

Correlations between physical and chemical defences in plants: tradeoffs, syndromes, or just many different ways to skin a herbivorous cat?

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Introduction

Herbivores consume over 20% of global annual net primary productivity (Agrawal, 2011) and strongly affect the standing biomass of primary producers in terrestrial and marine systems (Cyr & Pace, 1993; Poore *et al.*, 2012). As a result, plants have evolved a range of physical and chemical defences that reduce the nutritional quality of the foliage (e.g. tannins), make it difficult for herbivores to access or process foliage (e.g. spines, hairs, latex, leaf toughness), are toxic to herbivores (e.g. cyanide, alkaloids, phenolics), or attract insects that attack herbivores (e.g. extrafloral nectaries) (Duffey & Stout, 1996; Hanley *et al.*, 2007; Agrawal & Konno, 2009; Ness *et al.*, 2009; Ballhorn *et al.*, 2010; Spalinger *et al.*, 2010).

The assumption that defences are costly underlies theories to explain the distribution of defences among plant parts (e.g. optimal defence theory, Rhoades & Cates, 1976) and among individuals and species with varying access to resources (e.g. growth-differentiation balance hypothesis, Herms & Mattson, 1992) or with different life-history characteristics (such as growth rates, Coley, 1988). Consequently, allocation of resources to defences is predicted to trade off with other plant functions such as growth or reproduction (reviewed in Koricheva, 2002). Similarly, within a plant, defences are predicted to trade off against one another because a finite pool of resources is being divided between different types of defence (Janzen, 1966; Steward & Keeler, 1988; Eck *et al.*, 2001; Cornelissen *et al.*, 2009; Read *et al.*, 2009). These ideas have been used to suggest that there will be tradeoffs between physical and chemical defences (Steward & Keeler, 1988; Twigg & Socha, 1996; Cornelissen *et al.*, 2009; Read *et al.*, 2009), induced and constitutive defences (Morris *et al.*, 2006; Kempel *et al.*, 2011), tolerance and resistance to herbivores (Leimu & Koricheva, 2006; but see Núñez-Farfán *et al.*, 2007), and biotic defence (e.g. by ants) and direct chemical and/or physical defences (Janzen, 1966; Rehr *et al.*, 1973; Koricheva & Romero, 2012).

Summary

- Most plant species have a range of traits that deter herbivores. However, understanding of how different defences are related to one another is surprisingly weak. Many authors argue that defence traits trade off against one another, while others argue that they form coordinated defence syndromes.
- We collected a dataset of unprecedented taxonomic and geographic scope (261 species spanning 80 families, from 75 sites across the globe) to investigate relationships among four chemical and six physical defences.
- Five of the 45 pairwise correlations between defence traits were significant and three of these were tradeoffs. The relationship between species' overall chemical and physical defence levels was marginally nonsignificant ($P=0.08$), and remained nonsignificant after accounting for phylogeny, growth form and abundance. Neither categorical principal component analysis (PCA) nor hierarchical cluster analysis supported the idea that species displayed defence syndromes.
- Our results do not support arguments for tradeoffs or for coordinated defence syndromes. Rather, plants display a range of combinations of defence traits. We suggest this lack of consistent defence syndromes may be adaptive, resulting from selective pressure to deploy a different combination of defences to coexisting species.

Empirical evidence for a tradeoff between physical and chemical defences has been mixed. Cornelissen *et al.* (2009) found a triangular relationship between total phenolics and fibre across 34 subarctic species, with species showing high concentrations of phenolics with low fibre, high fibre with low concentrations of phenolics, or low quantities of phenolics and fibre, but never high quantities of both traits. Read *et al.* (2009) found positive or null correlations between leaf toughness and both total phenolics and tannin activity measured as protein precipitation across 125 species from New Zealand and New Caledonia. Twigg & Socha (1996) found strong negative correlations between fluoroacetate concentration and spines and toughness, but not hairs, in 28 species of *Gastrolobium*. There was no correlation between total phenolics and any mechanical property across six species of rainforest trees in Australia (Iddles *et al.*, 2003). Finally, a meta-analysis across five correlations from two studies found no significant correlation between physical and chemical defences (Koricheva *et al.*, 2004, although the sample size in this analysis was very small).

Plants generally allocate resources to several defensive traits simultaneously (Paul & Hay, 1986; Duffey & Stout, 1996; Agrawal & Fishbein, 2006; Read *et al.*, 2009). The absence of clear tradeoffs in previous research could be a result of these studies using a pairwise approach between single physical and chemical defences rather than integrating across the suite of chemical and physical defences employed by each species. Therefore, our first aim was to determine whether there is a tradeoff between the overall physical and chemical defence levels.

In addition to investigating the tradeoff between aggregated indices of physical and chemical defences, we aimed to provide a broad overview of the myriad correlations between defence variables. Defence strategies are often phylogenetically conserved (e.g. Cactaceae usually have spines, Myrtaceae have oil glands, and Euphorbiaceae tend to have latex; Mabblerley, 1997) and plant species tend to have a suite of different types of defence

(Duffey & Stout, 1996; Agrawal & Fishbein, 2006; Read *et al.*, 2009). To get a broad understanding of plant defence strategies, we therefore need to consider multiple traits simultaneously, across a wide range of species. However, most previous studies of plant defences focus either on one or two traits across a large number of species and taxa (e.g. Levin, 1976; Cornelissen *et al.*, 2009) or on multiple traits within taxonomically restricted groups (e.g. Steward & Keeler, 1988; Hanley & Lamont, 2002; Agrawal & Fishbein, 2006; Travers-Martin & Muller, 2008).

Although there has been considerable research on tradeoffs between plant defences, a second school of thought predicts that plants display coadapted complexes of defence traits, or defence syndromes (Feeny, 1976; Kursar & Coley, 2003; Agrawal & Fishbein, 2006). Much of the theory and evidence suggest that there is a gradient in defence allocation, ranging from species with high levels of defence to species with low levels of defence (Kursar & Coley, 2003). Under this school of thought, allocation to all defensive traits will often reflect ecological differences, such as the quality or type of habitat and/or plant apparency (Janzen, 1974; Feeny, 1976).

Empirical studies generally support the idea that plants can be categorized into defence syndromes. For example, hierarchical cluster analysis of seven traits suggested that 24 species of *Asclepias* could be characterized by three defence syndromes, representing species with high N and high amounts of latex and trichomes, species with high C : N ratios and tough leaves, and species with low C : N ratios and high concentrations of cardenolides (Agrawal & Fishbein, 2006). Hierarchical cluster analysis for seven species of Brassicaceae also revealed three groups: high N with high glucosinolates; high N with high trichome densities and high proteinase inhibitor activity; and low N, high C : N and low glucosinolates (Travers-Martin & Muller, 2008; though N is an important component of both glucosinolates and C : N ratio). Finally, in a larger comparative study, da Silva & Batalha (2011) showed that 61 species from a cerrado community in Brazil fell into five groups. Three of these groups had four or fewer species and were distinguished by a single trait. The two remaining groups represented a syndrome of low C : N and high specific leaf area, and a syndrome of high C : N, low specific leaf area (SLA) and presence of alkaloids, tannins and terpenoids.

In this study we aimed to extend our previous understanding of interspecific patterns in plant defences by asking how general these patterns are. Specifically, we asked whether tradeoffs and/or defence syndromes are seen across species from a broad range of taxonomic groups, from a wide range of habitats around the world. We collected a dataset of six physical and four chemical defence traits, measured on 261 species spanning 203 genera and 80 families, including a lichen, ferns, gymnosperms and angiosperms growing under natural conditions at a range of sites, to provide an unprecedented quantification of the tradeoffs and correlations among plant defences using both pairwise analyses and multivariate statistics.

In summary, our aims were to:

- test the hypothesis that there is a negative relationship between species' overall level of physical and chemical defence;
- quantify the correlations between a wide range of types of physical and chemical defences;

- test the hypothesis that plant species are clustered in defence syndromes.

Materials and Methods

Sampling

We worked at 75 study sites spanning every continent except Antarctica, and latitudes from 74.5°N to 51.5°S (Moles *et al.*, 2011). We generally studied the four species with the greatest leaf area index at each site (exceptions described in Moles *et al.*, 2011 and species list provided in Supporting Information, Table S1). One advantage of our method of selecting species is that it chooses species without consideration of their defence strategy, which means that we get general results that are distinct from, and complementary to, previous studies of clades with particularly interesting defence strategies. The final species list included 261 species spanning 80 plant families (see Table S2 for a summary of the taxonomic composition of our dataset), and included 106 tree species, 90 shrub species, 58 herbaceous species (broadly defined, and including the lichen) and seven climbing species.

We sampled fully expanded photosynthetic units (usually leaves or leaflets, but occasionally photosynthetic stems or phyllodes; henceforth 'leaves') from mature, outwardly healthy individuals of each species, as close to the peak growing season as possible (full details of material selection in Moles *et al.*, 2011). At least 40 g of fresh material was collected from at least five individuals of each species and placed in paper bags ready for oven drying. An additional three fresh leaves from each of five plants of each species were placed in plastic bags with damp tissue, and stored in a cooler or refrigerator until their SLA could be measured. Although we attempted to eliminate as many sources of variation as possible, there were some factors that were not controlled in our sampling, including leaf position, reproductive status and previous exposure to herbivores (see supplementary methods of Moles *et al.*, 2011 for further discussion).

Traits

We selected a range of traits that have been shown to make it difficult for herbivores to access or process leaf tissue, decrease leaf loss to herbivores, reduce growth or survivorship of herbivores, and/or increase the plants' survival or reproduction (evidence for each trait reviewed below and in Moles *et al.*, 2011). However, as we have not quantified the fitness advantage plants accrue from possessing each of our traits, they are formally resistance traits rather than defences *per se* (Strauss & Agrawal, 1999). These traits can affect different herbivores in different ways (Tanentzap *et al.*, 2011), and the fitness advantage conferred by a given trait depends on a range of factors, including the assemblage of herbivores present, other functions of the trait, the suite of other traits possessed by the plant, and the environment in which the plants grow (Koricheva, 2002).

Local environmental conditions (e.g. soil type, light, nutrient and moisture availability) and exposure to recent herbivory

(Coley *et al.*, 1985; Karban & Meyers, 1989) can affect the expression of defence or resistance traits in an individual plant. By considering several physical and chemical traits across a wide range of taxa and habitats, we aimed to quantify the relationships among traits given the inherent background variation in local environmental conditions. Thus, the observed relationships among traits are those currently occurring in the surveyed plants, with all other factors affecting the expression of defence traits varying naturally among plants or sites.

Methods for all trait measurements are described in Moles *et al.* (2011).

The physical traits quantified were as follows:

- *Specific leaf area.* Leaves with low SLA have higher physical toughness (Iddles *et al.*, 2003; Moles *et al.*, 2011), which is an extremely effective defence against herbivores (Choong *et al.*, 1992; Turner, 1994; Hanley *et al.*, 2007; Clissold *et al.*, 2009).
- *Presence/absence of hair on mature foliage.* Hairs make it difficult for invertebrates to access leaf tissue, decrease losses to herbivores (Moles & Westoby, 2000; Hanley *et al.*, 2007), reduce herbivore growth and survivorship (Haddad & Hicks, 2000; Agrawal & Fishbein, 2006), and reduce oviposition (Haddad & Hicks, 2000). We used a simple presence/absence instead of trichome density because the wide variety of different sizes and shapes of trichomes across our study species would make it very difficult to make meaningful quantitative comparisons. Further, all hairs, including glandular hairs, were counted as physical defences. There is certainly scope for a more detailed investigation of this trait in future studies.
- *Presence/absence of extrafloral nectaries.* Extrafloral nectaries occur in over 90 plant families, and the omnivorous invertebrates they attract (typically ants) attack herbivorous insects, thus increasing the host plant's survival and/or reproductive success (Ness *et al.*, 2009).
- *Presence/absence of spinescence.* Spines, thorns and prickles are an effective form of defence against a range of herbivores, particularly mammals (Hanley *et al.*, 2007).
- *Presence/absence of succulent leaves.* Succulence is primarily related to water availability. However, invertebrate herbivores may have difficulty processing succulent tissue. In a field study in Africa, succulent species lost only three-quarters of the leaf area lost by nonsucculent species, and grasshoppers removed almost twice as much leaf area from nonsucculent species than from succulent species in cafeteria experiments (Perez-Harguindeguy *et al.*, 2003).
- *Presence/absence of latex.* Latex deters chewing herbivores, particularly invertebrates, and experimental removal of latex increases herbivory (Agrawal & Konno, 2009). Although latex is a defence with both physical and chemical features, we have followed historical precedent in including it as a physical defence (Agrawal *et al.*, 2008).

The chemical traits quantified were as follows:

- *Lipid content.* The percentage of dry leaf tissue made up of oils (such as terpenes), cuticular waxes and resins. Many studies have shown that these traits deter or otherwise negatively affect herbivores (Lincoln, 1985; Peeters, 2002; Jones *et al.*, 2003; Marko *et al.*, 2008).

- *Ash content.* The percentage of dry leaf mass remaining after combustion at 600°C for 12 h. This is a measure of defences such as silica-based phytoliths and calcium oxalates. Silica is widespread and abundant in plant tissues, especially in grasses, which are typically 2–5% silica (Massey *et al.*, 2006; Cooke & Leishman, 2012). Calcium oxalate occurs in most plant families and is the most abundant insoluble mineral in plant tissue, accounting for 3–80% of plant dry mass (Franceschi & Nakata, 2005; Korth *et al.*, 2006). Both silica and calcium oxalates reduce feeding by a range of herbivores, including insects and mammals (Djamin & Pathak, 1967; Galimuhtasib *et al.*, 1992; Ward *et al.*, 1997; Korth *et al.*, 2006; Massey *et al.*, 2006; Hanley *et al.*, 2007).

- *Polyethylene glycol (PEG)-binding capacity.* We used Silanikove *et al.*'s (1996) PEG-binding assay as a measure of tannins. Tannins deter feeding in a range of herbivores (Furstenburg & Vanhoven, 1994; Fritz *et al.*, 2001; Roslin & Salminen, 2008). Tannins can reduce metabolic and growth efficiency in invertebrates (Roslin & Salminen, 2008), reduce protein and dry matter digestibility, cause endogenous nitrogen loss, and cause damage to the gastrointestinal tract, kidney and liver in mammals (Shimada, 2006; Spalinger *et al.*, 2010). We chose the PEG-binding assay rather than a traditional colorimetric assay for two reasons: it measures the degree to which tannins bind to plant protein (a functional trait that is relevant to animals) rather than the concentration of a suite of chemicals that can have varying effects on herbivores; the PEG-binding assay provides a straightforward quantitative index, while the different types of tannins found in different plant taxa produce different chromophores at similar concentrations and thus confound colorimetric assays (Mueller-Harvey, 2006). The use of PEG binding as a tannin assay is most applicable to mammalian herbivores, but predicting insect responses to tannins relies more on understanding the pro-oxidant activity of each tannin as well as the oxidative conditions in the gut (Barbehenn & Constabel, 2011). A larger project is underway to understand how the different approaches described intersect.

- *Cyanogenic glycosides.* The ability to release hydrogen cyanide in response to cell damage is a widespread defence trait that is effective against a wide range of invertebrate and vertebrate herbivores (Ballhorn *et al.*, 2010).

We also recorded the absolute and relative abundance of each of our sample species at each site. Absolute abundance was a simple estimate of the leaf area index of each of our study species. Relative abundance was calculated by dividing the leaf area index for each of our study species by the total leaf area index for the field site (details in Moles *et al.*, 2011). Finally, we placed each study species into coarse growth form categories (tree/shrub/herb/climber).

Data analyses

Unless stated otherwise, we analysed the data with R (R Development Core Team, 2007).

We began by performing cross-species analyses to quantify pairwise relationships between traits. These models included a random effect for site, to account for the fact that species sampled

at the same site are not independent, and weighted cases such that each species or subspecies received a total weight of 1 (that is, the 247 species or subspecies that occurred at only one site received weights of 1, while the 16 species or subspecies that occurred at more than one site received weights inversely proportional to the number of sites at which they occurred). R^2 values were calculated by sequential reduction in residual sum of squares on addition of the term (as usual), adding fixed-effects terms to the model before the random-effect term for site. SLA data were \log_{10} -transformed before analysis.

We performed phylogenetic analyses on all pairwise trait comparisons that involved at least one continuous variable. Phylogenetic trees were built using Phylomatic (<http://www.phylodiversity.net/phyloomatic/phyloomatic.html>) using a megatree based on the most recent Angiosperm Phylogeny Group classification (APG III 2009; Phylomatic megatree R20091110). We then used the analysis of traits module in Phylocom 4.1 (Webb *et al.*, 2008) to obtain values for each phylogenetically independent contrast. Species that occurred at multiple sites were given a mean value calculated across all the sites at which they occurred. Contrasts for two continuous variables were analysed using regressions through the origin (Garland *et al.*, 1992). For analyses between continuous and binary variables, contrasts for which the state of the binary variable changed were tested for difference from zero, using *t*-tests. It is not possible to run analyses of two binary variables in Phylomatic.

Defence indices

We used latent variable modelling (LVM) to calculate an index of chemical defence and an index of physical defence for each species. One benefit of LVM is that it explicitly recognizes the presence of underlying variables (in this case, overall chemical defence and overall physical defence) that are difficult or impossible to measure, but which are correlated with variables that we can measure (in this case, traits such as presence of spines, hairs and latex) (Sammel *et al.*, 1997; Regan *et al.*, 2002; Grace, 2006). We began by grouping variables based on their correlations. Traits with significant negative correlations were placed in separate groups, and traits with positive correlations were put in the same group. For chemical traits, ash and cyanide were placed in one group, with lipid and PEG binding in another group. For physical attributes, hair and latex made up one group with SLA, extrafloral nectaries, spines and succulent leaves in the other group. Quantile–quantile plots were constructed for each of the continuous variables. All plots indicated that each of these variables was approximately normally distributed. Therefore, for the purposes of a latent variable model, we assumed the continuous variables were normally distributed, with means related to the relevant sub-index via a simple linear model. Percentage variables were logit-transformed before analysis. For presence–absence data, we assumed a binary distribution and used logistic regression to relate the mean probability of presence to the sub-index. A separate sub-index was generated for each group, and indices for chemical and physical defence were calculated by adding scores from the component sub-indices. Additional details of

the methodology, and the R source code used for constructing the chemical index are provided in Methods S1.

Relationships between indices of chemical and physical defence were assessed using both cross-species analyses and phylogenetic regressions (methods as described earlier). We also performed a quantile regression using the *quantreg* package in R, to quantify the shape of the data cloud. A random effect for site could not be included in quantile regressions, but a fixed effect for site was not significant ($P=0.86$) in the fifth percentile analysis.

Multivariate analyses

We used categorical principal component analysis (CATPCA; Gifi, 1991) in SPSS 15.0 (SPSS Inc., Chicago, IL, USA) to explore the relationships among all traits simultaneously. This analysis allows the inclusion of categorical variables with numerical variables, and the existence of a nonlinear relationship between traits (Ellis *et al.*, 2006; Costantini *et al.*, 2009). Binary traits were treated as ordinal, while continuous traits were treated as numerical.

We used hierarchical cluster analysis (performed with SPSS 15.0) to determine whether our species were grouped in defence syndromes. Trait values were transformed so they ranged from 0 to 1. We used Euclidean distances as the dissimilarity measurement and UPGMA as the linking procedure (Legendre & Legendre, 1998), using. We tested for the presence of species groups in the *a priori* unstructured set of samples using the similarity profile routine (SIMPROF) in the software package Primer V.6. This routine contrasts the similarity profile (i.e. Euclidean distance between samples plotted against the rank order of similarities) of the observed data to that expected by chance (obtained by 9999 permutations, with the values for each variable independently permuted across all species; Clarke *et al.*, 2008).

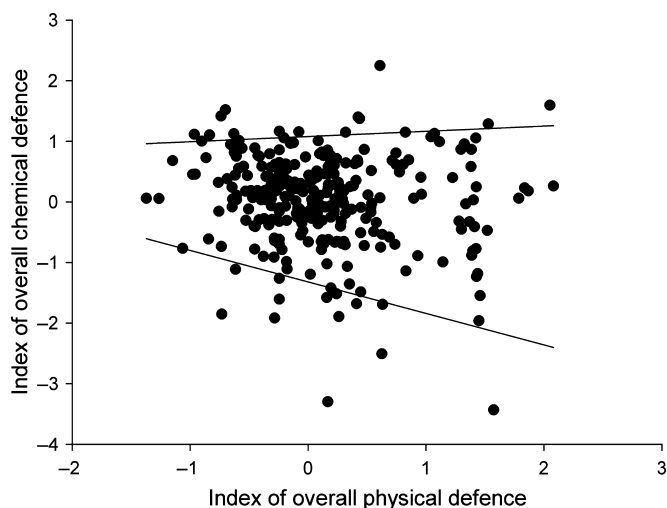


Fig. 1 The relationship between indices of physical defence, and chemical defence. Indices were generated using latent variable modelling (see Methods S1 for detailed methods). The lines show the fifth and the 95th quantiles, from quantile regression.

Results

The relationship between the index of overall physical defence and the index of overall chemical defence was marginally nonsignificant ($P=0.076$; slope = -0.13 ; $R^2=0.014$; Fig. 1). This result was not affected by excluding the lichen ($P=0.071$). There was no significant relationship between the indices of physical and chemical defence after accounting for phylogeny ($P=0.27$; slope = -0.12 ; $R^2=0.007$), ruling out the possibility that a significant relationship is being obscured by phylogenetic nonindependence of the data.

Next we added a term for growth form to investigate whether the inclusion of taxa of differing growth forms obscures a significant relationship between chemical and physical defences. However, growth form did not explain a significant proportion of the variation in this analysis ($P=0.26$).

Quantile regression showed that the slope of the 95th quantile was not significantly different from zero ($P=0.20$). The slope of the 10th quantile was not significant from zero either ($P=0.21$). However, the fifth quantile (lower line, Fig. 1) had a slope significantly different from zero ($P=0.03$; slope = -0.52); that is, our data cloud is triangular. There are species with high physical and chemical defence levels or high levels of either chemical or physical defence, but there is a lack of species with low levels of both chemical and physical defence.

Pairwise correlations between defence traits

Five of the 45 pairwise correlations between defence traits were statistically significant after applying a sequential Bonferroni (Rice, 1989). Three of these were consistent with tradeoffs between defences: a negative relationship between lipid content and ash content ($P<0.001$, $R^2=0.07$), a negative relationship between ash content and PEG-binding capacity ($P<0.001$, $R^2=0.17$), and a positive correlation between SLA and ash content ($P<0.001$, $R^2=0.19$; low SLA is related to higher toughness). There was a positive relationship between ash content and the likelihood of a species having hair ($P<0.001$, $R^2=0.05$), and species with extrafloral nectaries were disproportionately likely to have spines ($P<0.001$, $R^2=0.06$). A further six relationships had P -values <0.05 , but did not retain significance after sequential Bonferroni (Fig. 2).

The results corrected for phylogeny were qualitatively similar to the results of cross-species analyses in all but one case (Table S3). The exception was the relationship between lipid content and ash content, which was significant in cross-species ($P<0.001$) but not in phylogenetic analyses ($P=0.59$). In this case, the significant cross-species relationship is most likely driven by one or more divergences deep in the phylogeny. In the other cases, our results show that the cross-species results are neither obscured nor artificially strengthened by phylogenetic relationships among the study species.

Finally, we used categorical principal component analysis (PCA) to investigate multivariate relationships among our variables (Fig. 3a). The first axis explained 19.47% of the variation and separates species with high PEG-binding ability from species with

high values of ash and SLA. The second axis separates species with spines from those with high lipid content (14.07% of variation). Extrafloral nectaries were better correlated with axis 3 (12.72% of variation), succulent leaves and latex were correlated with axis 4 (10.15%), and cyanide was correlated with axis 5 (10.02%).

Evidence for defence syndromes

The low proportion of significant pairwise correlations between traits is not what we would predict if these traits fell into tightly coordinated syndromes. Consistent with this, arraying species in the multivariate space defined by the categorical PCA did not reveal any obvious clusters (Fig. 3b). We also applied hierarchical cluster analysis, a technique that has been previously used to argue for the existence of defence syndromes in plants (Agrawal & Fishbein, 2006). The phenogram for our data did not support the idea that our species were clustered in distinct groups (Fig. 4), and similarity profile analyses showed no evidence that the profile of between-species similarities differed from that expected under the null hypothesis of no structure (SIMPROF, $\pi=0.013$, $P=0.26$) – that is, our study species do not display coordinated defence syndromes.

Discussion

Across a taxonomically and geographically broad sample of plant species we found no evidence of a tradeoff between the overall chemical and physical defences against herbivores, and few negative relationships between pairs of defence traits; and no evidence for coordinated defence syndromes. Thus, it appears that while there can be correlations between pairs of traits, or syndromes of defence among closely related species (Agrawal & Fishbein, 2006; Travers-Martin & Muller, 2008), in general, plants employ a range of different combinations of defences against their herbivores.

Three of the 45 pairwise comparisons in our data revealed significant tradeoffs between defence traits. These results are broadly consistent with the findings of previous studies spanning multiple defences. Three of the 21 pairwise correlations among seven defence traits in 24 milkweeds (*Asclepias*) were statistically significant, with none of these being tradeoffs (Agrawal & Fishbein, 2006). There were negative relationships between phenolics and two traits, spines and leaf thickness; positive relationships between phenolics, SLA and leaf density, but no significant relationships between spines and SLA, leaf density or leaf thickness in seedlings of 14 species of *Hakea* (Hanley & Lamont, 2002). There were no significant correlations between alkaloids, mechanical defences and nectaries across 19 species of *Ipomoea* (Steward & Keeler, 1988). There was no correlation between indices of extrafloral nectaries and glands or trichomes across 31 species of the Gossypieae, but there was a negative relationship between glands and trichomes (Rudgers *et al.*, 2004). Finally, the only significant cross-species relationship from the 15 pairwise correlations between toughness, alkaloids, terpenoids, tannins, latex and trichomes across 61 species from a cerrado community was a negative relationship between trichomes and latex (da Silva & Batalha,

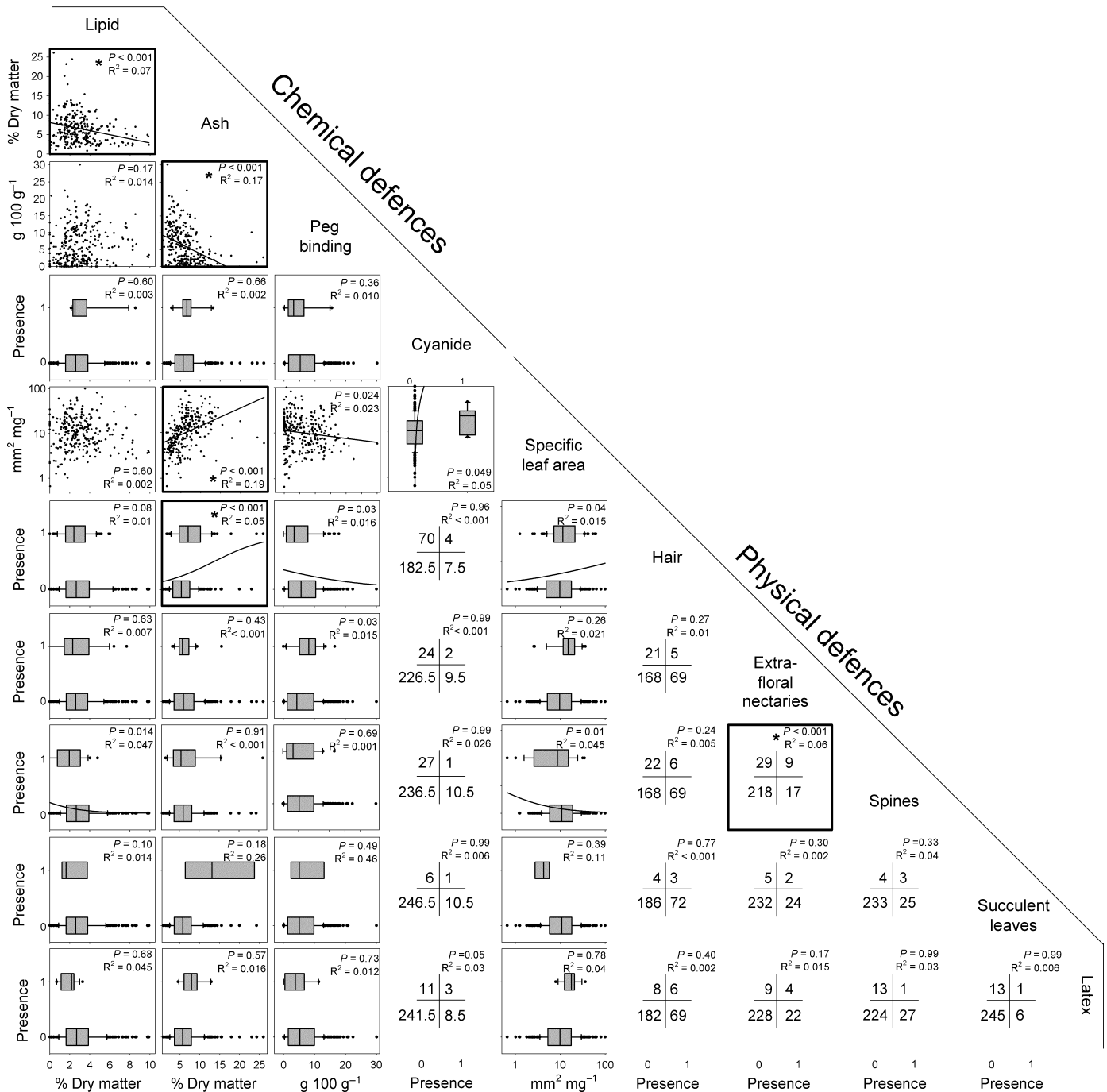


Fig. 2 Pairwise correlations between the four chemical and six physical traits. P -values and R^2 -values are from mixed-effects models including a random effect for site (see the Materials and Methods section for details). These were cross-species analyses, where each species was given a total weight of 1. The five correlations that remained significant after applying sequential Bonferroni are marked with asterisks and are surrounded by heavy boxes.

2011). Across these studies, there is a low incidence of significant correlations, and an even lower incidence of tradeoffs.

The low incidence of tradeoffs might be partly explained by the fact that it would not be possible for every trait in a study of more than two traits to be negatively related to all other traits, because if traits a and b are both negatively related to trait c, then traits a and b will be positively correlated. In addition, the theory that predicts tradeoffs between defences is about allocation of resources, rather than about levels of expression of traits. This

study, like the vast majority of the previous studies, measured expression of defence traits (which is biologically relevant, because this is what the herbivores encounter). Calculating the costs of producing and maintaining traits that use different currencies (e.g. carbon, silicon, nitrogen), and that have functions in addition to their roles in defence is well beyond what we can do here. However, addressing the mismatch between theory (allocation) and data (expression) is an important direction for the future. Another question for the future is whether traits that

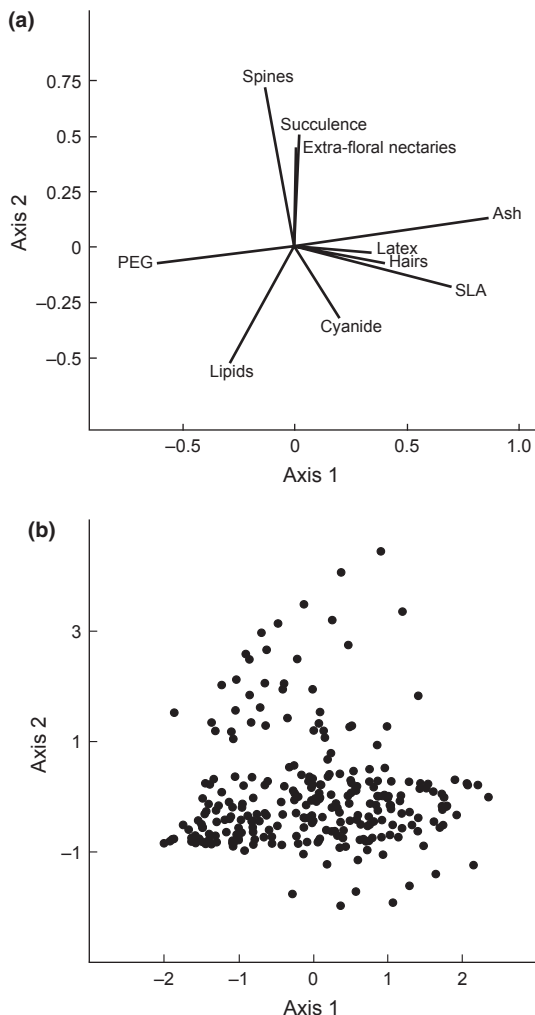


Fig. 3 (a) Biplot of the factor loadings for traits on the first two axes of the categorical principal component analysis (PCA), (b) biplot of species coordinates arrayed on the first two axes of the categorical PCA. PEG, polyethylene glycol; SLA, specific leaf area.

are built using the same resource (e.g. nitrogen-based defences or carbon-based defences) are more likely to display tradeoffs than are traits that use different resources (though this particular tradeoff could also appear as a result of a tradeoff between qualitative and quantitative defence). Finally, many innate and inducible chemical defences protect plants from biotrophic fungal, bacterial and viral microbes (Dangl & Jones, 2001; Dodds & Rathjen, 2010) as well as herbivores. If chemical defences are primarily

aimed at microbes, whereas physical defences primarily deter larger herbivores, these defence types would likely reflect the local composition of the herbivore and microbe communities, and would not necessarily occur as a tradeoff.

A broad comparative study such as ours necessarily incorporates variation from a wide range of sources, including habitat and abiotic pressures, the identity and abundance of herbivores, and characteristics of the species, such as life form and evolutionary history. We have attempted to control for some of these factors in this study, but many sources of variation remain. Significant relationships will be found only if they are strong enough to be detected against this background noise. In light of this, the five significant relationships between defence traits that we did detect are both strong and likely to apply across a wide range of ecosystems and species.

We have become accustomed to finding constellations of inter-related traits in comparative ecology. For instance, traits such as seed mass, plant size, time to maturity, longevity and wood density are related to seedling survival, seed production, relative growth rate and dispersal distance, and together characterize a species' life-history strategy (Weiher *et al.*, 1999; van Gelder *et al.*, 2006; Moles & Westoby, 2006). Traits such as SLA, leaf lifespan, leaf nitrogen and photosynthetic rate form the leaf economics spectrum (Wright *et al.*, 2004). However, defence traits are clearly different. It is well established that different defences deter different types of herbivores and that defences can act synergistically to reduce damage (Agrawal, 2007). Perhaps having a different combination of defences from coexisting plant species is also advantageous. If so, selection for unusual combinations of traits could contribute to the very weak correlations between different defences at the cross-species level. This question would be best addressed using data for a large number of defence traits across the suite of coexisting species within a community. It would also be interesting to compare the region of trait space occupied by the species within a community with that occupied by the species in this global study. In other words, are certain traits or values of traits absent at local scales because they are not adaptive (e.g. because certain types of herbivores are not present or because of environmental factors) or does selection for unusual combinations of traits maintain high degrees of variation among coexisting species?

Many of the traits we measured have functions in addition to their roles in deterring herbivores. For instance, scleromorphy protects leaves from damage from the abiotic environment, as well as from herbivores (Turner, 1994). The conflicting tradeoffs

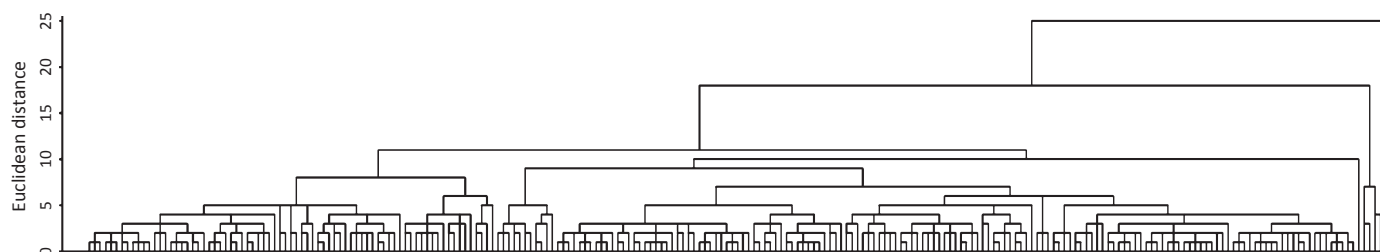


Fig. 4 Phenogram from hierarchical cluster analysis. Each branch at the bottom of the phenogram represents one species. Similarity profile analysis did not support the hypothesis that the structure of this tree was different from that expected under the null hypothesis of no structure ($P = 0.26$).

involved with the differing roles of the traits we measured might reduce the likelihood of plants displaying clear defence syndromes. However, this confounding reduces the chances of syndromes existing – it does not bias our analyses against finding syndromes if they are present. The argument that a high degree of allocation to one trait means that a plant will have fewer resources available for a second trait is also robust to the traits having multiple roles, so on this logic we would still expect to see tradeoffs between traits. However, the benefit of having a second function could make allocation to a second trait more advantageous than if the second trait acted solely as a defence. This would reduce our chances of finding tradeoffs among traits. Nonetheless, if tradeoffs between defence traits are weak enough that the vast majority are obscured by other functions, then it seems that tradeoffs among defences are not a major priority for plants in resource allocation.

Three results stand out from the pairwise analyses of correlations between defences. First, only one correlation involving SLA retained significance after sequential Bonferroni. Thus, although SLA is an indicator of leaf toughness (a highly effective plant defence), and also a central component of the leaf economics spectrum, it is a surprisingly poor predictor of a species' suite of chemical and physical defences. Secondly, four of the five significant pairwise relationships were between ash content and another defence. This suggests that phytoliths and calcium oxalates (traits that have often been overlooked in the defence literature) might form an important component of a plant's defence strategy. The negative relationship we found between ash content and PEG-binding capacity is consistent with Cooke & Leishman's (2012) recent demonstration of a negative relationship between silica content and total phenols. Thirdly, the only relationship between extrafloral nectaries and other defences was a positive association with spines. This relationship is largely the result of four species of *Acacia* and one *Caesalpinia* that possessed both spines and EFNs (extrafloral nectaries), though there were also species from the Rosaceae, Euphorbiaceae and Capparaeaceae that possessed both defences. Our finding does not support Janzen's (1966) hypothesis that species with biotic defence, such as ants, should have lower chemical or physical defences. Our results are in line with a recent meta-analysis, which showed that while species with food bodies and domatia had significantly lower direct defences, there was no effect of extrafloral nectaries on direct defences (Koricheva & Romero, 2012).

A novel finding from our analysis of the relationship between species' physical and chemical defences was the scarcity of species that had both low chemical defence and low physical defence. Most of our species had high chemical and physical defence levels, or high levels of either chemical or physical defence. It is possible that the lack of undefended plants is an artefact resulting from our selection of the four most abundant species in each ecosystem. This would occur if very rare species escaped from herbivores by being inconspicuous, rather than through defences (Feeny, 1976). However, our species had leaf area indices ranging from 0.002 to 3.92 m² m⁻², and represented between 0.3 and 88% of the total leaf area at the study sites, and neither absolute nor relative cover explained a significant proportion of the

variation when included in analyses of physical vs chemical defence (absolute cover, $P=0.82$; relative cover, $P=0.27$). Thus, we think it is more likely that our finding results from our inclusion of an unprecedented range of defence traits. There are many different ways for a plant to defend itself against herbivores, and although plants do not all invest in the same types of defences, most species invest in at least some types of defence. The existence of a suite of species that escape from herbivores through rapid growth and being inconspicuous is widely accepted (Feeny, 1976; Hay & Fenical, 1988; Kricher, 2011). However, a negative relationship between how conspicuous a plant is and defence does not mean that inconspicuous species are not defended. In fact, it is quite a challenge to think of plant species that do not have any form of defence against herbivores.

Our study asks whether there is evidence for tradeoffs or defence syndromes across a wide range of species and environments. It remains the case that there might be tradeoffs or syndromes at other levels of organization, such as within species, within genera or across coexisting species within habitats. If relationships do occur at lower taxonomic levels, or within particular communities, it will be interesting to ask whether these results are simply lost in the unavoidable noise associated with a global sample, or whether other ecological and evolutionary processes mask the relationships. It could also be that the tradeoff is not between particular types of defence, but rather between overall allocation to defences (both physical and chemical together) and nondefence factors such as growth and reproduction.

Our conclusions are limited to the most abundant species at each site, and to the set of physical and chemical traits we measured. It would be interesting to do a similar study on rare species, and incorporating additional traits would give a more comprehensive understanding of tradeoffs and correlations among plant defence traits. Our first suggestion would be to incorporate additional nitrogen-based defences.

There is still much to be learned about plant defences. In the near future, we plan to use our data to test hypotheses about factors such as the nutritional content of the foliage, apparency, habitat quality and climatic conditions that might drive selection for plant defences. Many of these hypotheses will be addressed using an aggregated index of defence, rather than data for individual defences, and it will be important to consider allocation to defence as part of a plant's ecological strategy rather than considering defences in isolation. However, these are only parts of the picture. Ultimately, we would like to provide an integrated analysis of the complex intercorrelations among plant defences, environmental conditions, herbivores and herbivory. For now, we have shown that across a broad sample of abundant plant species, the set of common defence traits we measured were neither grouped in syndromes nor arrayed as a series of tradeoffs. That is, our results are contrary to both of the major ideas in the literature.

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Supporting Information

Additional supporting information may be found in the online version of this article.

Table S1 Information about study sites, including sampling dates and species list

Table S2 Taxonomic composition of our dataset

Table S3 Results of phylogenetic analyses

Methods S1 An explanation of our use of latent variable modelling.

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