

Relationships between leaf lifespan and structural defences in a low-nutrient, sclerophyll flora

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Summary

1. The spectrum between species with low leaf mass per area (LMA), short-lived leaves and high photosynthetic rate to those with high LMA, long-lived leaves and low photosynthetic rate is one of the major spectra of variation between plant species, and is of particular relevance to the 'carbon-gain strategy' of plants.
2. In this study the relationship between physical properties of leaves and their lifespan was quantified for 17 sclerophyllous species from a nutrient-poor woodland in eastern Australia. Fracture properties of leaves (force of fracture, tissue toughness) and other leaf traits [LMA, thickness, dry-matter content (DMC), leaf area] were measured for each species and evaluated as predictors of leaf lifespan in cross-species and phylogenetic analyses, and for intercorrelation with one another.
3. The LMA, mean force of fracture, leaf thickness and leaf area each explained approximately 30–40% of variation in leaf lifespan. Leaf toughness explained 25% of variation in leaf lifespan, and DMC 12%. Leaf toughness and DMC were correlated with each other, but not with leaf thickness. Leaf thickness and toughness were related closely to LMA, while DMC and LMA were only marginally correlated.
4. Nutrients can be withdrawn prior to leaf death and redeployed elsewhere in the canopy when leaf death is initiated by a plant. However, when control is external to the plant these nutrients are lost. There may be advantages to increasing defence to give a high likelihood that the plant has control over the timing of leaf death.

Key-words: Leaf toughness, nutrient resorption, specific leaf area, tissue density

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Introduction

The net carbon gain from a leaf over its lifetime is determined by factors such as its construction cost, net photosynthetic rate and lifespan (Mooney & Gulmon 1982). Studies ranging across many continents and growth forms have shown that a major spectrum of variation exists between species with long-lived leaves, high leaf mass per leaf area (LMA) and slow net photosynthetic rate (mass basis) to species with short-lived leaves, low LMA and fast photosynthetic rate (Diemer 1998; Mulkey, Kitajima & Wright 1995; Reich, Walters & Ellsworth 1992; Reich, Walters & Ellsworth 1997; Reich *et al.* 1999). Leaf mass per leaf area is the product of leaf thickness and density (mass per volume); thus cross-species variation in either of these component traits must result in variation in LMA unless there is a counteracting trend in the other component (in which case leaf thickness and density would be negatively related; Witkowski & Lamont 1991). The strong relationship between leaf

lifespan and LMA across species suggests that structural reinforcement of leaves plays a major role in determining their lifespan, presumably by rendering them less susceptible to herbivory and other physical hazards.

A leaf may die in many ways: herbivory, temperature or water stress, damage by wind, rain or the thrashing action of neighbouring branches, etc. In these cases the initiation of death is external to the plant. Leaf death may also be initiated by a plant, for example in response to some phenological cue or to shading (whether self-shading, or from neighbouring plants).

An important difference between the plant having control over the timing of leaf death and having no control is the withdrawal of nutrients from leaves before they are shed (nutrient 'resorption'). On average, plants withdraw about 50% of leaf N and P prior to leaf abscission (Aerts 1996; Killingbeck 1996), with most retranslocated to well lit, newly developing leaves. Consequently, leaf deaths where the plant does not have the opportunity to withdraw nutrients could have a substantial impact on the nutrient pool in a plant.

Loss of leaf area to herbivory tends to be patchy in space and time. From a variety of habitats, estimates of

average leaf area loss to herbivory range between ≈ 7 and 70% over the life of leaves (Coley & Aide 1991; Coley & Barone 1996; Landsberg & Gillieson 1995; Lowman & Box 1983; Showalter, Hargrove & Crossley 1986). Some leaf features, such as the degree of sclerification of vascular bundles, presence of collenchyma strands or a thick epidermis, provide protection against herbivores and against other physical hazards (Cunningham, Summerhayes & Westoby 1999). Other features such as surface spines are more clearly associated with defence against herbivores. Because there is such a variety of potential hazards and herbivores, no single leaf property can summarize the extent of physical defence. Still, indices of leaf strength and toughness have gained acceptance as being ecologically meaningful as they are related to leaf palatability, food quality, growth rate of herbivores and wear of insect mouthparts (Cherrett 1968; Choong 1996; Coley 1987; Hochuli 1996; Ohmart, Stewart & Thomas 1985; Southwood, Brown & Reader 1986), as well as to leaf lifespan (Reich *et al.* 1991).

In this study we focused on the relationship between leaf lifespan and general physical properties of leaves. Undoubtedly, chemical defences can also play a major role in determining leaf palatability and thus lifespan. However, here we considered physical properties only – properties that potentially relate to defence against herbivores and other hazards alike. We set out to determine whether the force of fracture (from a cutting test across the lamina) was a better predictor of leaf lifespan than LMA across 17 co-occurring woody perennials. Force of fracture is itself the product of leaf thickness and leaf toughness, just as LMA is the product of leaf thickness and density. A complementary aim of the study was to assess the contribution of the various component attributes to variation in force of fracture and LMA, and the extent to which these attributes were themselves correlated. We evaluated these relationships using cross-species and correlated divergence regressions using phylogenetically independent contrasts (Felsenstein 1985; Harvey & Pagel 1991). These two types of analyses are complementary (Westoby *et al.* 1998). The first asks whether two traits are correlated across present-day species, thus characterizing trait combinations of species currently persisting within a habitat. The second asks whether divergences in one trait have been consistently correlated with divergences in another trait throughout evolutionary history.

Materials and methods

SITE AND SPECIES SELECTION

The study was undertaken on 17 perennial dicot species from an open woodland on nutrient-poor yellow-grey sandy soil derived from Hawkesbury sandstone, near Sydney, Australia (33°41'38" S, 151°08'35" E). Five random soil samples were taken (core ≈ 5 cm diameter, 15 cm deep), air-dried and analysed for total soil P

(94 $\mu\text{g g}^{-1}$, SD 28; analysis by solid fusion/XRF crystallography) and total N (0.030%, SD 0.001; analysis by mass spectrometry). Nutrient analyses were carried out at CSIRO Plant Industry, Canberra.

Rainfall is ≈ 1220 mm per year (average from >100 years' records from the nearest weather station), spread more-or-less evenly throughout the year. The vegetation is predominantly woody, sclerophyll, evergreen, fire-prone and species-rich. The 17 species were chosen randomly from a previously compiled species list for the site (65 vascular plant species in a 0.1 ha plot; M. Leishman, Macquarie University, Sydney, unpublished data). Climbing and twining species were excluded. A phylogeny was constructed for the species (Appendix Table 1), with nomenclature following Harden (1990), ordinal groupings based on Bremer *et al.* (1998), and relationships within Proteaceae based on Hoot & Douglas (1998).

MEASUREMENT OF LEAF PHYSICAL PROPERTIES

All leaf traits except lifespan (see below) were measured on young to medium-aged, fully expanded, outer-canopy leaves. Two leaves from each of three individual plants were used for measurement of force of fracture, thickness and toughness for most species (exceptions: four data points only for *Gompholobium glabratum*, five for *Hakea teretifolia*, eight for *Phyllota phlycooides*). Leaf thickness was measured at two to five points per leaf with a dial gauge micrometer. Major veins and the midrib were avoided on all but the smallest leaves. Midrib thickness was measured separately for several species.

Force of fracture for leaves (as determined by a shear test; Aranwela, Sanson & Read 1999; Wright & Vincent 1996) was determined using a purpose-built machine which was not intended to simulate any particular herbivore, but to provide a generalized measure of physical defence (Fig. 1; full details available from the first author). The machine measures the force required to cut a leaf at a constant cutting angle (20°) and speed, and is similar in principle to systems described by Aranwela *et al.* (1999) and Darvell *et al.* (1996). The cutting blade is supported by a cantilevered arm, which rises and falls according to the direction of rotation of a lead screw driven by a computer-controlled stepper motor. The leaf is placed on an anvil, providing a reference face against which sample shearing occurs. The force of fracture is concentrated in the centre of a double-concave, thin section of the cantilevered arm, and measured via paired strain gauges mounted on either side of the arm at this point. Output from the strain gauges is in the form of a series of force measurements taken at regular intervals as the blade traverses the sample (9.1 Hz, equivalent to every 0.03 mm along the edge of the anvil), giving a force (f) \times displacement (d) graph. The mean force of fracture for a sample was calculated as the average force registered across the cutting trajectory. Mean force of

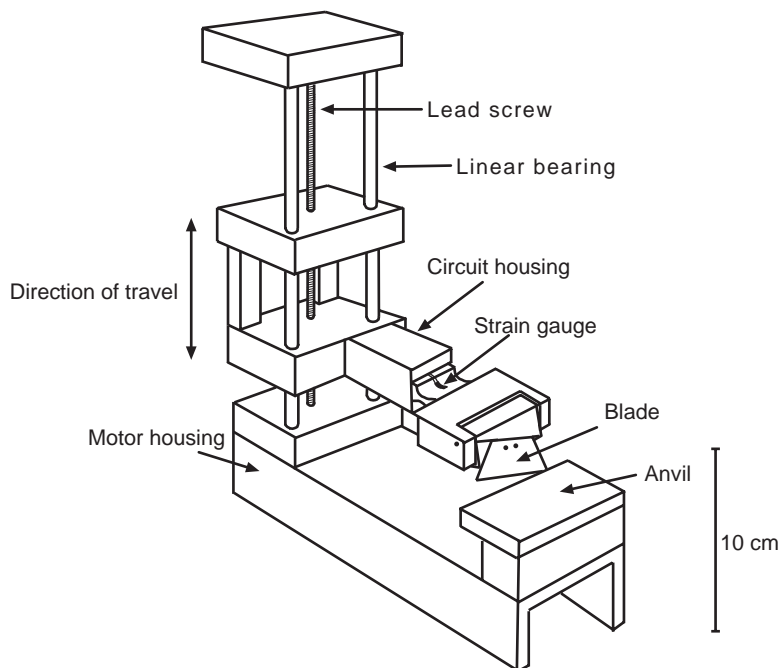


Fig. 1. Diagrammatic representation of the machine used to measure leaf force of fracture (safety guard, quick release sample holder and electronic control unit not shown).

fracture (N) divided by tissue thickness gives the tissue toughness (N m^{-1}). This is equivalent to the area under the $f \times d$ curve (work) divided by the cross-sectional area of the sample ($\text{J m}^{-2} = \text{N m}^{-1}$), and when derived as such is known as the specific work of fracture (Vincent 1990).

One cut at right angles to the midrib was made per leaf for measurement of force of fracture. Leaves were cut at the widest point along the lamina, or half-way between the leaf base and tip if the leaf had no obviously widest point. For species with prominent midribs and/or margins (e.g. *Corymbia gummifera*, Fig. 2a), these features could be discerned on the resulting $f \times d$ graph, and the toughness of the midrib and lamina were calculated separately in addition to that integrated over the whole leaf. For some species this was not possible, as the midrib was not sufficiently prominent relative to other features in the graph (e.g. *Pimelea linifolia*, Fig. 2b). For *Hakea dactyloides* it was possible but only partially informative, as the major veins required similar force to cut as the midrib (Fig. 2c). For others, the midrib was neither prominent nor relevant (species with needle or linear leaves, e.g. *Hakea teretifolia*, Fig. 2d; *Phyllota phyllicoides*, not shown).

The LMA and leaf dry-matter content (DMC) were measured on separate leaf samples taken from the same species at the same site, but not necessarily the same individuals. Five leaves from each of five individuals were collected. Projected leaf area was determined with a flat-bed scanner and DELTA-T SCAN software (Delta-T, Cambridge, UK). Leaves were placed between sheets of damp paper towel and stored at 4 °C overnight before being weighed (giving 'saturated' weight). Leaves were oven-dried for a minimum of 48 h at 65 °C and weighed

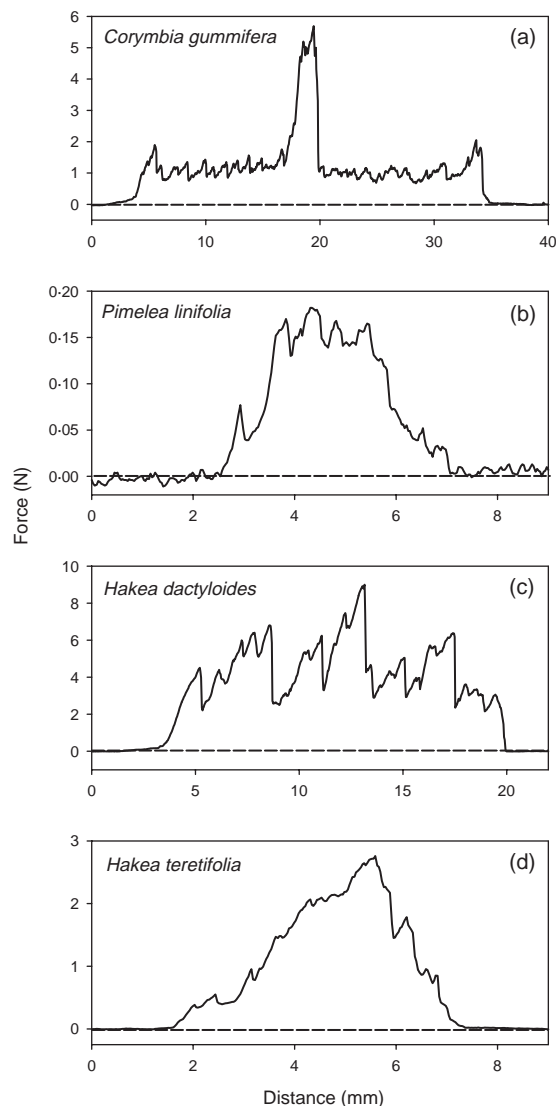


Fig. 2. Representative force–displacement curves for four species: (a) *Corymbia gummifera*; (b) *Pimelea linifolia*; (c) *Hakea dactyloides*; (d) *Hakea teretifolia*. Mean force of fracture was calculated as the average height (force) across the leaf, after first zeroing the data by subtracting the mean height for the sections before and after the leaf was cut (friction). Toughness was calculated by dividing the mean force of fracture by lamina thickness. Force of fracture and toughness calculated for the lamina only (ignoring midrib) were highly correlated with estimates which included the midrib (see text).

for the calculation of LMA and DMC (dry mass/saturated mass). Leaf DMC is an index of tissue density (Garnier & Laurent 1994; Niinemets 1999).

ESTIMATION OF LEAF LIFESPAN

For estimates of average leaf lifespan, 15 randomly chosen 'sun' twigs were chosen in July 1998 from individuals of each species. For each twig, a leaf sequence was defined by starting from the youngest primary leaf (>25% expanded) and tracing backwards along the twig to the oldest primary leaf found, whether this leaf was found on the same twig or, more commonly, on a branch one or more branching orders back from the

twig. The number of primary leaves in the sequence was counted. Five months later the number of leaves remaining in each sequence was counted (giving the number of leaf deaths), and new sequences were marked, using the same branches as previously where possible, or by tagging new branches where necessary (e.g. if the branch or plant had been damaged). Seven months later another census was undertaken using the same methods. A total of 14–32 branches (mean 19.7) from three to nine individuals per species were tagged and counted across the two census periods.

Average leaf lifespan was calculated as the inverse of the rate of leaf turnover (Ackerly 1996; Southwood *et al.* 1986). The rate of leaf death per year was calculated by pooling all branches for each species within each census period, and calculating a time-weighted average for each species from the two censuses, as the census periods were not evenly spaced (mid-points being 154 and 224 days). This method of calculating leaf lifespan assumes that a constant number of leaves die per unit time as opposed to, say, a constant proportional death rate. It is likely that the true average leaf lifespan is between the two estimates (P. Reich, University of Minnesota, unpublished data). However, the choice of method does not influence the results significantly (for these species the two indices were tightly correlated, $r = 0.99$).

TREATMENT OF DATA

Variance components analyses (ANOVA, type I sums of squares) indicated that 89–96% of variation in SLA, DMC, thickness, force of fracture and toughness was associated with differences between rather than within species (whether or not the within-species term was further decomposed into terms for between-individuals and leaves-within-individuals). Thus treating these variables as species means in subsequent analyses was strongly supported. The method of calculation for leaf longevity precluded variance components analysis on that trait, but variation is much greater between than within species for this trait also (Eckstein, Karlsson & Weih 1999; Reich *et al.* 1999).

Data were averaged for each species. Species means were log transformed before analyses, as most traits showed strongly right-skewed distributions across the 17 species (all transformed variables satisfied a standard test of normality: Shapiro–Wilks, $\alpha = 0.05$). For cross-species regressions, each species contributes a single data point. For correlated divergence analyses, each independent divergence or radiation contributes a single item of evidence (sometimes known as phylogenetically independent contrasts: Felsenstein 1985; Grafen 1989; Harvey & Pagel 1991). A contrast data set is created, in which the value assigned to each contrast is calculated as the difference between the trait values for the two nodes or species descending from the contrast node. Node values are themselves calculated as the average of trait values for the two immediately lower nodes

or species. The direction of subtraction in calculating contrasts is unimportant, providing all traits are treated in the same manner. Hence, in a graph of divergences in one trait against divergences in another, a data point indicating a positive divergence in both traits would have indicated negative divergences in each trait had the subtractions been performed the other way around. Due to this symmetry, regressions of contrast data have no intercept term. Multiple regressions were used to look at three-way relationships between leaf lifespan, toughness and thickness, and between leaf size, toughness and force of fracture (cross-species data only). Statistical analyses were run in SPSS ver. 8.01.

For the 11 species where the toughness of the midrib could be confidently measured separately from that of the leaf lamina (Appendix Table 1), it was clear that species with tougher laminae also had tougher midribs (correlation $r = 0.88$), with the ratio of midrib to lamina toughness ranging from 0.9 to 2.4 (mean = 1.5). Still, mean toughness (or force of fracture) integrated over the whole leaf was tightly correlated with lamina-only toughness or force of fracture (correlation $r = 0.95$ and 0.995, respectively; mean ratio of whole-leaf to lamina-only indices = 1.04), with little difference in the two estimates since, together, the midrib and margins (where discernible) made up a small proportion of the cutting path across the leaf. Consequently, the relationships reported were qualitatively identical whether reported on the basis of force of fracture (or toughness) of whole leaves, or for the force of fracture (or toughness) of the lamina only (data not shown).

Results

RELATIONSHIPS BETWEEN LEAF PHYSICAL PROPERTIES AND LEAF LIFESPAN

Average leaf lifespan and LMA varied sixfold across the 17 species (1–6 years, 0.06–0.34 mg mm²; Appendix Table 1); DMC and leaf thickness varied approximately twofold (0.27–0.55 mg mg⁻¹ and 0.24–0.57 mm, respectively); while mean force of fracture varied 40-fold (0.08–3.2 N) and leaf toughness almost 20-fold (335–6529 N m⁻¹) across the species.

Leaf lifespan and mean force of fracture were positively associated, both across species ($r^2 = 0.39$) and across evolutionary divergences ($r^2 = 0.32$; Fig. 3a,b). Force of fracture was more strongly correlated with leaf toughness than with thickness (correlation $r = 0.96$ versus 0.54; Table 1), with the two components (toughness and thickness) not associated directly. Thickness explained roughly the same amount of variation in leaf lifespan ($r^2 = 0.37$) as did force of fracture, while toughness explained somewhat less ($r^2 = 0.27$ and 0.20 in cross-species and divergence analyses, respectively; Fig. 3c–f).

The LMA and leaf lifespan were correlated to the same extent as were force of fracture and leaf lifespan in cross-species analyses ($r^2 = 0.38$), and slightly less so

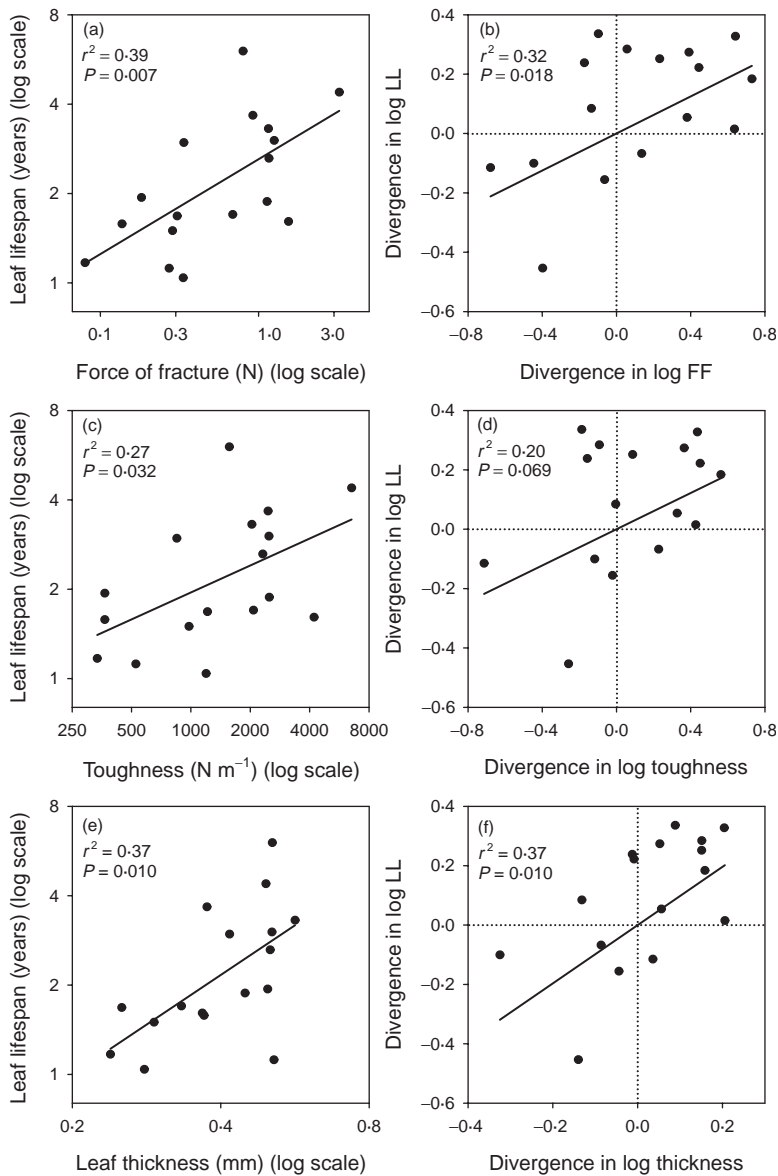


Fig. 3. Cross-species and correlated divergence regressions between leaf lifespan and force of fracture (a, b), toughness (c, d) and leaf thickness (e, f). Regression lines for cross-species regressions include an intercept term, while those for correlated divergence analyses do not (they are forced through the origin for reasons of symmetry; see text).

in divergence analyses ($r^2 = 0.27$; Fig. 4a,b). Variation in LMA was more closely associated with that in leaf thickness than in DMC (correlation $r = 0.78$ and 0.43 , respectively). Thickness and DMC were themselves unrelated, and DMC explained little variation in leaf lifespan ($r^2 < 0.14$ in both analyses; Fig. 4c,d).

Table 1. Leaf trait interrelationships: Pearson correlation r values shown for log-transformed leaf traits, with corresponding P values given in parentheses

Trait	Force of fracture (N)	Toughness (N m^{-1})	Thickness (mm)	LMA (mg mm^{-2})	DMC (mg mg^{-1})
Toughness	0.96 (<0.001)				
Thickness	0.54 (0.027)	0.29 (0.258)			
LMA	0.85 (<0.001)	0.72 (0.001)	0.78 (<0.001)		
DMC	0.64 (0.05)	0.69 (0.002)	0.12 (0.644)	0.43 (0.084)	
Leaf area	0.77 (<0.001)	0.72 (0.001)	0.48 (0.050)	0.54 (0.024)	0.43 (0.082)

Many of the physical properties of leaves were correlated with one another (Table 1). High-LMA leaves (thicker, tougher, denser) required greater force to cut, as might be expected. Toughness and DMC were correlated ($r = 0.69$, $P = 0.002$), suggesting that the two traits convey much the same kind of information. Variation in leaf thickness was more or less independent of variation in either of these two traits. Consequently, thickness and toughness together explained 50% of variation in leaf lifespan, with thicker leaves lasting longer at a given level of tissue toughness ($P = 0.025$), and tougher leaves lasting longer at a given leaf thickness ($P = 0.079$). Together, DMC and thickness explained 45% of variation in leaf lifespan, while other two-trait combinations explained less lifespan variation.

LEAF SIZE

Leaf size ranged between 15 and 3300 mm^2 , and was correlated with the other leaf traits such that the leaves of larger-leaved species tended to require greater force to fracture, and were tougher, thicker, and had higher LMA and DMC than leaves of smaller leaved species (Table 1). Leaf size was also correlated with leaf lifespan in cross-species and divergence analyses ($r^2 = 0.28$ and 0.37 , respectively; Fig. 5a,b). The portion of leaf lifespan variation accounted for by leaf size and the other traits overlapped substantially: controlling for leaf size (cross-species data only) rendered the partial regression coefficients for force of fracture and toughness nonsignificant ($P = 0.125$ and 0.376 , respectively), and those for thickness and LMA marginally significant ($P = 0.065$, 0.075). Conversely, the partial coefficient for leaf size was nonsignificant in each of these analyses ($P > 0.1$). Thus there was no indication that the relationships between leaf lifespan and leaf physical characteristics were merely secondary correlations due to larger-leaved species having more robust leaves.

OUTLIERS FROM MAJORITY TRENDS

Data points in correlated divergence graphs can fall into any one of the four quadrants that make up the graph (indicated by dotted lines in each of Figs 3b,d,f, 4b,d and 5b). The graphs can be read in terms of the number of points falling in each quadrant in order to identify outliers from majority trends, in addition to the more formal regression analysis (Westoby *et al.*

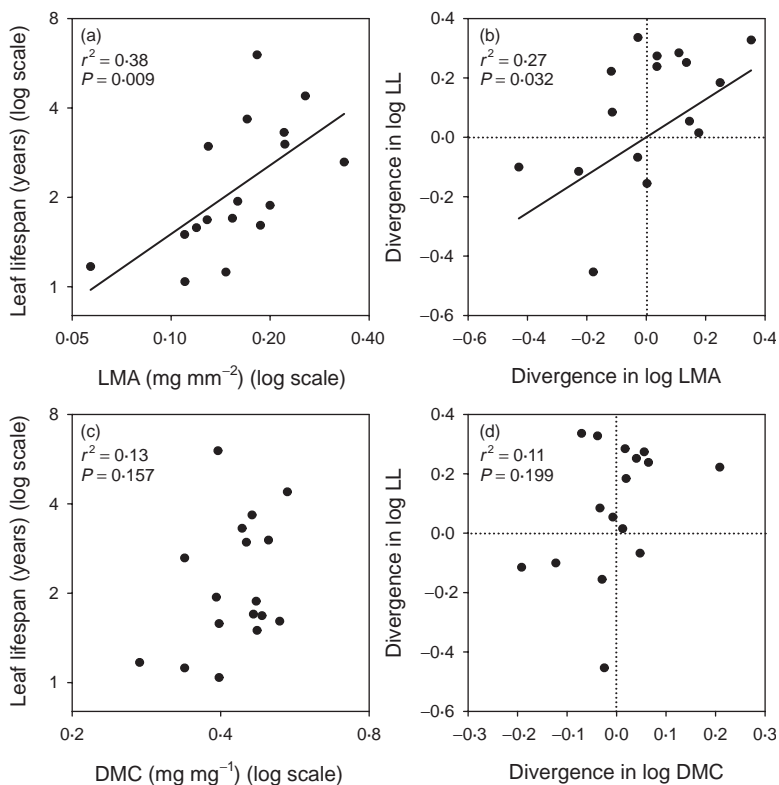


Fig. 4. Cross-species and correlated divergence regressions between leaf lifespan and leaf mass per area (LMA; a, b), and leaf dry matter content (DMC; c, d).

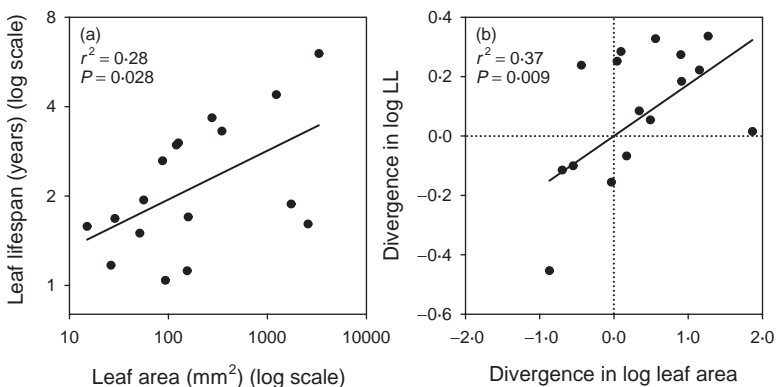


Fig. 5. Cross-species and correlated divergence regressions between leaf lifespan and leaf area.

1998). Typically, two to four of 16 divergences fell in the upper left or lower right quadrants for analyses where the majority trend (and, more formally, the best-fit regression) indicated a positive relationship between the traits (i.e. the majority of points fell in the lower left or upper right quadrants). In some cases the same divergence was an outlier from more than one overall trend in analyses between leaf lifespan and other traits, with two divergences being particularly notable in this respect (between *Banksia marginata* and *Lambertia formosa*, and between *Persoonia levis* and the rest of the Proteaceae). However, other outliers were spread across the phylogeny and were not concentrated in any one clade or at any particular taxonomic level.

Discussion

DETERMINANTS OF LEAF LIFESPAN

Across these 17 sclerophyllous species from a nutrient-poor habitat, up to 40% of between-species variation in leaf lifespan was explained by single, simple physical properties of the leaves, with up to 50% predictive power when analysed as two-trait combinations, such as leaf thickness and toughness, together. No one trait emerged as the single best predictor of leaf lifespan, with similar r^2 values for leaf thickness, force of fracture and LMA ($r^2 \approx 0.38$) in cross-species regressions, and r^2 values for thickness and leaf area equal highest in divergence analyses ($r^2 = 0.37$).

The extent to which herbivory contributed to leaf death was not formally measured. Evidence of leaf chewing or mining was generally minimal. Consequently, most leaves of these species appeared to survive to the stage of nutrient withdrawal (yellowing) and abscission, that is, the timing of death for most leaves was under endogenous control. Presumably a plant can increase the likelihood of controlling the timing of leaf death by constructing leaves of thick or dense fabric with greater physical resistance to herbivory and other damage. The construction cost per gram of such leaves may be similar to that of less reinforced leaves (Poorter & de Jong 1999), but a high LMA has indirect opportunity costs compared to a strategy of maximizing light-intercepting area per unit leaf mass. These costs could be amortized to some extent (if not completely) over the longer period of carbon fixation of a longer-lived leaf (Coley *et al.* 1985; Gulmon & Mooney 1986). Older leaves tend to be subject to self-shading, however, so there are limits to how long a leaf may be valuable in comparison to the alternative of withdrawing nutrients and redeploying them in well lit new leaves, despite half the nutrients being lost in this process, on average (Aerts 1996; Killingbeck 1996).

The duration of retention of leaf nutrients in a plant is largely a function of leaf lifespan and resorption efficiency (Escudero *et al.* 1992). In theory, there is greatest selective pressure in nutrient-poor habitats to maximize the duration of nutrient retention (Aerts & van der Peijl 1993). However, as resorption efficiency does not vary predictably with habitat quality (Aerts 1996; Killingbeck 1996), selective pressure for long-lived leaves in nutrient-poor habitats may be stronger than elsewhere. Still, the efficiency of nutrient resorption is only relevant for leaves where the timing of leaf death is initiated by the plant. Where leaves are lost to herbivores and other unexpected hazards, the resorption efficiency is more-or-less zero, depending on the extent to which nutrients were withdrawn over the life of a leaf (as it becomes increasingly shaded, for example; Kull & Niinemets 1998; Werger & Hirose 1991). Thus the premium paid for losing control over the timing of leaf death (inability to withdraw nutrients before leaf loss) may be greatest in habitats where nutrients are in

short supply. Consequently, the net cost of defending leaves to virtually ensure nutrient withdrawal prior to leaf death may be relatively less in these circumstances than in nutrient-rich habitats (the same conclusion is drawn from the more formal cost–benefit models presented by Coley 1987; Coley *et al.* 1985; Gulmon & Mooney 1986; Mooney & Gulmon 1982; but see Loreau & de Mazancourt 1999).

While these arguments concern reinforcement of mature leaves, the ability to defend young, expanding leaves structurally from herbivory is minimal, as these defences cannot be laid down until leaves have finished expanding (Coley & Barone 1996). Consequently, herbivory tends to be most severe for young leaves, being softer, and with higher nitrogen concentrations than fully expanded leaves (Coley & Kursar 1996; Feeny 1970; Lowman & Box 1983). This may also be the case for our study species, with 1–25% (median 5.0%) of leaf area lost to herbivory during leaf expansion (measurements made for eight of 17 species; Moles & Westoby 2000). These measurements were taken in nearby habitats, and suggest that the generally low levels of damage to mature leaves can be plausibly related to the defence of those leaves (whether physical or chemical), rather than an absence of herbivores.

MEASUREMENT AND INTERPRETATION OF FORCE OF FRACTURE AND LEAF TOUGHNESS

By definition, an increase in leaf thickness or density increases the LMA of a leaf, unless thickness and density are themselves negatively correlated. Similarly, variation in force of fracture is determined by underlying variation in thickness and toughness. From the limited data available, it seems that leaf thickness and toughness vary more-or-less independently across species, while toughness is generally correlated with traits such as the proportion of vascular tissue, fibre or sclerenchyma, and tissue density (Choong *et al.* 1992; Wright & Illius 1995; present study).

Penetrometer tests measuring the force required to force a blunt rod or needle through leaf tissue (a measure analogous to force of fracture) have indicated a negative relationship between leaf robustness and palatability (e.g. Cherrett 1968; Coley 1987; Lowman & Box 1983; Reich *et al.* 1991; although for young leaves only in Lowman & Box's study). However, it is not clear whether thickness or toughness contributed most to the patterns in those studies. Even where force of fracture is decomposed into thickness and toughness, it is difficult to compare measurements made with different instruments. In particular, the cutting angle may affect the measured toughness in studies using a guillotine-like apparatus as used here (Aranwela *et al.* 1999). Still, leaf toughness ranged from ≈ 1900 – 6500 N m^{-1} for the species studied here, overlapping with the range reported by Edwards, Read & Sanson (2000) for 19 Australian sclerophyll species (430 – 6000 N m^{-1}). Two species occurred in both studies (*Acacia*

suaveolens and *Banksia marginata*), with leaves 1.5 to four times tougher in that study than found here. The results of both studies extend to a higher range than those reported previously (80 – 1100 N m^{-1} for 42 tropical tree species, Choong *et al.* 1992; 270 – 2000 N m^{-1} for 33 species from three sclerophyll communities, Turner *et al.* 1993). However, in both these studies the toughness reported was that of the 'least tough path', a cutting path that avoided secondary veins. Whether the secondary veins, margins or midrib should be included in estimates of fracture properties may vary with the plant species, the purpose of the study or the herbivore being modelled (if that is the aim). Certainly, no single physical attribute can indicate the susceptibility of a leaf to damage by wind, rain, wilting, freezing, folivores, skeletonizers and sap-sucking insects. General measures of leaf reinforcement tend to be correlated with one another and with outcomes such as palatability and leaf lifespan, and thus provide informative indices by which different species can be compared.

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Appendix Table 1. Phylogeny and data for 17 species

Species	Family	Leaf life-span (years)	Force of fracture (N)	Toughness (N m ⁻¹)	Leaf thickness (mm)	LMA (mg mm ²)	DMC (mg mg ⁻¹)	Leaf area (mm ²)
<i>Acacia suaveolens</i> *	Mimosaceae	3.3	1.15	2036	0.57	0.22	0.44	347
<i>Gompholobium glabratum</i>	Fabaceae	3.0	0.34	848	0.42	0.13	0.45	120
<i>Phyllota phyllicoides</i>	Fabaceae	1.6	0.14	366	0.37	0.12	0.40	15
<i>Pimelea linifolia</i>	Thymeleaceae	1.2	0.08	335	0.24	0.06	0.27	26
<i>Boronia ledifolia</i>	Rutaceae	1.9	0.18	366	0.50	0.16	0.39	56
<i>Eriostemon australasius</i> *	Rutaceae	1.1	0.27	526	0.51	0.15	0.34	155
<i>Corymbia gummifera</i> *	Myrtaceae	1.6	1.55	4213	0.37	0.19	0.53	2570
<i>Eucalyptus haemastoma</i> *	Myrtaceae	1.9	1.13	2498	0.45	0.20	0.47	1738
<i>Leptospermum trinervium</i>	Myrtaceae	1.7	0.31	1213	0.25	0.13	0.48	29
<i>Hibbertia bracteata</i> *	Dilleniaceae	1.0	0.33	1193	0.28	0.11	0.40	93
<i>Persoonia levis</i> *	Proteaceae	6.0	0.80	1567	0.51	0.18	0.39	3311
<i>Hakea dactyloides</i> *	Proteaceae	4.4	3.24	6529	0.49	0.26	0.55	1230
<i>Hakea teretifolia</i>	Proteaceae	2.6	1.16	2314	0.50	0.34	0.34	87
<i>Grevillea speciosa</i> *	Proteaceae	1.7	0.69	2075	0.33	0.15	0.47	158
<i>Grevillea buxifolia</i> *	Proteaceae	1.5	0.29	978	0.29	0.11	0.47	51
<i>Banksia marginata</i> *	Proteaceae	3.7	0.92	2459	0.38	0.17	0.46	275
<i>Lambertia formosa</i> *	Proteaceae	3.0	1.26	2485	0.51	0.22	0.50	126

LMA, leaf mass per area; DMC, dry matter content.

* Force of fracture and toughness were estimated for the midrib and lamina separately (data not shown), as well as across the entire leaf.