

Putting plant resistance traits on the map: a test of the idea that plants are better defended at lower latitudes

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Summary

- It has long been believed that plant species from the tropics have higher levels of traits associated with resistance to herbivores than do species from higher latitudes. A meta-analysis recently showed that the published literature does not support this theory. However, the idea has never been tested using data gathered with consistent methods from a wide range of latitudes.
- We quantified the relationship between latitude and a broad range of chemical and physical traits across 301 species from 75 sites world-wide.
- Six putative resistance traits, including tannins, the concentration of lipids (an indicator of oils, waxes and resins), and leaf toughness were greater in high-latitude species. Six traits, including cyanide production and the presence of spines, were unrelated to latitude. Only ash content (an indicator of inorganic substances such as calcium oxalates and phytoliths) and the properties of species with delayed greening were higher in the tropics.
- Our results do not support the hypothesis that tropical plants have higher levels of resistance traits than do plants from higher latitudes. If anything, plants have higher resistance toward the poles. The greater resistance traits of high-latitude species might be explained by the greater cost of losing a given amount of leaf tissue in low-productivity environments.

Introduction

The idea that there is a latitudinal gradient in traits associated with resistance to herbivores, where plants from low latitudes are more strongly defended against herbivores than are plants from high latitudes, is very widely accepted (Schemske *et al.*, 2009). The dominant theory is that low-latitude species experience more intense herbivory than do species at higher latitudes, and have thus been under selective pressure to evolve higher levels of resistance traits (Dobzhansky, 1950; MacArthur, 1972; Coley & Aide, 1991; Coley & Barone, 1996; Van Alstyne *et al.*, 2001). These ideas are central to our understanding of global patterns in species richness and the factors that shape latitudinal gradients in plant traits.

Some studies have provided support for the idea that plants from lower latitudes have higher levels of traits that are thought to confer resistance to herbivores. For example, studies have reported latitudinal gradients in the proportion of species that contain alkaloids (Levin, 1976; Levin & York, 1978), latex (Lewinsohn, 1991), and extrafloral nectaries (Pemberton, 1998), and others have shown latitudinal gradients in leaf toughness and tannin and total phenolic concentrations both within (Siska *et al.*, 2002) and across species (Coley & Aide, 1991; Hallam & Read, 2006). However, many studies do not show higher levels of resistance traits at lower latitudes, either within species (Lesage *et al.*, 2000; O'Neill *et al.*, 2002; Gaston *et al.*, 2004; Stark *et al.*, 2008; Adams *et al.*, 2009; Martz *et al.*, 2009) or across species (Ardon *et al.*, 2009; Graca & Cressa, 2010; Steinbauer, 2010). A recent review found that

only nine of the 56 latitudinal comparisons of plant chemical traits available in the literature showed higher levels of resistance traits at lower latitudes (Moles *et al.*, 2011). Meta-analysis showed that the average effect size from these studies was indeed significantly different from zero, but in the opposite direction from that predicted by much of the literature. On average, chemical resistance traits were *c.* 40% higher levels at the high-latitude end of the comparisons (Moles *et al.*, 2011). A review of the literature also failed to support the idea that physical resistance traits would be greater in lower latitude plants. Only 13 of the 25 latitudinal comparisons of physical resistance traits found higher levels of resistance at lower latitudes, and meta-analysis showed no significant difference between the average effect size and zero (Moles *et al.*, 2011). Of course, the result of this meta-analysis does not invalidate any of the studies that have shown latitudinal gradients in resistance traits – it simply provides an estimate of the combined weight of evidence on the question of whether there is a latitudinal gradient in traits associated with resistance to herbivores.

On the surface, it appears that we should immediately reject the idea that plants at low latitudes have higher levels of traits that confer resistance to herbivores. However, Moles *et al.* (2011) caution that the data on which their analysis is based are far from perfect, with most studies contrasting sites in just two or three regions rather than from a range of latitudes, applying imperfect or inconsistent methods, spanning only a fraction of the latitudinal gradient and/or including relatively small numbers of species. To provide a conclusive answer to the question of whether

there is a general latitudinal gradient in plant resistance traits, we need a study that applies consistent methods to a range of species from sites spanning as much of the latitudinal gradient as possible. The main aim of the present paper was to report on such a study.

We quantified a broad array of physical and chemical traits that are thought to affect vulnerability to herbivores (putative resistance traits), across 301 species from 216 genera and 85 families. These were the four most abundant species (in terms of leaf area displayed) from each of 75 relatively natural sites from all around the world, including rainforests in the Republic of Congo, Panama, China and Mexico, deserts in Israel, Australia and the USA, tundra in Greenland and Alaska, savannas in Zambia, South Africa and Australia, and temperate forests in Sweden, the USA and Australia (Fig. 1). Our aim was to determine whether plants from low latitudes have higher levels of resistance traits than do plants from high latitudes.

Materials and Methods

Site location and species selection

We worked at 75 study sites, distributed from 74.5°N to 51.5°S (Fig. 1; Supporting Information Table S1). Sites were selected to sample the dominant vegetation types at a wide range of latitudes. Details of site selection are given in Methods S1, and a list of sites is presented in Table S1, but the primary criterion was that the levels of herbivory, disturbance regime and plant community composition should be relatively natural (i.e. as close as possible to those with which the plant traits we are measuring are thought to have evolved). At each site, we sampled the four most abundant species (exceptions are outlined in Methods S1).

Leaf sampling

We sampled fully expanded photosynthetic units for each species (usually leaves or leaflets, but occasionally photosynthetic stems or phyllodes). All of these photosynthetic units are henceforth referred to as 'leaves', for convenience. Leaves from at least five mature, outwardly healthy individuals of each study species were sampled as close to the peak growing season as possible. Full details of leaf selection are given in Methods S1.

A total of at least 40 g of fresh leaves was collected from at least five individuals of each species, and placed in paper bags ready for oven-drying, and 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 they could be measured.

Leaf traits

We did not investigate the precise nature of the chemistry for each species, or try to account for the feeding preferences of herbivores in different parts of the world. Instead, we tried to select a wide range of broad physical and chemical traits that would capture as much information about the species' likely vulnerability to herbivores as possible. These traits are referred to as 'resistance traits' for convenience throughout the paper. These traits are among the most commonly identified traits involved in resistance, and it is generally believed that these traits increase plant fitness in the presence of herbivores (but see Carmona *et al.*, 2011). Most of these traits have multiple functions, and, while our correlative approach shows global patterns in traits, it cannot resolve the causes of latitudinal gradients. However, we see obtaining a firm quantification of the global patterns as an important first step in understanding global patterns

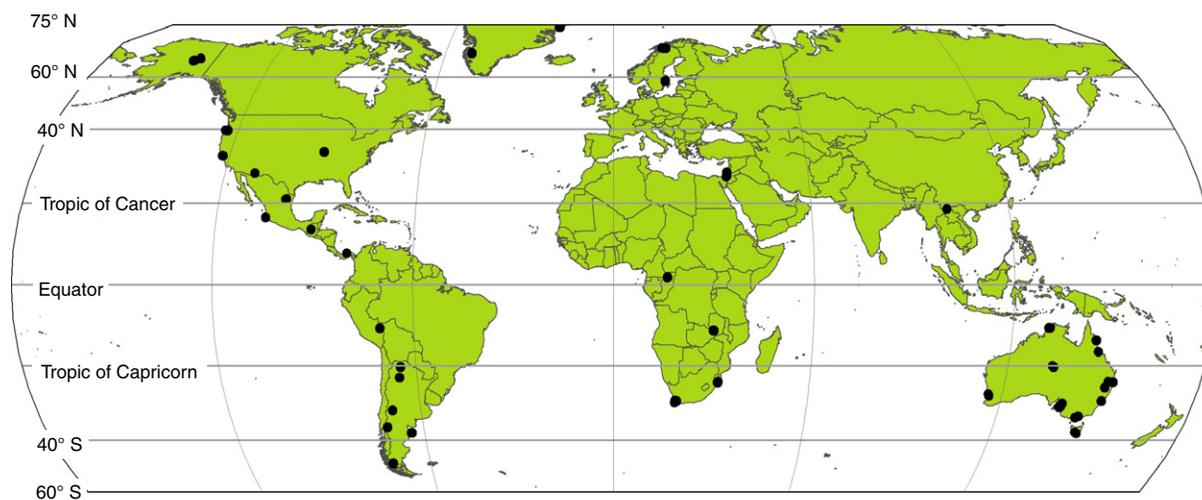


Fig. 1 World map, showing study site locations. Some points represent more than one site (this occurs where two or more different ecosystems were close in geographic space).

in plant–animal interactions. A rationale for the inclusion of each trait is given in the following sections, along with an explanation of our measurement techniques.

Leaf toughness and size We began by measuring physical toughness, an extremely important form of resistance against herbivores (Choong *et al.*, 1992; Turner, 1994; Hanley *et al.*, 2007; Clissold *et al.*, 2009). We used the leaf slicing machine designed by Wright & Cannon (2001) to measure the force required to push a blade through 10 fresh leaves (mean force of fracture (N); a detailed description is given in Wright and Cannon, 2001). Our data set for force of fracture is smaller than for other traits, because of the difficulty of transporting the instrument to the different sites to use on fresh leaf material. We therefore complemented the fracture data with measures of specific leaf area (SLA), which is negatively related to force of fracture (standardized major axis slope = -0.76 ; $R^2 = 0.53$). We used a flat-bed scanner to scan 15 fresh leaves, with a ruler or grid for scale. Images were analyzed using IMAGE-J (National Institute of Health, Bethesda, MD, USA) to calculate leaf area. These leaves were dried at 55 – 65°C (the vast majority at 55°C), for 24–48 h, and weighed for dry mass. Specific leaf area (SLA) was calculated by dividing mean leaf area by mean dry mass.

Although leaf size is not a resistance trait *per se*, it does affect the vulnerability of the leaf to herbivores. Larger leaves have been shown to experience greater degrees of herbivory within a habitat (Moles & Westoby, 2000), probably because of their greater apparency to herbivores (Feeny, 1976), and/or because of their greater expansion times (Moles & Westoby, 2000).

For the remaining traits, we used dried, ground leaves. Samples were oven-dried at temperatures between 55 and 65°C (the vast majority at 55°C). With very few exceptions, samples were placed in a drying oven within 6 h of collection. No method of drying leaf tissue is optimal and every method (including freeze-drying and prolonged air-drying) may change plant chemistry. We chose oven-drying at low temperatures as a reasonable compromise between speed of dehydration (and so reduction in plant enzyme activity) and ability to be performed consistently over all sites. A key measure in our study was polyethylene glycol (PEG)-binding capacity, a measure of tannin activity, and this measure has been found not to differ between oven-dried and freeze-dried material (Silanikove *et al.* 1996).

Dry samples were packed in plastic sample vials, and transported to Sydney, Australia. Samples from outside Australia were gamma-irradiated at 50 kGray, according to quarantine requirements (this does not affect the gross chemical composition of the samples, except by rendering them biologically sterile). All samples were ground to pass a 1-mm sieve on an Udy Cyclone Sample Mill (Udy Corporation, Fort Collins, CO, USA), and stored refriger-

ated in the dark until analyses could be performed. All chemical analyses except that of ash content were performed in duplicate. Analyses of any pair of samples whose values were too different (usually a $\text{CV} > 5\%$) were repeated in duplicate.

Cyanogenic glycosides The ability to release hydrogen cyanide in response to cell damage is a widespread resistance trait that is known to be effective against a wide range of invertebrate and vertebrate herbivores (Ballhorn *et al.*, 2010). To test for cyanogenesis, we added $150\ \mu\text{l}$ of phosphate buffer (pH 5.0; 0.1 M) to $0.20\ \text{g}$ ($\pm 0.01\ \text{g}$) of dried, ground leaf in a sealed Vacutainer (Becton-Dickinson, Franklin Lakes, NJ, USA) with a strip of Feigl-Anger paper (which turns blue in the presence of cyanogenic glycosides; Feigl & Anger, 1966) suspended from the top. If no activity was observed within 24 h, the assays were stopped (because of the possibility of false results from cyanogenic bacteria). New assays were initiated for all species that showed a negative response, in which $1.12\ \text{units}\ \text{ml}^{-1}$ β -glucosidase from almond (*Prunus dulcis*) (Sigma-Aldrich) was added to the buffer to determine whether each species could release cyanide if enzymes were supplied from a source other than the plant (for example, if a glucosidase was available during digestion inside a herbivore). This method is the same as that used by Marsh *et al.* (2007). Eleven species responded positively in the presence of the glucosidase, while nine species responded positively with buffer only. We report the results from the trials with glucosidase throughout.

We used near-infrared reflectance spectroscopy (NIRS) to predict some values for the remaining chemical traits (Foley *et al.*, 1998). We followed the standard procedures recommended by the American Society for Testing and Materials for all quantitative NIRS analyses (A.S.T.M., 1995; Foley *et al.*, 1998). In brief, after collecting duplicate spectra on a FOSS NIR-Systems 6500 spectrophotometer (FOSS, Hillerød, Denmark), we selected 100 species based on their spectral variability. We conducted full chemical analyses on these samples and then developed partial least squares regression models to relate chemical and spectral variation. After validation, we used these models to predict the chemical composition of unmeasured samples from their spectra. Details of these models can be found in Methods S1 and Table S2.

Lipids We measured total lipid content, to quantify resistance traits such as oils (including terpenes), cuticular waxes, and resins. Several studies have shown that these traits deter or otherwise negatively affect herbivores, probably through a combination of physical and chemical effects (Lincoln, 1985; Peeters, 2002; Jones *et al.*, 2003; Marko *et al.*, 2008). For instance, cuticle thickness was negatively correlated with densities of a range of invertebrate herbivores, including sessile phloem feeders, rostrum chewers, and

external chewers (Peeters, 2002). Juniper (*Juniperus Communis*) individuals with higher concentrations of essential oils are less damaged by herbivores (Marko *et al.*, 2008), and kangaroos avoid plant species with volatile essential oils (Jones *et al.*, 2003). Finally, the growth of butterfly larvae was inhibited by high concentrations of leaf resin in *Diplacus aurantiaeus* (Lincoln, 1985).

To measure lipid content, we added 10 ml of petroleum spirit (bp 40–60°C; also known as petroleum ether) to 1 g (\pm 0.01 g) of dried, ground leaf sample, in a pre-weighed 10-ml centrifuge tube. Samples were shaken vigorously, placed in a sonicator for 20 min and then left to stand for 2 h. Previous trials showed that longer periods of mixing and standing did not increase weight loss from the sample. Samples were centrifuged at 4000 g for 5 min and then the lipid extract was decanted quantitatively. The remaining sample was oven-dried at 50–60°C for 5–6 d and reweighed to calculate the lipid loss. Ether-extractable lipids have previously been shown to be negatively correlated with herbivore preferences across a range of herbivores (Bryant & Kuropat, 1980).

Tannins Tannins have been found to deter feeding in a range of herbivores, including mammals, slugs and insects (Furstenburg & Vanhoven, 1994; Fritz *et al.*, 2001; Roslin & Salminen, 2008). In mammals, tannins reduce protein and dry matter digestibility, cause endogenous nitrogen loss, and can cause damage to the gastrointestinal tract, kidney and liver (Shimada, 2006; Spalinger *et al.*, 2010). Tannins have also been shown to reduce metabolic and growth efficiency in invertebrates (Roslin & Salminen, 2008). There is also increasing evidence that the oxidative capacity of tannins might be an important component of their role as resistance traits (Salminen & Karonen, 2011).

We employed PEG-binding capacity, determined using Silanikove *et al.*'s (1996) PEG-binding assay, as a measure of tannins. We chose the PEG-binding assay over more traditional colorimetric assays of phenolics for two reasons. First, it measures the degree to which tannins bind the plant protein. That is, it measures a functional trait that is relevant to animals (particularly mammals), rather than quantifying the concentration of a suite of chemicals that can have varying effects on herbivores. Second, the PEG-binding assay does not rely on extraction of tannins from the plant matrix and their subsequent quantification using external standards. These two issues are major limitations of most tannin analyses and preclude comparison of data for widely differing taxa because different tannins produce different chromophores at similar concentrations, making interpretation of colorimetric methods problematic across different species without detailed knowledge of their chemistry (Mueller-Harvey, 2006).

PEG-binding capacity was measured by mixing 0.50 g of dried ground leaf with 7.5 ml of a solution containing

33.33 g l⁻¹ PEG 4000 spiked with [¹⁴C]-labeled PEG 4000. Samples were incubated for 24 h and centrifuged, and 75 μ l of supernatant was combined with 10 ml of scintillant and counted (as in DeGabriel *et al.* 2008).

Nitrogen digestibility, carbon (C) : nitrogen (N) ratio and N fixation Leaves with low N content and/or high C : N ratios are generally considered lower quality food for herbivores (Mattson, 1980; Schmitz, 2008). Although N content is not a resistance trait, we consider it here because herbivores have been shown to balance N intake against the defensive properties of their food (Bryant & Kuropat, 1980; Galimuhtasib *et al.*, 1992). Leaf C and N contents were measured using a LECO C : N : S analyser (LECO, St. Joseph, MI, USA) at the Environmental Analysis Laboratory at Southern Cross University, Lismore, Australia.

We determined *in vitro* N digestibility using the method of DeGabriel *et al.* (2008). The assay involved digesting the samples in porous bags (Ankom F57; Ankom Technology, Macedon, NY, USA), first with pepsin (24 h) and then with cellulase (48 h). We analyzed the residue at the end of the assay to quantify the digestibility of the N (%). We were interested in measuring the effects of tannins on available N, so at the start of the digestion we incubated two bags per sample for 24 h either with 33.3 g l⁻¹ PEG 4000 (in 0.05 M Tris-BASE buffer) or with buffer alone (both 25 ml per sample). We then thoroughly washed the bags before drying them to constant mass at 50°C and weighing them.

We scored each plant for presence/absence of the ability to fix N, based on information from the published literature.

Ash We measured ash content, in order to gain information about resistance traits such as silica-based phytoliths and calcium oxalates. Calcium oxalate is present 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). Silica is also widespread and abundant in plant tissues, especially in grasses, which are typically 2–5% silica (Massey *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). These minerals increase the abrasiveness of leaf material, and reduce herbivore growth rates and digestion efficiency (Korth *et al.*, 2006; Massey *et al.*, 2006). There was a highly significant positive correlation ($P = 0.001$; $R^2 = 0.50$) between log₁₀ ash content and log₁₀ silica content across 27 terrestrial species (data analyzed were from Lanning & Eleuterius, 1985).

To calculate ash content, 1.00 g of dry sample was combusted at 600°C for 12 h before weighing the residual ash.

Other traits We used observation in the field, published records and/or local knowledge to score each species for possession of a range of additional traits: delayed greening, latex, hair on adult and/or juvenile leaves, extrafloral nectaries, and spines. Each of these traits is thought to reduce damage by herbivores. Delayed greening (possession of red, white or pink young leaves that have low chlorophyll content) is associated with a low N content, which may reduce the attractiveness of young foliage to herbivores and reduce the cost of herbivory on juvenile leaves (Kursar & Coley, 1992). Latex is present in over 20 000 plant species, and several lines of evidence suggest that it acts to deter chewing herbivores, particularly invertebrates (Agrawal & Konno, 2009). The presence of hair on leaves is thought to make it more difficult for invertebrates to access leaf tissue, and has been shown to decrease losses to herbivores (Moles & Westoby, 2000; Hanley *et al.*, 2007). Extrafloral nectaries are found 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). Spines (including thorns) have been shown to be an effective form of resistance against a range of herbivores, particularly mammals (Hanley *et al.*, 2007).

Statistics

Variables were \log_{10} -transformed as appropriate. We used linear mixed-effects models (Venables & Ripley, 2002) for each of the traits described in the preceding sections, with a fixed-effect term for latitude and a random effect term for site. We included an interaction term for hemisphere in the analyses, to allow for different relationships in the Northern and Southern Hemispheres. The random-effects term for site was included to account for site-to-site variation in leaf traits that was not explained by latitude. This term also allowed us to determine what percentage of the unexplained variation lay within vs across sites. Models were fitted using restricted maximum likelihood via the R package lme4 (Bates *et al.*, 2008). With data for multiple sites, R^2 for a term in the model (fixed or random) was calculated by the usual method (sequential reduction in residual sum of squares on addition of the term), but adding fixed-effects terms to the model before the random-effects term. Binary variables (such as presence or absence of spines) were analyzed using logistic regression with a random site term via lme4 (Bates *et al.*, 2008).

We also quantified the relationships between species' cover (absolute and relative) at each site and each of the resistance variables, to determine the extent to which our selection of the four most abundant species might have affected our results. Absolute cover values are estimates of the leaf area index for each species in a series of three to seven quadrats, while relative cover divides the cover of the

focal species by the total cover in the quadrats. Both cover metrics were \log_{10} -transformed before analysis for normality, and trait variables were log-transformed where appropriate, as for previous analyses. Continuous traits were analyzed with ordinary regressions, while binary traits were analyzed with logistic regressions, both in PASW 18 (formerly SPSS, IBM Corporation, Somers, NY, USA).

Results

The PEG-binding capacity (a measure of tannins) of species in the tropics was lower than that of species at high latitudes ($P = 0.047$; $R^2 = 0.02$; Fig. 2a). Species found $> 60^\circ$ from the equator had a mean PEG-binding capacity 1.6 times higher than that of species growing in the tropics (7.5 vs 4.7 g 100 g⁻¹, respectively). The highest PEG-binding capacity was found in *Eucalyptus miniata*, from Darwin, Australia (30 g 100 g⁻¹), followed by *Crassula rupestris* from near Stellenbosch, South Africa (22 g 100 g⁻¹), and *Pistacio lentiscus* from Israel (21 g 100 g⁻¹).

Our analyses of *in vitro* N digestibility support the idea that tannins are decreasing the availability of N to herbivores at high-latitude sites. There was no latitudinal gradient in N digestibility in the absence of PEG ($P = 0.17$), but when PEG (which binds to tannins, rendering them inactive) was added to the samples before digestion, high-latitude species had significantly higher N digestibility ($P = 0.04$; $R^2 = 0.02$; Fig. 2b).

There was a striking latitudinal gradient in lipid concentration ($R^2 = 0.21$; Fig. 2c). Species found $> 60^\circ$ from the equator had a mean lipid concentration more than twice as high as that of species growing in the tropics (5% vs 2.1%). The highest lipid concentration in our samples was 9.9% in *Picea mariana* from Alaska, followed by three species of *Eucalyptus* from near Adelaide in Australia that ranged from 9.8 to 8.7% lipid. One possible explanation for the higher lipid concentrations at high latitudes is that the lipids might protect cells from freezing damage. Plants from colder climates tend to have higher unsaturation of lipids (Badea & Basu, 2009), but there has been little focus on the relationship between total lipid concentration and cold tolerance.

Ash content was significantly higher in tropical species ($P = 0.014$; $R^2 = 0.03$; Fig. 2d). We wondered whether the higher ash content in tropical species might result from the inclusion of several grass species from tropical savannas, as species in the *Poales* are known to have high silicon content (Hodson *et al.*, 2005). We therefore added a term for grass/nongrass to the analysis. There was no significant interaction between this term and latitude ($P = 0.42$), indicating that the slope of the relationship was not significantly different between grasses and nongrasses. However, as expected, grasses did have a significantly higher intercept than did nongrasses ($P = 0.003$).

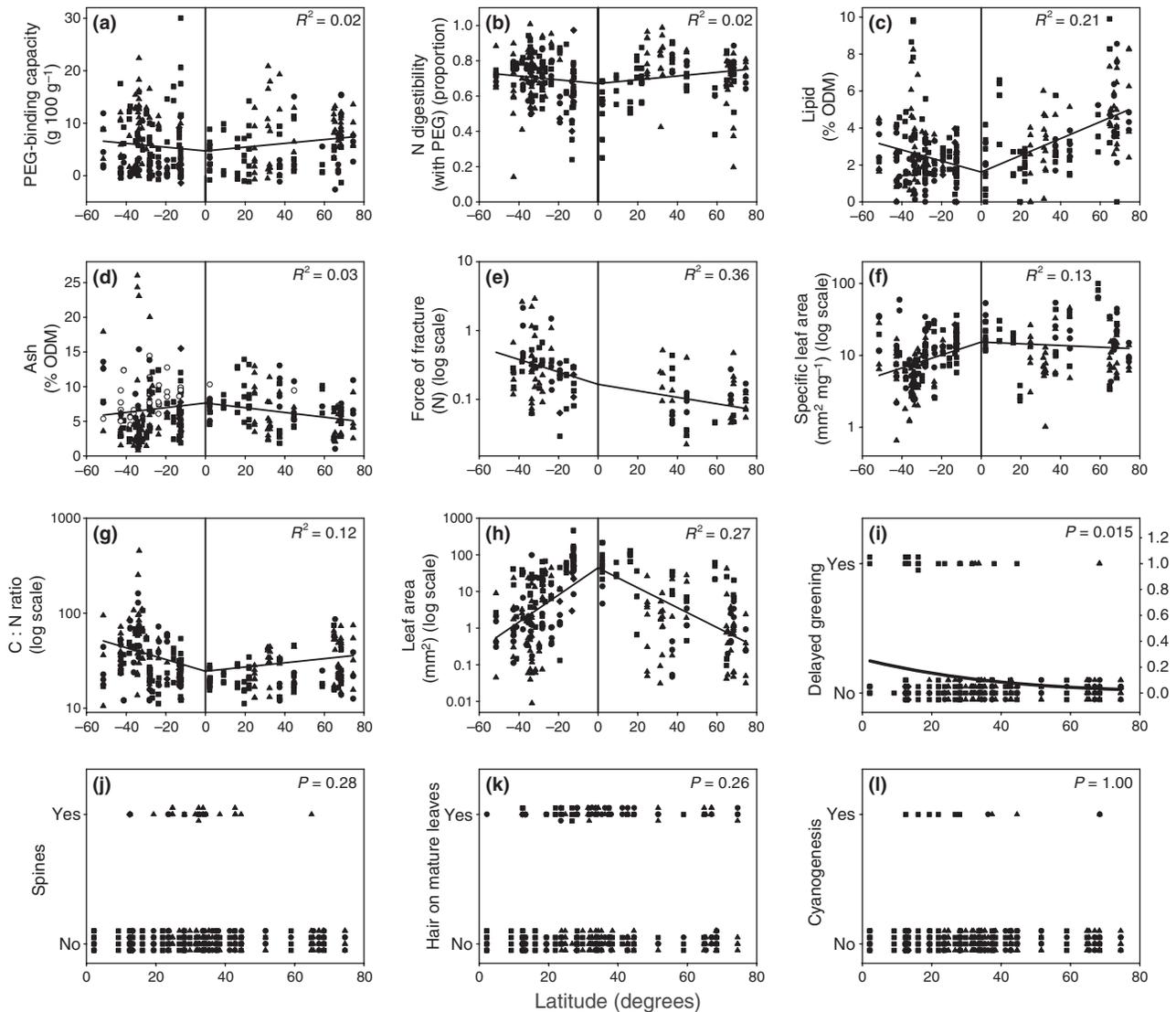


Fig. 2 Relationships between latitude and resistance traits. (a) Polyethylene glycol-binding capacity (a measure of tannin), (b) N digestibility in the presence of polyethylene glycol, which renders tannin inactive, (c) Lipid concentration, (d) Ash content, (e) Force of fracture, (f) Specific leaf area, (g) C : N ratio, (h) Leaf area, (i) Delayed greening, (j) Possession of spines, (k) Presence of hair on juvenile leaves. Each point represents one species at a site. Symbols represent growth forms: trees, squares; shrubs, triangles; herbs (including grasses), circles; climbers, diamonds. In (d), grasses are shown as open circles while all other taxa are closed. Relationships for continuous traits are from linear mixed-effects models, and are plotted with Northern and Southern Hemispheres separately (Northern Hemisphere, positive latitudes; Southern Hemisphere, negative latitudes). Relationships for binary traits are from random-effects logistic regressions. The line in (i) shows the probability of a species from a given latitude possessing delayed greening (scale on right y-axis). As there was no significant difference in the slope of the relationship between the two hemispheres for any binary trait, these relationships are plotted with absolute latitude (distance from equator) on the x-axis. ODM, over dry matter.

Leaf toughness, measured as force of fracture, decreased toward the pole in the Northern Hemisphere, but increased toward the pole in the Southern Hemisphere ($R^2 = 0.36$; Fig. 2e). There was no relationship between SLA and latitude in the Northern Hemisphere ($P = 0.74$), but there was a significant relationship in the Southern Hemisphere, with SLA increasing toward the equator ($P < 0.001$; $R^2 = 0.10$; Fig. 2f; the SLA at 60°S ($4.44 \text{ mm}^2 \text{ mg}^{-1}$) was only 29% that at the equator ($15.3 \text{ mm}^2 \text{ mg}^{-1}$). Taking force of frac-

ture and SLA together, tropical leaves seem to have slightly less physical resistance to herbivores than do leaves from high latitudes. A likely explanation for the difference in SLA and force of fracture between the Northern and Southern Hemispheres is the greater prevalence of deciduous leaves in the Northern Hemisphere, and thus longer leaf lifespans in the Southern Hemisphere.

There was a substantial latitudinal gradient in C : N ratio ($R^2 = 0.12$; Fig. 2g), with species growing $> 60^\circ$ from the

equator having a mean C : N ratio 1.7 times higher than that of species growing in the tropics. This gradient was generated by both lower concentrations of C ($R^2 = 0.07$) and higher concentrations of total N ($R^2 = 0.12$) in tropical leaves. We also found that significantly more of the dominant species in tropical communities are able to fix N ($P = 0.04$; $R^2 = 0.04$). These data, combined with those for tannins and N availability, suggest that tropical species have higher nutritional value, and are thus likely to be more attractive to herbivores.

There was a strong relationship between latitude and leaf area ($R^2 = 0.27$; Fig. 2h), with leaves at the equator averaging 43 times the size of leaves at 60°N. Although the existence of a latitudinal gradient in leaf size has been known for some time (e.g. Webb, 1959), our study is the first global-scale quantification of this important trait.

Delayed greening was present in a significantly higher proportion of species toward the tropics ($P = 0.016$; $R^2 = 0.05$; Fig. 2i). The fact that a high proportion of tropical species have delayed greening has been noted before (Coley & Kursar, 1996; Dominy *et al.*, 2002), but, contrary to some previous reports (Coley & Barone, 1996), delayed greening was not restricted to tropical species. Delayed greening seems mostly to be a mechanism for reducing the amount of N lost to herbivores, rather than a resistance trait *per se*, but this remains one trait whereby tropical species are less vulnerable to herbivores than are their temperate counterparts.

There was no significant relationship between latitude and the presence of spines (Fig. 2j), hairs on either juvenile or adult leaves (Fig. 2k), extrafloral nectaries, or latex (all $P > 0.2$; Table S3). Eleven of the 286 species tested positive for cyanogenic capacity, but this ability was not significantly related to latitude (Fig. 2l). Nonsignificant results for some traits (especially extrafloral nectaries and latex) might be a consequence of the small number of species in our data set that possessed the trait (Table S3).

Data considerations

To determine whether any of the relationships between latitude and resistance traits investigated here were affected by our inclusion of species with different growth forms, we conducted a series of analyses including terms for growth form (tree, shrub, herb (including grasses) or climber). In no case was there a significant interaction between growth form and latitude (all $P > 0.1$), indicating that the slopes of the relationships between resistance traits and latitude were not significantly different among species with different growth forms. In only two cases was the main effect of a growth form term significant ($P < 0.05$). Thus, in most relationships, the intercepts of the lines for species with different growth forms did not differ significantly. The exceptions were that herbs had a significantly lower inter-

cept in the relationship between latitude and C concentration ($P = 0.003$), while shrubs had a significantly lower intercept in the relationship between latitude and \log_{10} leaf area (i.e. they had smaller leaves at a given latitude; $P = 0.02$). Despite these minor differences, it is clear that the relationships between resistance traits and latitude are neither obscured nor artificially strengthened by the inclusion of species with different growth forms.

We quantified the relationship between the resistance traits and the relative and absolute cover of each species, to determine whether our selection of the four most abundant species at each site might have influenced our results (because these abundant species might make up a higher proportion of the total cover at high-latitude sites than at low-latitude sites; Kleidon *et al.*, 2009). Only four out of 34 relationships between resistance traits and cover were significant at $P < 0.05$, and the highest R^2 was 0.031 (Table S4). Thus, the relationships between abundance and plant resistance traits are too weak to be driving our findings regarding the latitudinal gradient in resistance. The significant relationships were a positive relationship between absolute cover and leaf area ($P = 0.008$), negative relationships between relative cover and both lipid content ($P = 0.048$) and specific leaf area ($P = 0.026$), and a positive relationship between relative cover and C : N ratio ($P = 0.021$).

We performed phylogenetic analyses on all of the relationships investigated in this study (Methods S1, Table S5). The phylogenetic and cross-species analyses gave qualitatively similar results for 14 of 17 traits. The three exceptions were relationships that were significant in cross-species analysis but nonsignificant in phylogenetic analysis. Crucially, there was no trait for which cross-species analyses showed no significant relationship between latitude and resistance or significantly higher resistance traits toward the poles where the phylogenetic analysis showed higher resistance traits at lower latitudes. That is, the phylogenetic analyses rule out the possibility that the expected relationship between resistance traits and latitude (higher levels of resistance traits in the tropics) is being obscured by phylogenetic history.

One of the strengths of our global sampling is that we have data from six different continents. However, different trends in the different continents could have obscured global relationships between latitude and resistance traits. We therefore ran models that included a term for continent. Some of the terms for continents were significant (Table S6). However, the overall significance and direction of the relationships between latitude and resistance traits were generally similar between models that included a term for continent and those that analysed data at the cross-continental scale. The exceptions were N fixation and PEG-binding capacity, which moved from being marginally significant ($P = 0.04$ and $P = 0.047$, respectively) to being marginally nonsignificant

($P = 0.08$ and $P = 0.075$, respectively), and leaf area, which became nonsignificant ($P = 0.19$) once a term for continent had been included (Table S6).

The relationships between latitude and plant resistance traits had relatively low R^2 values (mean R^2 for continuous traits = 0.16; max = 0.36; min = 0.02). However, a great deal of the unexplained variation lay at the within-site level (mean R^2 unexplained within sites = 0.45; max = 0.64; min = 0.31). That is, much of the unexplained variation was between coexisting species, and thus could not possibly be explained by latitude. Latitude explained an average of 29% of the between-site variation in plant resistance traits.

Discussion

Our data do not support the idea that plants from low latitudes have higher resistance to herbivores than do high-latitude species. If anything, the trend appears to be for greater resistance traits at higher latitudes. We measured six traits for which high-latitude species have higher levels of resistance than do low-latitude species: PEG-binding capacity, lipid concentration, leaf size, specific leaf area, force of fracture, and C : N ratio. We measured six resistance traits for which there is no significant relationship between resistance traits and latitude: ability to synthesize cyanide, presence of hairs on juvenile and mature leaves, extrafloral nectaries, spines, and latex. We measured only two resistance traits for which low-latitude species have higher levels of resistance than do high-latitude species: delayed greening and ash content. Our findings are based on the most comprehensive and consistent data set collected to date.

It is possible that, if we had measured additional traits, we might have found more evidence of higher resistance to herbivores at lower latitudes. However, it would take a lot of additional traits behaving differently from those we did measure to change the overall conclusion of our study, and we have measured a selection of the most important known resistance traits.

The results from the present study, which used consistent methods to quantify latitudinal gradients in 14 resistance traits in 301 species from 75 sites world-wide, are consistent with the findings of the recent meta-analysis of data from the published literature (Moles *et al.*, 2011). The congruence of evidence from a large-scale empirical study and a comprehensive synthesis of data from the literature strongly suggests that traditional ideas in this field need to be overturned.

The main reason ecologists predicted stronger resistance traits at lower latitudes is that low-latitude species were thought to experience more intense herbivory than species at higher latitudes, thus being under selective pressure to evolve higher degrees of resistance (Dobzhansky, 1950; MacArthur, 1972; Coley & Aide, 1991; Coley & Barone, 1996; Van Alstyne *et al.*, 2001). However, it is not clear

that herbivory is actually more intense at lower latitudes. Meta-analysis of data from the literature did not support the idea that there is a latitudinal gradient in herbivory (Moles *et al.*, 2011). This result is consistent with findings from palaeoecology. Damage diversity on fossil leaves tracks mean annual temperature tightly through time, suggesting a greater diversity of herbivores in warmer times (Currano *et al.*, 2010). However, damage frequency is much more weakly related to mean annual temperature, and this correlation becomes nonsignificant once serial autocorrelation is removed (Currano *et al.*, 2010). If there is no latitudinal gradient in herbivory, then the fact that plant resistance traits are not stronger at lower latitudes is considerably less surprising. Performing a field study that quantifies the latitudinal gradient in herbivory using appropriate and consistent methods that account for differences in leaf life-span at sites at a wide range of latitudes around the world is a top priority for understanding patterns in plant–animal interactions through both space and time.

Both Moles *et al.*'s (2011) meta-analysis and the present empirical study show that, if anything, plant resistance traits actually tend to be higher at high latitudes. One possible explanation is that the cost of losing leaves in the relatively high-productivity environments at low latitudes might be lower than the cost of losing leaf area at higher latitudes, where productivity is lower. A latitudinal gradient in the cost of losing leaf area would favor higher levels of resistance in low-productivity/short growing-season environments, including high-latitude systems. This idea is a basic extension of the resource availability hypothesis (Coley *et al.*, 1985; Hallam & Read, 2006) and Janzen's suggestion that plants should produce better protected leaves in low-productivity environments (Janzen, 1974).

Another possibility is that the latitudinal gradients in leaf chemical and physical traits are driven not by herbivory, but by abiotic conditions. Many traits are known to have dual roles in protection against herbivores and the environment. For instance, scleromorphy protects leaves from damage from the abiotic environment, as well as from herbivores (Turner, 1994). Environmental factors such as soil fertility and exposure to UV are known to influence traits such as leaf toughness, resins and the phenolic content of leaves (Wainhouse *et al.*, 1998; Close *et al.*, 2003; Jordan *et al.*, 2005). If latitudinal gradients in resistance traits are driven by abiotic conditions rather than herbivory, then either abiotic factors are more important drivers of selection on leaf traits than is herbivory, or degrees of herbivory do not vary as substantially across the globe as do abiotic factors. These are interesting possibilities that definitely merit attention. However, latitudinal gradients in traits such as phenolics have long been accepted as evidence for a latitudinal gradient in resistance to herbivores (e.g. Schemske *et al.*, 2009). Moving the goalposts because the present evidence does not support traditional ideas would be disingenuous.

Our findings raise a host of interesting questions about large-scale patterns in plant resistance traits and plant–animal interactions. In the near future, we plan to quantify correlations among resistance traits, determine which environmental factors underlie latitudinal gradients in each trait, and investigate phylogenetic patterns in the evolution of plant resistance traits (including asking whether gymnosperms have higher levels of resistance traits than do angiosperms). There are also important questions that cannot be addressed with the present data, the most pressing of which is quantifying the effects of leaf longevity on plant resistance traits. We hope that the many questions raised by our findings will stimulate a burst of research on global patterns in plant–animal interactions and plant resistance traits.

Our results have implications beyond the understanding of plant–animal interactions. The world-wide distribution of compounds associated with resistance to herbivores has important implications for the global C cycle. Globally, the effect of leaf chemistry is the predominant control of decomposition rates (Cornwell *et al.*, 2008), and as such the patterns of leaf resistance traits described here may represent crucial information for understanding rates of terrestrial C cycling across latitude. One theory is that C that is not well defended and is thus consumed by herbivores is rapidly respired back to the atmosphere, while better defended tissue will have a longer residence time on the plant and as litter.

This is the first study to examine large-scale patterns in the relationships between plant cover and resistance traits across a range of species and sites. Across the 34 relationships between cover and resistance traits in this study, the highest R^2 was 0.031 and only four of these regressions were significant at $P < 0.05$. That is, we found no biologically relevant relationship between percentage cover and investment in resistance traits. One might have expected that resistance would increase with plant apparency and thus percentage cover (Feeny, 1976). However, there are reasons to predict null or even negative relationships. For example, there is often a trade-off between somatic growth and investment in resistance traits (e.g. Coley *et al.*, 1985; Yamamura & Tsuji, 1995; VanDam *et al.*, 1996; see Hanley *et al.*, 2007 for caveats), suggesting that across species percentage cover could decline with increases in resistance traits. Alternatively, as productive sites are expected to benefit species with little investment in resistance traits, while species with greater herbivore resistance traits should dominate in unproductive sites (Herms & Mattson, 1992; Yamamura & Tsuji, 1995; see Hanley *et al.*, 2007 for caveats), an interaction between a resource supply effect and growth–defence trade-offs (e.g. Fine *et al.*, 2004) might lead to no relationship between percentage cover and resistance traits when sites that differ in productivity are pooled.

Our results, combined with findings from a recent meta-analysis (Moles *et al.*, 2011), strongly suggest that plants at low latitudes do not have higher levels of traits associated

with resistance to herbivores than do plants at high latitudes. These findings cast serious doubt on traditional ideas about global patterns in plant resistance traits, and leave ecologists in a position to formulate new theories about the factors that shape plant strategy, species coexistence, and plant–animal interactions in different environments. Our results also suggest that we should ask whether other ‘well-known’ relationships might not be supported by empirical data. There are exciting times ahead.

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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

Table S2 Settings used to develop partial least squares regression models to relate reflectance spectra from leaf samples to chemical traits

Table S3 Relationships between binary traits and latitude

Table S4 Relationships between species abundance and each of the traits included in this study

Table S5 Results of phylogenetic analyses

Table S6 Analyses including a term for continent

Methods S1 Selection of sites, study species and individuals, near-infrared spectroscopy methods and phylogenetic analyses.

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