Oikos 125: 126–136, 2016
doi: 10.1111/oik.02325

Subject Editor: Regino Zamora. Editor-in-Chief: Dries Bonte. Accepted 8 April 2015

Defence against vertebrate herbivores trades off into architectural and low nutrient strategies amongst savanna Fabaceae species

Kyle W. Tomlinson, Frank van Langevelde, David Ward, Herbert H.T. Prins, Steven de Bie, Ben Vosman, Everardo V. S. B. Sampaio and Frank J. Sterck

K. W. Tomlinson (orcid.org/0000-0003-3039-6766)(kylewtomlinson@gmail.com), Centre for Integrative Conservation, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, CN-666303 Menglun, Yunnan, PR China. – KWT, F. van Langevelde, H. H. T. Prins and S. de Bie, Resource Ecology Group, Wageningen Univ., PO Box 47, NL-6700 AA Wageningen, the Netherlands. – HHTP and D. Ward, School of Life Sciences, Univ. of KwaZulu-Natal, Private Bag X01, Scottsville 3209, South Africa. – B. Vosman, Wageningen UR Plant Breeding, Wageningen Univ. and Research Center, PO Box 16, NL-6700 AA Wageningen, the Netherlands. – E. V. S. B. Sampaio, Univ. Federal de Pernambuco, Av. Prof. Luis Freire 1000, Recife, PE 50740-540, Brazil. – F. J. Sterck, Forest Ecology and Management Group, Wageningen Univ., PO Box 47, NL-6700 AA Wageningen, the Netherlands.

Herbivory contributes substantially to plant functional diversity and in ways that move far beyond direct defence trait patterns, as effective growth strategies under herbivory require modification of multiple functional traits that are indirectly related to defence. In order to understand how herbivory has shaped plant functional diversity, we need to consider the physiology and architecture of the herbivores and how this constrains effective defence strategies. Here we consider herbivory by mammals in savanna communities that range from semi-arid to humid conditions. We posited that the saplings of savanna trees can be grouped into two contrasting defence strategies against mammals, namely architectural defence versus low nutrient defence. We provide a mechanistic explanation for these different strategies based on the fact that plants are under competing selection pressures to limit herbivore damage and outcompete neighbouring plants. Plant competitiveness depends on growth rate, itself a function of leaf mass fraction (LMF) and leaf nitrogen per unit mass (N_m). Architectural defence against vertebrates (which includes spinescence) limits herbivore access to plant leaf materials, and partly depends on leaf-size reduction, thereby compromising LMF. Low nutrient defence requires that leaf material is of insufficient nutrient value to support vertebrate metabolic requirements, which depends on low N_m. Thus there is an enforced tradeoff between LMF and N_m, leading to distinct trait suites for each defence strategy. We demonstrate this tradeoff by showing that numerous traits can be distinguished between 28 spinescent (architectural defenders) and non-spinescent (low nutrient defenders) Fabaceae tree species from savannas, where mammalian herbivory is an important constraint on plant growth. Distributions of the strategies along an LMF-N_m tradeoff further provides a predictive and parsimonious explanation for the uneven distribution of spinescent and non-spinescent species across water and nutrient gradients.

Plant fitness depends on the ability to capture and retain sufficient resources to allow growth to reproductive maturity (Grime 1977). Resource retention depends on both the plant's ability to reduce the loss of resources during senescence of plant organs (Berendse and Elberse 1989) and the ability to minimise losses to defoliation (Grubb 1992, Hanley et al. 2007). Herbivory constitutes a widespread and persistent defoliation pressure on plants. Plants have evolved a range of defence traits against herbivory and these are categorised in different ways. Here we classify them into two broad groups that basically describe the general defence approach: architectural defences and direct leaf defences. Architectural defences, such as spinescence (possession of thorns, spines and prickles), divaricate branching and stem hairs, retard leaf and stem removal by herbivores by inhibiting or slowing access to edible parts such as leaves and meristems (Cooper and Owen-Smith 1986, Bond et al. 2004, Belovsky et al. 2015). Direct leaf defences, including low nutrient content (Lundberg and Åström 1990, Agrawal and Fishbein 2006), physical toughness (Kitajima and Poorter 2010), and tissue chemicals that are thought to retard digestion (e.g. polyphenols) or actively poison herbivores (e.g. alkaloids) (Herms and Mattson 1992, Agrawal and Fishbein 2006, Barton and Koricheva 2010, Broekgaarden et al. 2011, Fine et al. 2013), dissuade or retard herbivory directly. We use the term 'architectural defence' here rather than 'structural defence' to emphasise that the strategy requires whole-plant morphological modification to ensure an effective defence strategy whereas 'structural defence' is used more generically and covers purported defence traits that could form part of direct leaf defence (e.g. physical toughness of leaves).

There is now substantial evidence suggesting that plants are selected for distinct defence syndromes that include defence traits and other functional trait modifications

(Bond et al. 2004, Fine et al. 2004, Agrawal and Fishbein 2006), suggesting that plants are selected for multiple traits simultaneously to render effective growth strategies. Thus herbivory contributes substantially to plant functional diversity and in ways that move far beyond direct defence trait patterns. It is also clear that plant defence traits change in effectiveness across resource gradients (Coley et al. 1985, Endara and Coley 2011). Thus, the context of herbivory both in terms of the physiology and architecture of the herbivores and available environmental resources are critical to understand the plant functional diversity we see, and broad-scale comparisons that are not specific on context may not detect patterns (Carmona et al. 2011, Moles et al. 2013).

In this paper we concentrate on plant defences against vertebrate mammals and we ask the question whether woody plant species with low nutrient contents in their leaves, a type of leaf defence (Lundberg and Åström 1990, Hartley and Jones 1996), represent a distinct defence strategy from species with spinescence (thorns, spines and prickles), a type of architectural defence effective against mammals, in terms of their physical, chemical and physiological traits. It has long been understood that mammals are limited by low nutrient contents in plant material (Owen-Smith 1982) and that their feeding is slowed by spinescence (Cooper and Owen-Smith 1986), but it has yet to be clearly demonstrated that these two defence types form part of alternative strategies possessing an array of functional modifications. Spinescence in plants includes at least three types of sharp-pointed mechanical projections that impede feeding vertebrates, namely spines (modified leaf stipules, petioles, midribs or veins), thorns (modified stems) and prickles (projections from the epidermis or cortex) (Grubb 1992, Hanley et al. 2007). In savannas, stipular spines (which remain attached to stems after the leaves have senesced), stem prickles and thorns are most abundant (Coates-Palgrave 2002). We refer to all types generically as ‘spines’ for the remainder of this article.

Here we lay out a mechanistic explanation as to why two alternative defence strategies against mammals (architectural defence, low nutrient defence) might have evolved. We proceed following the arguments of Coley et al. (1985), Gulmon and Mooney (1986) and Herms and Mattson (1992) that plant species are under selection pressure both for reducing the negative effects of vertebrate herbivory and for out-competing other plants for resources, and that these pressures select for opposite traits. Thus all defence strategies are subject to the constraints on growth encapsulated in the relative growth rate (RGR, $\mathrm{g\ g^{-1}\ d^{-1}}$) formulation of Evans (1972):

$$\mathrm{RGR} = \mathrm{LMF} \times \mathrm{SLA} \times \mathrm{ULR} \qquad (1)$$

where LMF ($\mathrm{g\ g^{-1}}$) is the fraction of total biomass that is leaf, SLA ($\mathrm{m^2\ g^{-1}}$) is the specific leaf area per unit mass and ULR is the unit leaf rate, which indicates the rate of increase in plant biomass per unit leaf area ($\mathrm{g\ m^{-2}}$), thought to be closely related to assimilation rate per unit area (A_a) (Poorter and Van der Werf 1998). If SLA and ULR are combined, this yields the rate of increase in plant biomass per unit leaf mass ($\mathrm{g\ g^{-1}}$), which in turn is related to the assimilation rate per unit mass (A_m), itself related to leaf nitrogen concentration (N_m) (Reich et al. 2003). In the absence of herbivory, the equation implies that RGR may vary across species through the entire combined range space of LMF and N_m, and that maximum RGR can be achieved through a combination of high LMF and high N_m. However, when vertebrate herbivory is included, we reason that species cannot have both high N_m and high LMF, as the two posited anti-herbivory strategies (architectural defence, low nutrient defence) depend on restrictions of either component and thus vertebrate herbivory pressure itself enforces a tradeoff between LMF and N_m. Species selected for architectural defences must have small leaves that are difficult to access beyond spines or divaricate stems (Cooper and Owen-Smith 1986, Bond et al. 2004) such that harvesting them may be energetically limiting to herbivores (Belovsky et al. 2015). This reduced leaf size comes at a direct cost to their LMF. They can compensate their carbon production for growth under this restriction through allocating to higher N_m arranged in high SLA leaves to facilitate high A_m and high photosynthetic nitrogen use efficiency (Reich et al. 2003). Their reduced requirement for carbon in leaves simultaneously increases their ability to allocate carbon to stems or roots, leading to greater root or stem mass fractions (RMF, SMF) relative to species with low nutrient defence. By contrast, species selected for low nutrient defence must have low N in the leaves, as a low N:C ratio ensures an unsustainable diet for herbivores that renders them undesirable as forage. Low N_m compromises leaf A_m, which these species can compensate for by allocating more carbon to leaves (high LMF) to increase photosynthetic surface to support growth rate. They may also have low SLA leaves, which allows for low N_m while sustaining high nitrogen per unit area (N_a) and hence high assimilation per unit area (A_a). These different

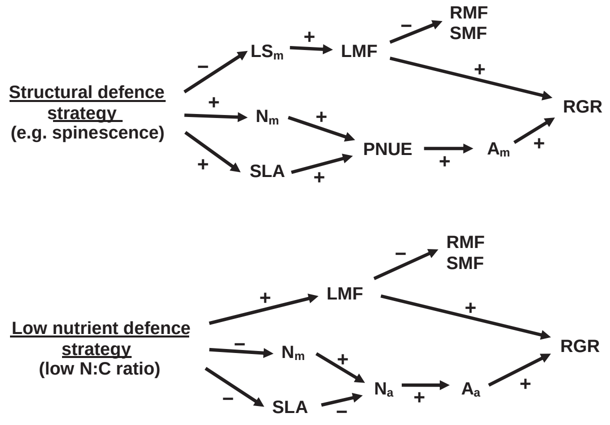

Figure 1. Path diagram indicating plant traits expected to change under selection for two defence strategies against vertebrate herbivores, and selection to maintain competitive growth rates (RGR). Species selected for structural defence must have small leaves that are difficult to access, which restricts their leaf biomass fraction (LMF), and hence photosynthetic surface. They compensate their carbon production through high photosynthetic capacity (A_m) due to high leaf nitrogen (N_m) arranged in high SLA leaves (facilitating high photosynthetic nitrogen use efficiency, PNUE). Species selected for low nutrient defence must have low leaf N_m to ensure an unsustainable diet for herbivores, which compromises leaf A_m. They compensate their carbon production through photosynthetic surface (large LMF). They may also have low SLA leaves, which allows for low N_m while sustaining high nitrogen per unit area (N_a) and hence high assimilation per unit area (A_a).

trait selection processes are illustrated in a path diagram in Fig. 1.

We compared organ biomass partitioning and leaf physiology of 20 week-old juvenile trees of 28 tree species (all Fabaceae), 16 non-spinescent and 12 spinescent, which are abundant in the semi-arid and humid savannas on southern continents, Africa, Australia and South America. Large vertebrate herbivores are common in all continental savannas although their diversity has been substantially reduced following human migrations (Sandom et al. 2014). Therefore common evolved trait responses to vertebrates should be observed in all three communities. We concentrated on juvenile trees because this is the stage at which plants are very sensitive to defoliation impacts by ground-foraging vertebrates (Brooks and Owen-Smith 1994, Gowda and Palo 2003), and hence evolved responses to herbivory pressure should be apparent. We formulated three predictions on traits of spinescent versus non-spinescent species: 1) spinescent species have small leaves and LMF relative to non-spinescent species; 2) non-spinescent species have low leaf N_m and SLA relative to spinescent species; 3) consequent on predictions 1 and 2, spinescent and non-spinescent species are found at opposite ends of an LMF–N_m tradeoff enforced by vertebrate herbivory.

Material and methods

Species selection

As part of a larger experiment (Tomlinson et al. 2012, 2013), we sampled dominant or very abundant tree species from humid and semi-arid savannas in southern Africa (coastal and inland savannas in South Africa and Zimbabwe) (Coates-Palgrave 2002, Mucina and Rutherford 2006), northeastern Australia (coastal and inland woodlands in Queensland) and eastern South America (Cerrado and Caatinga biomes in Brazil) (Cole 1986). Species were classified as spinescent or non-spinescent based on whether they possess spines as saplings. Of those species for which we were able to germinate sufficient seedlings for our experiment, spinescence was almost exclusively restricted to Fabaceae and was most abundant among African species. Therefore, to reduce phylogenetic effects on plant traits, we compared Fabaceae species only. A full species list is provided in the Supplementary material Appendix 1 Table A1, including their continent of origin and whether they are spinescent or not.

We constructed our 28-species phylogeny of Fabaceae species used in the experiment by pruning down a much larger 301-terminal tree that we had previously constructed based on plastid matK nucleotide sequences (described in Tomlinson et al. 2012). The phylogenetic relationships between the species are shown in Fig. 2.

Spinescent species are notably more abundant towards drier environments (Cole 1986, Pennington et al. 2000, Mucina and Rutherford 2006). Consequently, it can be argued that some of the trait patterns we are predicting as differences caused by selection under herbivory may instead be due to selection against water stress under lower water regimes, e.g. small leaf size (Westoby et al. 2002). Therefore, to exclude this explanation for differences between spinescent and non-spinescent species in savanna trees, we have included estimates of the mean annual precipitation (MAP, mm $year^{-1}$) in the regions where the seed was collected for each species as a covariate in the

Table 1. Regression models testing for significant effects of spinescence and the covariates sapling mass (Mass) and mean annual precipitation (MAP) on organ biomass allocation and leaf morphological, chemical and physiological traits for 27 savanna tree species (Fabaceae) grown under common conditions (*). Model results are presented for best-fitting models (either phylogenetic or non-phylogenetic (see Supplementary material Appendix 2 Table A2 for full statistical results).

Trait	n	Regression estimates					
		Intercept	Mass†	MAP	Spines	Mass × Spines	MAP × Spines
Biomass allocation							
RMF	27	0.286	–	–	0.118	–	–
SMF	27	0.213	–	–	0.073	–	–
LMF	27	0.481	–	–	−0.177	–	–
Leaf morphology							
LS_a‡	27	0.887	0.675	0.001	−0.910	–	–
LS_m‡	27	−1.492	0.966	–	−1.773	–	–
SLA	27	10.446	–	0.005	7.491	–	–
LAR	27	3.902	–	0.004	–	–	–
Leaf chemistry							
N_m	27	28.187	−0.851(NS)	–	21.392	−4.440	–
N_a	27	2.861	–	−0.001	−0.636	–	–
Leaf physiology							
A_a	23	11.599	-1.251	−0.002	2.897	–	–
A_m	23	115.202	−9.673(NS)	–	229.601	−39.917	–
PNUE	23	3.793	–	–	1.571	–	–
g_s	23	–	–	–	–	–	–
PWUE	23	0.123	−0.010	–	0.051	–	–
Growth rate							
RGR	26	–	–	–	–	–	–

Lysiphyllum hookeri was excluded from the analyses

†Mass is ln-transformed.

‡LS_a and LS_m are ln-transformed.

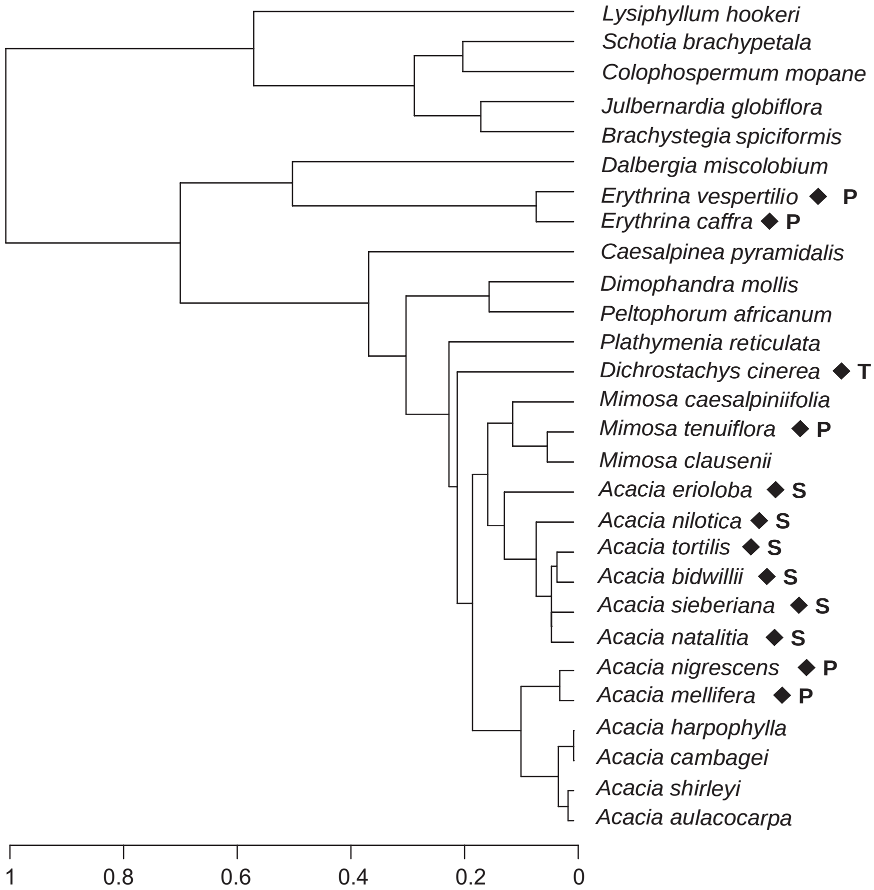

Figure 2. Phylogenetic relationships of considered species resulting from pruning a wider 301-terminal angiosperm eudicot phylogeny, based on plastid-encoded matK DNA sequences (Tomlinson et al. 2012). Spinescent species are indicated (◆) , as well as the type of spinescence possessed (S = stipular spines; T = stem thorns, P = epidermal prickles). Sequence data were collected directly from individuals of most species used in the study. The following placeholder species were used where no DNA could be extracted: *Acacia shirleyi* (substitute: *Acacia aulacocarpa*), and *Lysiphyllum hookeri* (substitute: *Lysiphyllum gilvum*). Branch to tip length is scaled to 1 unit. This tree was cut from a much larger phylogenetic tree constructed for savanna species across the angiosperm clade. That tree is provided in the supplementary materials of Tomlinson et al. (2012).

models, and have also compared the water use efficiency of the two groups.

Greenhouse experiment

To control for any variability between the different sites (whether inter- or intra-continental), the plants were grown in a controlled greenhouse compartment at Unifarm, Wageningen University, the Netherlands (51°59'17"N, 5°39'45"E) between September 2008 and October 2010. Temperature in the greenhouse was set at 28°C for 12 h (day time) and 23°C for 12 h (night time). These temperatures were chosen to lie within the range of mean minimum temperatures (17.4–24.2°C) and mean daily temperatures (24.8–30.3°C) during the warmest month of the locations where seed was collected for the experiments. Daily variation of light in the greenhouse was from 150 to 450 μmol m^{-2} s^{-1}, supplied from sunshine and supplementary light. Supplementary light (150 μmol m^{-2} s^{-1}) was provided for 12 to 16 h (seasonally adjusted) to ensure that the daily supply of photon flux density exceeded 10 mol m^{-2} d^{-1}. Other studies have shown that the total daily photon flux density, rather than peak irradiance, is most important for plant growth (Poorter and Van der Werf 1998). Although the total amount of light is low by comparison with full sun environments in the tropics, plant growth was substantial in some species and varied substantially among species (Supplementary material Appendix 1 Table A1), indicating that available light was sufficient to distinguish growth abilities of different species.

Plants were grown in plastic tubes of 10 cm diameter and 100 cm length, to allow the roots more space to grow in an effort to reduce the effects of pot limitation on plant allocation (Poorter et al. 2012). Pots were filled with river sand mixed with slow-release fertiliser (18-6-12 N-P-K, 8–9 month mixture) at a concentration of 5 kg fertiliser per m^3 river sand. Water was supplied through irrigation three times per day at a rate of 40 ml per pot per day, equivalent to 800 mm of rainfall over 20 weeks of growth. This was a much more even water supply than most of these species would receive in their natural environment, but the intent was to ensure that all the plants were well-watered while they grew, so that water limitation did not contribute to any variation in trait expression between species.

Plants were grown across three batch repetitions of the experiment to include all species evaluated in the experiment. Individual seedlings were planted into pots following germination, and then grown for a further 5 or 20 weeks before harvesting at each age. Each species was grown in one or, more usually, two of the three batch repetitions, leading to a maximum replication of 10 individuals harvested at each age (minimum replication was 5 individuals). Some seedlings did not thrive after transplanting into pots due to poor handling and those individuals were removed from the experiment. Actual replications per species per age group per trait measured are provided in Supplementary material Appendix 1 Table A1.

Data collection

Plants were harvested at 5 and 20 weeks after planting to estimate the relative growth rate over the period of 5 to 20 weeks (RGR). Plants harvested after 20 weeks were additionally used to measure biomass allocation and leaf functional traits that reflect photosynthetic production. Most of the larger species had reached the bottom of the pot by this stage and consequently there was evidence that taproot growth was hindered by pot length. In spite of this limitation, most species had very little lateral root development (*Mimosa caesalpiniifolia* was the exception). One to two weeks prior to harvest, photosynthetic parameters were measured from standing plants.

Individual leaf area (LS_a, cm^2) was estimated by taking the mean area of up to six fully expanded fresh leaves, scanned with a flatbed scanner. This included the midrib and all leaflets for all species including the compound-leafed species. Individual leaf mass (LS_m) was calculated as LS_a divided by specific leaf area (SLA, described below). Total plant mass (g dry matter) and organ mass fractions of plants ($g\ g^{-1}$ dry matter; leaf mass fraction, LMF; stem mass fraction, SMF; root mass fraction, RMF) were estimated. Spines were retained on stems and therefore contributed to the biomass of stems.

We used net assimilation (A) as a measure of leaf photosynthetic production. Leaf morphological and chemical traits were measured that are thought to affect carbohydrate production rates (Reich et al. 2003), including specific leaf area (SLA, mm^2 leaf area mg^{-1} dry leaf) and leaf nitrogen content. SLA was calculated using the dry mass of the scanned leaves used to estimate leaf area. Leaf chemical and physiological traits were estimated per individual using the following procedures: Leaf nitrogen per unit mass (N_m, mg N g^{-1} dry leaf) was estimated by a single digestion, following Novozamsky et al. (1983). Leaf nitrogen per unit area (N_a, mg N cm^{-2} fresh leaf) was calculated by dividing mass-based estimates by associated SLA ($N_a = N_m \times SLA$).

Area-based assimilation rates (A_a, $\mu mol\ CO_2\ m^{-2}\ s^{-1}$) were measured with an infra-red gas-exchange system (IRGA) on the youngest fully developed leaves on plants 1 to 2 weeks prior to harvesting. Leaves were measured under an irradiance of 600 $\mu mol\ m^{-2}\ s^{-1}$ provided by a PLC6 leaf cuvette. Readings were taken when plants were fully induced and photosynthetic rates had stabilised. Mass-based assimilation rate (A_m, nmol $CO_2\ g^{-1}\ s^{-1}$) was calculated directly as $A_m = A_a \times$ SLA. Photosynthetic nitrogen-use efficiency (PNUE, $= A_a / N_a$, $\mu mol\ CO_2\ s^{-1}\ g^{-1}$ N) was also estimated. Photosynthetic measurements were only taken for the first two batches (the IRGA was under repair during the harvesting period of the third batch) and thus cover 23 species and exclude five species only grown in the third batch. Readings were taken at 600 $\mu mol\ m^{-2}\ s^{-1}$ because maximum A_a measurements stabilised around this light supply rate, as indicated by the photosynthesis–light response curves that we measured for several of our species. This may have occurred because of the low peak-growth light levels that we were able to supply to our plants in the greenhouse (up to 450 $\mu mol\ m^{-2}\ s^{-1}$). The light-saturated A_a values we recorded (range: 3–13 $\mu mol\ m^{-2}\ s^{-1}$) (Supplementary material Appendix 1 Table A1) are similar to ranges of values often recorded on savanna adults (see comparisons in Tomlinson et al. 2013).

RGR ($g\ g^{-1}\ d^{-1}$) of each individual at 20 weeks was calculated as the difference between the final mass of that individual at 20 weeks (ln $Mass_{t2}$) and the mean initial mass of all individuals of the same species harvested at five weeks (ln $Mass_{t1}$) divided by the interval of growth (days) (adapted from Hoffmann and Poorter 2002).

$$RGR = \frac{\ln Mass_{t2} - \overline{\ln Mass_{t1}}}{t_2 - t_1} \qquad (2)$$

Mean trait values per species are provided in Supplementary material Appendix 1 Table A1.

One of the non-spinescent species we studied (*Lysiphyllum hookeri*) had the smallest leaves of all species sampled and possessed a highly branched growth form (much more than any of the other species). This suggested that the species falls under the architectural defence strategy (possibly divaricate branching) (Bond et al. 2004), which we could not accommodate in our deliberate dichotomous comparison between spinescent and non-spinescent species. Therefore the species was dropped from all the analyses described below, although it is plotted in all the figures that present individual species data, to show where it is positioned relative to the other species.

Statistical analysis

We assessed whether spinescent and non-spinescent species differed in biomass partitioning, leaf structural and physiological traits, and RGR (prediction 1 and 2) by comparing species' mean estimates of each trait in ANCOVA. Plant biomass partitioning and defence traits can change with ontogeny and plant size (Barton and Koricheva 2010). Therefore, the natural logarithm of species' mean mass (g) (Mass) was included as a covariate to correct for size effects on partitioning. In doing this, we treat sapling mass as a species trait. Sapling mass achieved by individuals arises from inherent growth rate (RGR) and initial mass, which is related to seed mass in our study and varies by two orders of magnitude. In addition, estimates of mean annual precipitation (MAP) taken near where the seed for each species was collected was used as a covariate in the model to account for the effects of environmental water stress on the plant traits. The full ANCOVA model tested on all variables was as follows:

$$y = \beta_0 + \beta_1\ Mass + \beta_2\ MAP + \beta_3\ Spinescence + \beta_4\ Mass \times Spinescence + \beta_5\ MAP \times Spinescence + \varepsilon \qquad (3)$$

As our species were all taken from Fabaceae and in addition a number were from the same genera, we tested both phylogenetically-adjusted and unadjusted models to account for the role of phylogenetic relatedness in the trait expression by spinescent and non-spinescent species (Ackerly 2009). A full description of the phylogenetic methods applied is provided in Supplementary material Appendix 2. All regression models were analysed using the Regressionv2.m program (Lavin et al. 2008). Traits that differed significantly between defence groups were usually identical for phylogenetically-adjusted and unadjusted models (see derived models in Supplementary material Appendix 2 Table A2). Best-fitting models for each trait, either phylogenetically-adjusted or unadjusted models depending on which had the lower Akaike information criterion (Lavin et al. 2008, Freckleton 2009), are discussed further (Table 1, Fig. 3).

To test whether biomass partitioning to leaves (LMF) is positively related to leaf size, we first regressed LS_m against LS_a using simple linear regression to show that they are highly correlated. Then we regressed LMF against LS_m, corrected for mean sapling mass.

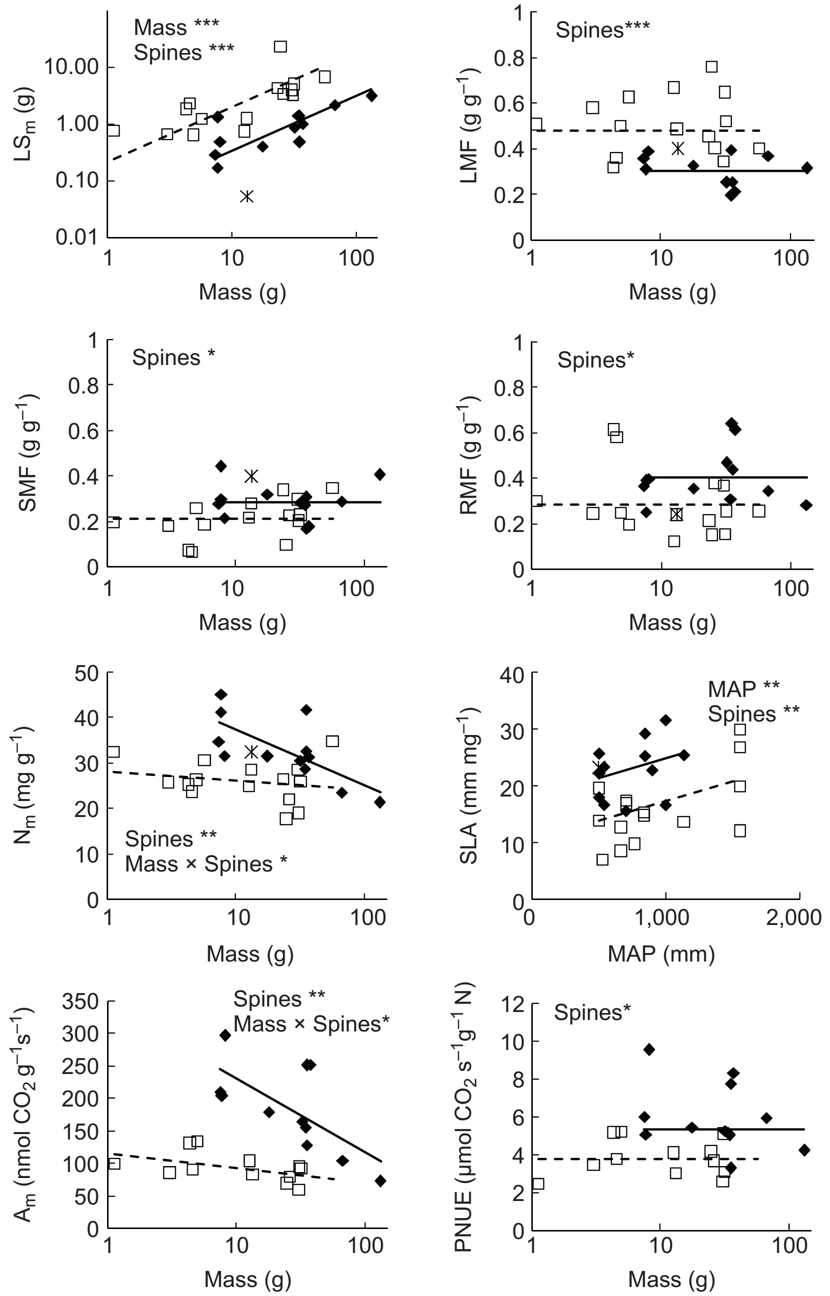

Figure 3. Biomass allocation and leaf traits plotted against plant mass (Mass) or mean annual precipitation (MAP) for spinescent (◆) and non-spinescent (☐) savanna tree species. *Lysiphyllum hookeri*, which is excluded from the regression analyses, is also indicated with a star. LS_m = individual leaf mass, RMF = root mass fraction, LMF = leaf mass fraction, N_m = leaf nitrogen content, SLA = specific leaf area, A_m = leaf assimilation rate, PNUE = photosynthetic nitrogen use efficiency. Significance of plant size and leaf habit effects are indicated on the figures (significance levels: "*" = $p < 0.05$, "**" = $p < 0.01$, "***" = $p < 0.001$) (see Supplementary material Appendix 2 Table A2 for full statistical results). Model results are presented for the best model.

$$\text{LMF} = \beta_0 + \beta_1\ \text{Mass} + \beta_2\ \text{LS}_m + \beta_3\ \text{Mass} \times \text{LS}_m + \varepsilon \quad (4)$$

To test whether allocation to leaf (LMF) is negatively related to leaf nitrogen content (N_m) and assimilation rate (A_m) across all species, and whether the two defence groups are associated with opposite ends of LMF–N_m tradeoff (prediction 3), we calculated correlations between the three traits and plotted them against one another, with different symbols used for each defence group. We then tested whether the of species of each defence group were significantly different from one another using permutation analysis of variance (PERMANOVA) (Anderson 2001). The PERMANOVA was run using the 'vegan' package of R (Oksanen et al. 2013).

To test whether architectural versus low nutrient defence was a dichotomy explaining differences in trait values among the selected species, we applied linear discriminant analysis using the 'Mass' package in R. We used a misclassification test to assess how well the predicted groups were able to assign each species to the correct group. Here we also applied an overall PERMANOVA test to establish that the groups were significantly distinct from one another. Because LDA uses a regression procedure we dropped RMF, LS_m and PNUE from these analyses as those variables were highly correlated with other variables included in the model. We also used transformed variables to satisfy the normality requirement of LDA. Because we did not have complete data for all traits, this multivariate analysis was conducted on a subset of species with complete records for all traits. It should be noted that our misclassification test should be view circumspectly as we were tested classification ability using the same species that we had used to derive the predictor function in the LDA. This was necessary because we simply did not have enough species to make a training set and a testing set.

Results

Trait differences between spinescent and non-spinescent species

Biomass partitioning and leaf chemical, physiological and structural traits differed significantly between spinescent and non-spinescent species in support of prediction 1 and 2 (Table 1, Fig. 3). Spinescent species had smaller leaves (LS) and allocated 38% less biomass to leaves (LMF), 41% more biomass to roots (RMF), and 34% more biomass to stems (SMF), than non-spinescent species. Spinescent species also had greater SLA than non-spinescent species. Among leaf chemistry traits, N_m was significantly greater and N_a was significantly lower for spinescent species than non-spinescent species. Among leaf physiology traits, assimilation parameters (A_a, A_m) and photosynthetic nitrogen use efficiency (PNUE) were both greater for spinescent species than non-spinescent species. Spinescent species had greater photosynthetic water use efficiency (PWUE) than non-spinescent species, although there was no significant different between the groups with respect to stomatal conductance (g_s) (Table 1). Area-based leaf size (LS_a) and LAR increased, and N_a and A_a decreased with MAP (Table 1).

Across all species, leaf mass fraction was positively related to individual leaf mass ($\text{LMF} = 0.567 - 0.060 \ln \text{Mass} + 0.057 \ln \text{LS}_m$; $R_a^2 = 0.21$, $F_{2,25} = 4.586$, $p = 0.020$) (interaction term in Eq. 4 was non-significant). Individual leaf mass was itself positively related to individual leaf area ($\ln \text{LS}_m = -2.970 + 1.029 \ln \text{LS}_a$; $R_a^2 = 0.90$, $F_{1,26} = 237.4$, $p < 0.001$).There was wide overlap in RGR ranges for each defence group (Supplementary material Appendix 1 Table A1), and hence no differences in mean RGR of each group (Table 1). However, the slowest-growing species were all non-spinescent species while the fastest-growing species were all spinescent species (Supplementary material Appendix 1 Table A1).

For all traits, PERMANOVA significantly distinguished spinescent and non-spinescent species (pseudo $F = 5.42$, $p < 0.001$, $R^2 = 0.21$). Further, when we ran the misclassification test on the discriminant function we derived using LDA, our model correctly allocated all spinescent species to the spinescent group and all non-spinescent species to the non-spinescent group. Thus the derived discriminant function was 100% successful in allocating species to the correct group, further supporting our expectation that these are two different defence strategies.

Tradeoffs in allocation to leaf mass versus leaf productivity

There was strong evidence for a tradeoff between leaf biomass allocation and leaf productivity across species (prediction 3). Spinescent and non-spinescent species were associated with opposite ends of this tradeoff (prediction 2) (Fig. 4). Across all species, LMF was negatively correlated with leaf assimilation rate (A_m) and leaf nitrogen content (N_m). A_m and N_m were positively correlated with one another (Pearson's $r = 0.59$, $p < 0.01$). The defence groups were significantly different from one another across the LMF–N_m tradeoff and the LMF–A_m tradeoff (Fig. 4).

Discussion

We have shown that saplings of savanna trees with and without spinescence have distinct plant trait suites that may indicate contrasting defence strategies against vertebrate mammalian herbivores, namely architectural defence versus low nutrient defence. We proceeded with the reasoning that plant species are under dual and opposing selection pressures for reducing the negative effects of vertebrate herbivory and for outcompeting other plants for resources (Herms and Mattson 1992). Competitive ability is associated with RGR (Grime 1977), which depends on both LMF and N_m (Eq. 1). We predicted that spinescent species (architectural defenders) must have small leaves and low LMF that make leaf harvesting difficult (prediction 1) while non-spinescent species (low nutrient defenders) have low N_m to ensure leaf material is of insufficient nutritive value for herbivores (prediction 2). Consequently, we expected that there would be a direct tradeoff between species' LMF and N_m enforced by vertebrate herbivory pressure on these groups (prediction 3) that fundamentally constrains RGR. Our data provided support for all three predictions (Table 1, Fig. 3–5). In this Discussion we interpret our findings in the wider context of the functional and ecological significance of architectural versus

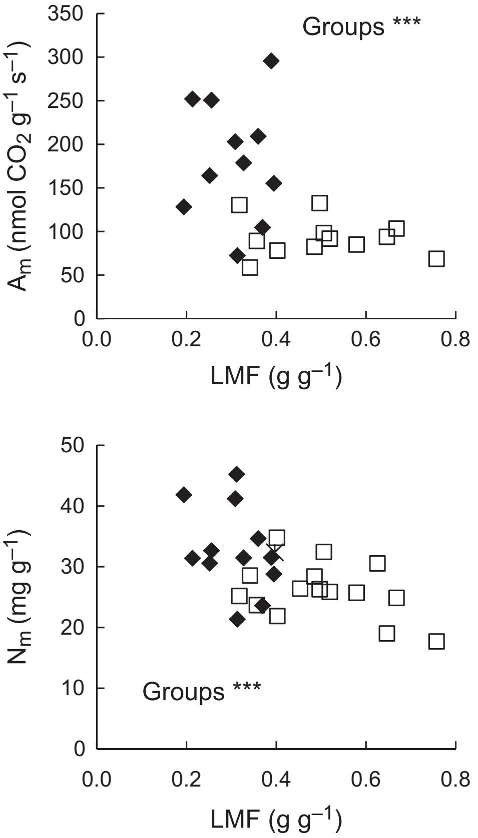

Figure 4. Pearson correlations of leaf mass fraction (LMF) with leaf assimilation rate per unit mass (A_m) and with leaf nitrogen content per unit mass (N_m). Species are categorised by defence group (◆ spinescent; □ non-spinescent). (*Lysiphyllum hookeri* is also indicated with a star.) Correlation between LMF and N_m is –0.55 ($p < 0.01$). Correlation between LMF and Am is –0.49 ($p < 0.05$). (Correlation between N_m and A_m is 0.59, $p < 0.001$.) Significant differences in the mean distributions of species of each group across the biplots were tested using PERMANOVA (Anderson 2001). Significant differences between the groups are also indicated on the plots.

low nutrient defences strategies, and also comment on effective ways to increase our understanding of functional trait correlations in plant defence.

For architectural defence vertebrate architecture is critical

Effective architectural defence is likely to differ according to architecture of the dominant vertebrate herbivores in the system. This has already been ably demonstrated by Bond and colleagues (Bond et al. 2004, Bond and Silander 2007), who have shown that architectural defence traits differ between continental systems where dominant herbivores are mammals (spines are useful) versus island systems where dominant herbivores were birds (spines are irrelevant). The same is true for spinescence and mammal architecture.

In this article we have concentrated on continental savanna systems where large terrestrial mammals dominate the vertebrate biomass. In these systems mammals approach trees externally and feed on branch tips while standing on the ground. Under these conditions small leaf size is necessary so that thorns on terminal branches can protect them (Cooper and Owen-Smith 1986). Possession of small leaves also reduces the loss of plant resources to herbivores because LMF is lower so more biomass can be saved by diversion to stem and root matter, which are less favoured by mammals. Other experiments on spinescent *Acacia* species in savannas have shown that Acacias increase terminal branch spinescence and reduce leaf size when subjected to increased defoliation pressure (Milewski et al. 1991, Rohner and Ward 1997) but revert to lower spinescence and larger leaves when this defoliation pressure is removed (Young et al. 2003). A parsimonious explanation is that this plasticity allows adjustment between allocation to resistance against herbivory and allocation to growth in accordance to the herbivory pressure applied (Karban 2011).

In systems where arboreal vertebrates are more important herbivores than terrestrial vertebrates, small leaf size may not form part of the trait complex expressed by architectural defenders and positioning of spinescence might also differ. For instance, in forests many tree and liana species possess large spines and large leaves (e.g. rattan palms, Grubb 1992; *Acacia karroo*, Ward 2011), and many forest species only have thorns on their main stems and not their branches. Large thorns inhibit the ability of arboreal vertebrates to climb onto the plants to feed on their leaves and shoots (Cooper and Ginnett 1998), and hence small leaf size is not required as an additional component to reduce herbivory in forest trees. As a consequence, forest spiny species may be capable of substantially faster growth rates than savanna species because their leaf mass is unrestricted. Thus different animal architectures modify both architectural defences and growth potential of woody plants.

Is low leaf N the main characteristic of low nutrient defence?

Our second posited defence strategy rests on the idea that certain plants make leaves which are of insufficient nutritional value to support the metabolic requirements of herbivores and are therefore likely to be avoided. We have concentrated on leaf nitrogen content as a measure of leaf nutritional value to plants. This is a long-recognised strategy (Coley et al. 1985, Lundberg and Åström 1990), but it is usually extended to consider the dual roles of low nutrient value and high secondary carbon metabolites. Quantitative metabolites such as tannins are known to precipitate proteins inhibiting their digestion by vertebrates (Van Soest 1982), and hence it is believed that tannin content in leaves limits the nutrient value of leaves to vertebrate herbivores by limiting digestibility of nitrogen-bearing materials. Thus the low nutrient defence mechanism may also depend on secondary carbon metabolite quantity (Scogings et al. 2014). As we did not measure any secondary metabolites we are not in a position to assess this properly. However, the fact that spinescent and non-spinescent species were statistically distinguishable with respect to this trait suggests that low nitrogen content may be the primary determinant of the differences in the two broad strategies employed against vertebrate herbivores. We are not implying that secondary metabolites are not important; certainly recent work on invertebrate herbivory indicates that there may be different defence syndromes that involve different secondary metabolites, either quantitative or qualitative (Agrawal and Fishbein 2006, Travers-Martin and Müller 2008, Fine et al. 2013). Possibly there are also multiple sub-strategies of low nutrient defence against vertebrates, but this remains to be established. Such a situation might explain why a previous broad-scale meta-analysis failed to detect an overall association between secondary metabolite concentrations and susceptibility to vertebrate herbivory (Carmona et al. 2011) rather than there being no relationship of significance.

Relative resource supply and anti-herbivory defence

We have proposed that two alternative anti-herbivory strategies are selected for under vertebrate herbivory because competition selects for greater LMF and N_m while vertebrate herbivory selects for smaller leaves and low LMF (architectural defence) or for low N_m (low nutrient defence), as illustrated in Fig. 5a. Species with high N_m can be slow-growing if they have very low LMF while species with low N_m can be fast-growing as long as they have large LMF e.g. compare *Acacia erioloba* and *Mimosa claussenii* in Supplementary material Appendix 1 Table A1. *Acacia erioloba* is heavily defended by thorns. The fact that species can have high N_m, high defences and low RGR, challenges previous assumptions that the level of quantitative plant defences is negatively correlated with leaf nitrogen (Coley et al. 1985, Gulmon and Mooney 1986). As we have argued, leaf N may relate more closely to the 'type' of defence that predominates i.e. architectural defences versus leaf defences (though it should be noted that Coley et al. (1985) 'do' hypothesise about differences in types of chemical defences between high- and low-RGR plants, for which there is now strong evidence (Fine et al. 2013).

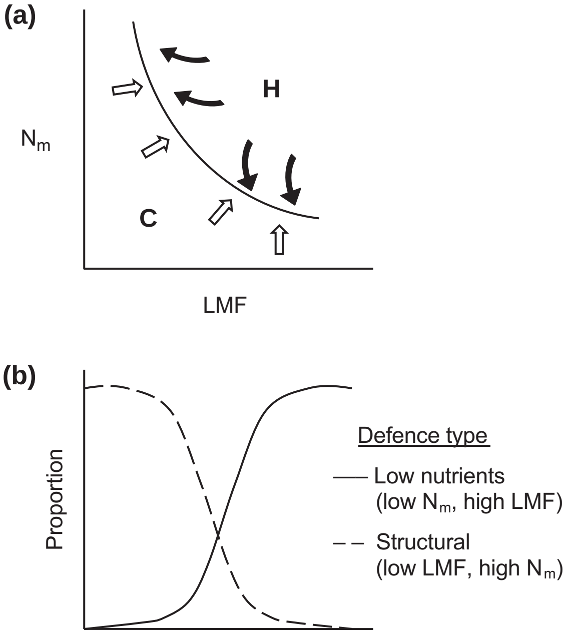

Figure 5. Hypothesised shifts in the tradeoff between allocation of carbon to leaf mass fraction (LMF) versus allocation of nitrogen to leaf (N_m) resulting from dual selection pressures of competition and vertebrate herbivory under different resource scenarios. (a) Illustration of selection pressure from competition (C) to increase LMF and N_m, and selection pressure from vertebrate herbivory (H) to decrease LMF or N_m. (b) Shifts in the abundance of architectural versus leaf defence across water: soil nutrient gradient. When soil nutrients are more limiting than water (high H_2O: nutrient ratio), then defence against vertebrates is predominantly low nutrient defence (low N_m). When water is more limiting than soil nutrients (high H_2O: nutrient ratio), then defence against vertebrates is predominantly architectural defence.

The exact position of the LMF–N_m tradeoff line may be determined by the productivity of the local environment, which determines the optimal position of the tradeoff between allocation to defence versus growth, as proposed by Coley et al. (1985). In more productive systems, plants can replace biomass lost to herbivory more rapidly and hence the level of defence may decline (Endara and Coley 2011). Under this scenario the LMF–N_m tradeoff line may shift towards greater LMF and N_m.

The LMF–N_m tradeoff and its link with the two defence strategies can also explain the distribution of architectural defenders and low nutrient defenders across water and nutrient gradients in savannas (Fig. 5b). In savannas in Africa and South America, spinescent species and other small-leafed species are associated with fertile soils in semi-arid environments and increase in abundance as climates become more arid, whereas large-leafed species are associated with infertile soils in semi-arid savannas and increase in abundance towards humid dystrophic savannas (Cole 1986, Venter et al. 2003, Mucina and Rutherford 2006). These patterns can be interpreted in terms of the relative supply of soil nutrients versus water, which affects the viability of each defence strategy. In high rainfall systems, carbohydrate production rate exceeds soil nitrogen supply, and regrowth is severely limited by soil nutrient supply. Hence low nutrient defenders with high LMF and low N_m (non-spinescent, broad-leafed species) predominate (Fine et al. 2004). In low rainfall regions, soil nitrogen supply exceeds carbohydrate production leading to high leaf N_m, and nutrients lost to herbivory can be recompensed if water is available to support growth. Hence architectural defenders (spinescent species and small-leafed species with divaricate branching) predominate. In intermediate rainfall conditions, dominance by either defence strategy is determined by soil type (Venter et al. 2003): architectural defender species dominate on fertile soils and leaf defender species dominate on infertile soils.

Plant family bias?

We have only considered differences between extant Fabaceae species, which are known for their ability to form mutualisms that fix N and are thought to have high leaf N in general (Sprent 2007). Nitrogen-fixing is not a universal trait in legumes, and even in species that do fix nitrogen, older plants may not do so as much as young plants (Santiago De Freitas et al. 2015) However, it is clear that it is a trait possessed by both spinescent and non-spinescent species in our sample. As such, it might be argued that the patterns we observed are true only for Fabaceae. Evidence from studies of co-occurring spinescent and non-spinescent *Hakea* species (Proteaceae) growing in southwestern Australia do support our findings: Rafferty et al. (2005) found a negative relationship between leaf N and spine density, and Hanley and Lamont (2002) found a negative relationship between leaf area and spinescence, among species of the genus. More formal comparison under common conditions is required to properly confirm the consistency of the tradeoff, and it is our intention to extend our research to cover species from a wider range of woody families.

On methods of identifying and evaluating defence-related traits

Agrawal (2007) noted that the lack of quantitative evidence supporting single trait tradeoffs in plant defence probably reflected by the fact that defence is rarely, if ever, effective as a single trait. Rather, defence traits can be most effective when acting together, leading to the likely existence of plant defence syndromes, which could trade off if they represent alternative adaptive strategies. Agrawal and Fishbein (2006) relied on multivariate methods to identify multiple defence syndromes in Asclepiadaceae, avoiding a priori predictions of tradeoffs, and assuming that traits group together on the basis of minimised costs for maximised defence. Their method is useful for identifying potential groups/ syndromes. The strength of our approach is that it relies on the conceptual understanding of constraints on plant growth (defence versus competition) and constraints on vertebrate feeding (food quality and accessibility) to predict which plant traits might change to satisfy these limits, which in turn can then be tested formally with an appropriate data set. Our approach has also allowed us to provide a mechanistically based prediction as to where each of the two anti-herbivory strategies is likely to predominate across landscape scales, confirming observed patterns, and, importantly explaining why two defence strategies were selected in the first place instead of just one. We have thus propounded a clear and testable theory that links observed trait patterns with environmental constraints. We do not propose that architectural defence and low nutrient defence are the only strategies selected in response to vertebrate herbivory, but our data suggests that it may be a very important basis for the link between the defence syndromes. Further data are required to confirm this relationship. Subsequently analyses must refine how other defence characteristics may have enhanced defence (e.g. ant mutualisms). Importantly, we hope this foundation will allow progress to be made on the relationships between defences selected against vertebrates and those selected against invertebrates.

Acknowledgements –We wish to thank the staff of UNIFARM and members of the Resource Ecology Group, both of Wageningen University, for technical support to the experiment, especially Anne-Marie van den Driessche, Leo Goudzwaard, Jia Wu, Ainara Ballesteros-Larranaga and André Maassen. This project and KWT were funded by a research grant from Shell Research Foundation.

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Supplementary material (available online as Appendix oik-02325 at <www.oikosjournal.org/appendix/oik-02325>). Appendix 1: Species' trait data. Appendix 2: Phylogenetically corrected and uncorrected analyses of trait differences between spinescent and non-spinescent species