al. 2005). It is thus possible that higher concentration of alkaloids serves as an anti-mammalian defence in highland *Ficus*. Furthermore, several phenanthroindolizidines, such as antofine, show strong anti-fungal activities (Mogg et al. 2008). Fungal pathogens of plants generally decrease in abundance with elevation (Geml et al. 2014). However, the relative costs of compensating for damage by fungal pathogens increases with the elevation too (Brown and Vellend 2014), as with the relative costs of herbivory, possibly making anti-pathogen defences more important. There are very likely several biotic factors driving the elevational increase in *Ficus* alkaloids (and indeed other compound groups). More data on mammalian herbivores, *Ficus* leaf pathogens, and the activity of leaf extracts would be needed to identify their relative contribution to the observed trends.

Although we observed an elevational increase in alkaloids and flavonoids this trend was not universal across all the metabolite groups studied. For example, populations of several *Ficus* species from mid elevations were high in procyanidins and showed high protein precipitation capacity. The ability of procyanidins to precipitate proteins is low in alkaline conditions as found in the digestive tract of many caterpillars (Barbehenn et al. 2008; Roslin and Salminen 2008; Salminen and Karonen 2011). We did not find any correlation of procyanidins or protein precipitation capacity to the insect community structure, in agreement with studies of lowland fig species (Volf et al. 2018). The mid-elevational populations of *Ficus* also shared the highest number of insect herbivores, suggesting that high procyanidin concentration did not strongly restrict host preferences of the studied insects. On the other hand, procyanidins have been shown to affect feeding preferences and reduce apparent N digestibility in mammalian

herbivores, which have low to neutral pH in their digestive system (Foley et al. 1999). The increase in procyanidins towards mid elevations might be an adaptive response to increased pressure from mammalian herbivores (Flannery 1995; Tallowin et al. 2017). However, unlike mammalian species richness and abundance, procyanidins concentration and diversity decreased between middle and high elevations. Procyanidins may thus serve another function in this system, be driven by a combination of several factors, or simply show levels of interspecific variation that are too high for detecting as a simple elevational trend. Relatively low concentrations and high interspecific variation may also explain the limited responses to elevation of other polyphenol groups despite their known biological effects on leaf-chewing insects (Segar et al. 2017a; Volf et al. 2018).

In agreement with Defossez et al. (2018) and Moreira et al. (2018), we suggest that instead of universal directional trends, plant traits can show contrasting elevational trends depending on their function. Using analyses based on multiple traits and linking them to datasets on herbivores or pathogens is thus necessary to understand elevational trends and interactions in plant defences (Defossez et al. 2018; Escobar-Bravo et al. 2017). Additionally, overall elevational trends in plant defences may be largely dependent on the gradient studied and, in particular, its span (Moreira et al. 2018). Unfavourable conditions can stimulate investment into defensive traits (Givnish 1999; Salgado et al. 2016) but truly adverse conditions can limit investment into secondary metabolites. This effect has been reported from plants exposed to extreme conditions above the tree line (e.g. Pellissier et al. 2014). In turn, the levels of defensive traits may be highest at elevations where conditions are adverse enough to increase the relative costs of compensating for biomass loss, but not adverse enough to hamper secondary metabolite production: resulting in the increase along the forested gradient studied here.

Interspecific variability between *Ficus* species can also play an important role in elevational trends. We found some elevational increase in alkaloids and certain polyphenols in most of the species. Exceptions to this rule included *F. copiosa*, which was relatively undefended at all sites. Several previous studies have suggested that closely related species of host-plants often diverge in their defences to avoid sharing insect herbivores (e.g. Becerra 2007; Kursar et al. 2009; Volf et al. 2018, 2019). Based on some of our results, it seems that closely related host-plant species may differ in their investment in defences along elevational gradients. As pointed out by Moreira et al. (2018), it would be interesting to analyse whether this can be driven by the costs imposed by herbivores and resulting divergent selection. Indeed, continuously forested gradients provide fascinating systems for studying the biotic and abiotic selective pressures imposed on plants. While generalities are emerging, we suggest that comparative multi-species studies sensitive to variation in herbivore and pathogen diversity are needed.

Acknowledgements We thank the staff of the New Guinea Binatang Research Centre in Papua New Guinea, especially Bradley Gewa and Grace Luke, for their assistance and Nicholas Silverson and Julia Englund at the Smithsonian who harvested most of the Lepidoptera for sequencing at the Biodiversity Institute of Ontario, as part of the International Barcode of Life project. MV acknowledges funding from Czech Academy of Sciences, and Programme for Research and Mobility Support of Starting Researchers (MSM200962004), Alexander von Humboldt Foundation and the Federal Ministry for Education and Research (Ref.3.3-CZE-1192673-HFST-P), and Grant Agency of the Czech Republic 20-10543Y. KS acknowledges the ERC grant BABE 805189. FV acknowledges support by the Grant Agency of the University of South Bohemia (GAJU n. 048/2019/P). JPS acknowledges funding from the Academy of Finland (grant no 258992), and the help of Saku Valkamaa, Atte Tuominen, Anne Koivuniemi, and Valtteri Virtanen in the chemical analyses. VN acknowledges the ESF grant 669609 and Grant Agency of the Czech Republic 17-23862S and 19-28126X. STS acknowledges funding from a USB Postdoc project reg.no. CZ.1.07/2.3.00/30.0006 (funded by the European Social Fund and the Czech State Budget) and Grant Agency of the Czech Republic 15-24571S. He also acknowledges departmental support from Harper Adams University. We thank the Papua New Guinea Forest Research Institute, in particular Kipiro Damas, for assistance granting export permits.

References

Barbehenn R, Weir Q, Salminen JP (2008) Oxidation of ingested phenolics in the tree-feeding caterpillar *Orgyia leucostigma* depends on foliar chemical composition. J Chem Ecol 34:748–756

Bates D, Maechler M, Bolker B, Walker S (2015) Fitting Linear Mixed-Effects Models Using lme4. J Stat Softw 67:1–48

Baumgartner B, Erdelmeier CA, Wright AD, Rali T, Sticher O (1990) An antimicrobial alkaloid from *Ficus septica*. Phytochemistry 29:3327–3330

Becerra JX (2007) The impact of herbivore-plant coevolution on plant community structure. Proc Natl Acad Sci USA 104:7483–7488

Berg C, Corner E (2005) Moraceae (*Ficus*). In: Flora Malesiana, Series I (Seed plants), vol 17. National Herbarium of the Netherlands, Leiden

Brown CD, Vellend M (2014) Non-climatic constraints on upper elevational plant range expansion under climate change. Proc R Soc Lond B Biol Sci 281:20141779

Bruneton J, Shamma M, Minard RD, Freyer AJ, Guinaudeau H (1983) Novel biogenetic pathways from (+)-reticuline. Three dimeric alkaloids:(+)-vanuatine,(+)-vateamine, and (+)-malekulatine. J Org Chem 48:3957–3960

Cruaud A et al (2012) An extreme case of plant-insect codiversification: Figs and fig-pollinating wasps. Syst Biol 61:1029–1047

Cui L et al (2004) On-line identification of phenanthroindolizidine alkaloids in a crude extract from *Tylophora atrofolliculata* by liquid chromatography combined with tandem mass spectrometry. Rapid Commun Mass Spectrom 18:184–190

Damu AG, Kuo PC, Shi LS, Li CY, Kuoh CS, Wu PL, Wu TS (2005) Phenanthroindolizidine alkaloids from the stems of *Ficus septica*. J Nat Prod 68:1071–1075

Defossez E, Pellissier L, Rasmann S (2018) The unfolding of plant growth form-defence syndromes along elevation gradients. Ecol Lett 21:609–618

Engström MT, Palijarvi M, Fryganas C, Grabber JH, Mueller-Harvey I, Salminen J-P (2014) Rapid qualitative and quantitative analyses of

proanthocyanidin oligomers and polymers by UPLC-MS/MS. J Agric Food Chem 62:3390–3399
Engström MT, Pälijärvi M, Salminen J-P (2015) Rapid fingerprint analysis of plant extracts for ellagitannins, gallic acid, and quinic acid derivatives and quercetin-, kaempferol- and myricetin-based flavonol glycosides by UPLC-QqQ-MS/MS. J Agric Food Chem 63: 4068–4079
Escobar-Bravo R, Klinkhamer PG, Leiss KA (2017) Interactive effects of UV-B light with abiotic factors on plant growth and chemistry, and their consequences for defense against arthropod herbivores. Front Plant Sci 8:278
Ezedin Z, Weiblen G (2019) Additions and changes to *Ficus* (Moraceae) in New Guinea with comments on the world's largest fig. Gardens' Bulletin Singapore 71:197–216
Flannery TF (1995) Mammals of New Guinea, 2nd edn. Comstock/ Cornell, Ithaca
Foley W, Iason G, McArthur C Role of plant secondary metobolites in the nutritional ecology of mammalian herbivores: how far have we come in 25 years? In: 5th Internaional Symposium on the Nutrition of Herbivores (1999) pp 130–209
Garibaldi LA, Kitzberger T, Chaneton EJ (2011) Environmental and genetic control of insect abundance and herbivory along a forest elevational gradient. Oecologia 167:117–129
Geml J et al (2014) Large-scale fungal diversity assessment in the Andean Yungas forests reveals strong community turnover among forest types along an altitudinal gradient. Mol Ecol 23:2452–2472
Givnish TJ (1999) On the causes of gradients in tropical tree diversity. J Ecol 87:193–210
Hagerman AE, Butler LG (1978) Protein precipitation method for the quantitative determination of tannins. J Agric Food Chem 26:809–812
Han G, Liu Y, Wang Q (2013) Total synthesis of phenanthroindolizidine alkaloids through an amidyl radical cascade/rearrangement reaction. Org Lett 15:5334–5337
Harborne JB, Williams CA (2000) Advances in flavonoid research since 1992. Phytochemistry 55:481–504
Kanowski J, Irvine A, Winter J (2003) The relationship between the floristic composition of rain forests and the abundance of folivorous marsupials in north-east. Qld J Anim Ecol 72:627–632
Khan IA, Rali T, Sticher O (1993) Alkaloids from *Ficus pachyrhachis*. Planta Med 59:286–286
Konno K, Hirayama C, Nakamura M, Tateishi K, Tamura Y, Hattori M, Kohno K (2004) Papain protects papaya trees from herbivorous insects: role of cysteine proteases in latex. Plant J 37:370–378
Kursar TA et al (2009) The evolution of antiherbivore defenses and their contribution to species coexistence in the tropical tree genus *Inga*. Proc Natl Acad Sci U S A 106:18073–18078
Lee Y-Z et al (2011) Isolation and biological activities of phenanthroindolizidine and septicine alkaloids from the Formosan *Tylophora ovata*. Planta Med 77:1932–1938
Malisch CS, Salminen J-P, Kölliker R, Engström MT, Suter D, Studer B, Lüscher A (2016) Drought effects on proanthocyanidins in sainfoin (*Onobrychis viciifolia* Scop.) are dependent on the plant's ontogenetic stage. J Agric Food Chem 64:9307–9316
Miller JS, Feeny P (1983) Effects of benzylisoquinoline alkaloids on the larvae of polyphagous Lepidoptera. Oecologia 58:332–339
Mogg C et al (2008) Tests of the antibiotic properties of the invasive vine *Vincetoxicum rossicum* against bacteria, fungi and insects. Biochem Syst Ecol 36:383–391
Moilanen J, Sinkkonen J, Salminen J-P (2013) Characterization of bioactive plant ellagitannins by chromatographic, spectroscopic and mass spectrometric methods. Chemoecology 23:165–179
Moore B, Marsh K, Wallis I, Foley W (2005) Taught by animals: how understanding diet selection leads to better zoo diets. Int Zoo Yearb 39:43–61
Moreira X, Petry WK, Mooney KA, Rasmann S, Abdala-Roberts L (2018) Elevational gradients in plant defences and insect herbivory: recent advances in the field and prospects for future research. Ecography 41:1485–1496
Novotny V et al (2005) An altitudinal comparison of caterpillar (Lepidoptera) assemblages on *Ficus* trees in Papua New Guinea. J Biogeogr 32:1303–1314
Novotny V et al (2010) Guild-specific patterns of species richness and host specialization in plant-herbivore food webs from a tropical forest. J Anim Ecol 79:1193–1203
Novotny V, Toko P (2015) Ecological research in Papua New Guinean rainforests: insects, plants and people. In: Bryan JE, Shearman PL (eds) The State of Forests of Papua New Guinea: Measuring Change over the Period 2002–2014. University of Papua New Guinea, Port Moresby, pp 71–85
Pellissier L, Roger A, Bilat J, Rasmann S (2014) High elevation *Plantago lanceolata* plants are less resistant to herbivory than their low elevation conspecifics: is it just temperature? Ecography 37:950–959
Perrigo A, Hoorn C, Antonelli A (2020) Why mountains matter for biodiversity. J Biogeogr 47:315–325
Pinheiro J, Bates D, DebRoy S, Sarkar D, Team RDC (2019) nlme: Linear and Nonlinear Mixed Effects Models. R package version 31–140, https://CRANR-projectorg/package=nlme
Rasmann S, Pellissier L, Defossez E, Jactel H, Kunstler G (2014) Climate-driven change in plant–insect interactions along elevation gradients. Funct Ecol 28:46–54
Ratnasingham S, Hebert PD (2007) BOLD: The Barcode of Life Data System (http://www. barcodinglife. org). Mol Ecol Resour 7:355–364
Ratnasingham S, Hebert PD (2013) A DNA-based registry for all animal species: the Barcode Index Number (BIN) system. PloS One 8:e66213
Richards LA, Dyer LA, Forister ML, Smilanich AM, Dodson CD, Leonard MD, Jeffrey CS (2015) Phytochemical diversity drives plant–insect community diversity. Proc Natl Acad Sci U S A 112: 10973–10978
Roslin T, Salminen JP (2008) Specialization pays off: contrasting effects of two types of tannins on oak specialist and generalist moth species. Oikos 117:1560–1568
Salgado AL, Suchan T, Pellissier L, Rasmann S, Ducrest A-L, Alvarez N (2016) Differential phenotypic and genetic expression of defence compounds in a plant–herbivore interaction along elevation. R Soc Open Sci 3:160226
Salminen JP (2014) The chemistry and chemical ecology of ellagitannins in plant–insect interactions: from underestimated molecules to bioactive plant constituents. Recent Adv Polyphenol Res 4:83–113
Salminen JP, Karonen M (2011) Chemical ecology of tannins and other phenolics: we need a change in approach. Funct Ecol 25:325–338
Sam K et al (2020) Insect herbivory and herbivores of *Ficus species* along a rainforest elevational gradient in Papua New Guinea. Biotropica. https://doi.org/10.1111/btp.12741
Sam K, Koane B, Bardos DC, Jeppy S, Novotny V (2019) Species richness of birds along a complete rain forest elevational gradient in the tropics: Habitat complexity and food resources matter. J Biogeogr 46:279–290
Segar ST et al (2017a) Variably hungry caterpillars: predictive models and foliar chemistry suggest how to eat a rainforest. Proc R Soc Lond B Biol Sci 284:20171803
Segar ST et al (2017b) Speciation in a keystone plant genus is driven by elevation: a case study in New Guinean *Ficus*. J Evol Biol 30:512–523
Shanahan M, So S, G GS, RICHARD G (2001) Fig-eating by vertebrate frugivores: a global review. Biol Rev 76:529–572
Sourakov A, Emmel TC (2001) On the toxic diet of day-flying moths in the Solomon Islands (Lepidoptera: Arctiidae). Trop Lepidoptera Res 12:5–6
Souto-Vilarós D et al (2018) Pollination along an elevational gradient mediated both by floral scent and pollinator compatibility in the fig and fig-wasp mutualism. J Ecol 106:2256–2273

Souto-Vilarós D et al (2019) Faster speciation of fig-wasps than their host figs leads to decoupled speciation dynamics: snapshots across the speciation continuum. Mol Ecol 28:3958–3976

Stærk D, Christensen J, Lemmich E, Duus J, Olsen CE, Jaroszewski JW (2000) Cytotoxic activity of some phenanthroindolizidine N-oxide alkaloids from *Cynanchum vincetoxicum*. J Nat Prod 63:1584–1586

Tallowin O, Allison A, Algar AC, Kraus F, Meiri S (2017) Papua New Guinea terrestrial-vertebrate richness: elevation matters most for all except reptiles. J Biogeogr 44:1734–1744

Ter Braak CJ, Smilauer P (2012) Canoco Reference Manual and User's Guide: Software for Ordination (version 5.0). Microcomputer Power, Ithaca

Toussaint EF, Sagata K, Surbakti S, Hendrich L, Balke M (2013) Australasian sky islands act as a diversity pump facilitating peripheral speciation and complex reversal from narrow endemic to widespread ecological supertramp. Ecol Evol 3:1031–1049

Volf M et al (2018) Community structure of insect herbivores is driven by conservatism, escalation and divergence of defensive traits in *Ficus*. Ecol Lett 21:83–92

Volf M, Hrcek J, Julkunen-Tiitto R, Novotny V (2015) To each its own: differential response of specialist and generalist herbivores to plant defence in willows. J Anim Ecol 84:1123–1132

Volf M, Salminen J-P, Segar ST (2019) Evolution of defences in large tropical plant genera: perspectives for exploring insect diversity in a tri-trophic context. Curr Opin Insect Sci 32:91–97

Whitfeld TJ, Weiblen GD (2010) Five New *Ficus* Species (Moraceae) from Melanesia. Harv Pap Bot 15:1–11

Wilson JJ (2012) DNA barcodes for insects. In: Kress WJ, Erickson DL (eds) DNA Barcodes: Methods and Protocols. Springer, New York, pp 17–46

Xiang Y, Abliz Z, Li L, Xs H, Yu Ss (2002) Study of structural characteristic features of phenanthriondolizidine alkaloids by fast atom bombardment with tandem mass spectrometry. Rapid Commun Mass Spectrom 16:1668–1674