Relationships among leaf traits of Australian arid zone plants: alternative modes of thermal protection

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Abstract. Despite the importance of leaf traits that protect against critically high leaf temperatures, relationships among such traits have not been investigated. Further, while some leaf trait relationships are well documented across biomes, little is known about such associations within a biome. This study investigated relationships between nine leaf traits that protect leaves against excessively high temperatures in 95 Australian arid zone species. Seven morphological traits were measured: leaf area, length, width, thickness, leaf mass per area, water content, and an inverse measure of pendulousness. Two spectral properties were measured: reflectance of visible and near-infrared radiation. Three key findings emerged: (1) leaf pendulousness increased with leaf size and leaf mass per area, the former relationship suggesting that pendulousness affords thermal protection when leaves are large; (2) leaf mass per area increased with thickness and decreased with water content, indicating alternative means for protection through increasing thermal mass; (3) spectral reflectance increased with leaf mass per area and thickness and decreased with water content. The consistent co-variation of thermal protective traits with leaf mass per area, a trait not usually associated with thermal protection, suggests that these traits fall along the leaf economics spectrum, with leaf longevity increasing through protection not only against structural damage but also against heat stress.

Introduction

Broad relationships among leaf traits and the environment are well documented, demonstrating robust patterns in trait variation across multiple biomes (Reich *et al*. 1997; Wright *et al*. 2002, 2004). The 'leaf economics spectrum' compares the photosynthetic return of an investment against its cost (Wright *et al*. 2004), suggesting alternative strategies among species. Consistent relationships between leaf traits, for example leaf mass per area (LMA) and leaf life span, are viewed as a spectrum of variation representing the structural trade-offs made among species globally. Less is known, however, about whether these global patterns occur within a single biome, where leaf survival strategies are likely to be based on a narrower range of specific environmental stressors.

Desert biomes are typified by unpredictable rainfall and temperature extremes, where high daytime temperatures in summer represent a major threat to leaf survival. Without mechanisms that help limit or regulate heat load, leaves can heat to damaging temperatures within minutes (Forbes and Watson 1992; Singsaas and Sharkey 1998). Latent heat loss through transpiration is one of the most effective ways for leaves to avoid excessive heating (Gates 1980; Gutschick 1999; Nobel 2005) but many plants reduce stomatal conductance to avoid excess water loss in hot dry conditions (Barradas *et al*. 1994; Hamerlynck *et al*. 2000), eliminating a

major source of cooling. Alternatively, heat stress can be avoided via the production of heat shock proteins (Heckathorn *et al*. 1998; Knight and Ackerly 2001) and isoprenes (Sharkey and Yeh 2001; Sharkey *et al*. 2008). However, these heat protection mechanisms incur a sizable metabolic cost and often afford short-term benefits only (Sharkey and Yeh 2001; Sharkey *et al*. 2001). Therefore, for arid zone plants, particularly under conditions of nutrient poverty, intrinsic leaf traits that confer long-term structural thermal protection are particularly important. There is an array of structural leaf traits possessed by desert plants that help to reduce excessively high leaf temperatures. Despite the importance of such traits for the survival of leaves in high temperature environments, the relationship between leaf traits common to a single biome has rarely been investigated. Among the range of thermal protective leaf traits found in plants in arid or desert regions worldwide, discernible variation in these traits exists among species, even within a single family in a given region. Assuming that the production of such traits carries some cost, variation in a given trait may represent a trade-off for, or replacement with, another thermal protective trait. Alternatively, traits may co-vary to increase protection by multiple means.

Among these thermal protective traits is leaf size. It has long been known that reduced leaf size (width across the lamina) can assist in maintaining average leaf temperatures close to ambient

temperature due to increased rates of convection across the boundary layer (Gates 1980). Indeed, small leaves are common in hot, dry regions, indicating that many plants in these areas reduce the incidence of thermal damage by maintaining comparatively low leaf temperatures (Parkhurst and Loucks 1972; Thuiller *et al*. 2004). Even so, while the average width of leaves in desert biomes is relatively small, e.g. <10 mm^2 in deserts of southern USA (Gibson 1998), size can vary in relative terms, such that a 'large' desert leaf may be an order of magnitude bigger than this. Therefore, reduced size is clearly not the only way desert plants minimise leaf thermal damage.

Avoiding excessive heat loads can be aided by reducing incident radiation, i.e. through changes in leaf orientation. For example, leaves that are positioned vertically have less midday solar radiation reaching their surface (Valladares and Pugnaire 1999; Falster and Westoby 2003) than those positioned horizontally. If leaves are positioned vertically through being pendulous, they not only avoid direct radiation during the hottest part of the day, but can also flutter, even at relatively low wind speeds, aiding convectional cooling (Roden and Pearcy 1993). Thus, pendulousness can serve a dual function in protecting against excessive heat loads in high temperature environments. Little is known about pendulousness, however, either in terms of its relative occurrence in desert biomes, or whether it predictably occurs in association with other leaf traits.

A less obvious thermal protective trait is leaf thickness. Thicker leaves, via increased LMA and/or water content (succulence), have greater thermal mass and therefore a longer thermal time constant (τ, response time to a change in temperature). Increased leaf thickness can damp rapid excursions to extreme high temperatures caused, for example, by sudden sun flecks or lulls in wind speed (Ball 1988; Leigh *et al*. 2012). The proportion of species with high LMA (Wright *et al*. 2005), succulent (von Willert *et al*. 1992), and thick (Wright and Westoby 2002) leaves tends to increase away from temperate areas towards semi-arid and arid regions, suggesting that these traits are particularly useful in regulating temperature in hot dry climates. LMA does not consistently vary with leaf thickness (Poorter *et al*. 2009) and it is not known whether leaf thickness is more closely associated with LMA or water content among desert species. A positive correlation between LMA and thickness would indicate a role for high LMA desert leaves in protecting against rapid temperature spikes.

Species can also differ in the amount and type of radiation that is absorbed by leaves. Absorption by leaves of infrared wavelengths (thermal radiation) can vary depending on water content (Woolley 1971), whereas epidermal waxes and hairs can reflect visible wavelengths at the leaf surface before they can be absorbed by the leaf pigments, leading to considerable reduction in leaf heat loads (Mooney *et al*. 1977; Ehleringer 1981; Skelton *et al*. 2012). Although reflective leaves are common in desert biomes they are not universal, presumably because leaf hairs and waxes are costly to produce and reduce carbon gain (Ehleringer and Björkman 1978; P'yankov *et al*. 2001). Less reflective leaves are therefore considerably more vulnerable to heat stress without other means of thermal protection.

The aim of this study was to investigate whether different leaf traits contributing to protection against excess radiation consistently occur in the presence or absence of another trait. If a leaf's morphology was such that it would normally sustain a high average temperature, we might expect a relationship with another trait that would serve to counteract excessive leaf temperatures. For example, the temperature-damping benefit conferred by thick leaves (either via increased LMA or succulence) is predicted to be particularly high for absorptive leaves or for large, wide leaves (Leigh *et al*. 2012). Therefore, a positive relationship between leaf thickness and leaf size or a negative relationship between leaf thickness and reflectance could suggest a replacement for thickness with reduced size or high reflectance in protecting against thermal damage. Similarly, the coupling of large leaves with a pendulous habit would reduce incident heat load and increase convectional cooling of large leaves.

This study investigated bivariate relationships between nine leaf traits that afford thermal protection, including traits not normally considered in multi-trait datasets: leaf spectral reflectance and pendulousness. The study is also novel in focusing on the more subtle trait variation inherent within a single ecosystem, rather than across multiple biomes. The investigation incorporated 95 species from a wide range of plant families native to arid Australia. Given that at least two-thirds of the Australian continent is semi-arid or arid, surprisingly little research has been conducted on the vegetation of this region. In contrast to deserts elsewhere, the Australian arid region represents a model system in which to investigate the question of leaf thermal protection and survival as it is typified by particularly low nutrient soils and predominantly evergreen plants (Beadle 1954), indicating that strategies for enhancing leaf survival are especially important.

Methods

Study area and species selection

The study was conducted in north-west New South Wales, Australia, in an area bounded by the Barrier and Byngnano Ranges at Mutawintji National Park in the south and the Grey Range at Sturt National Park in the north (Fig. 1). The sampling region has a hot, arid climate with an average maximum summer temperature reaching >44°C and annual precipitation averaging <250 mm (Australian Government Bureau of Meteorology 2011).

The study employed a limited environmental and geographical context as this is most suitable when the level of replication is species (Brown and Maurer 1987). Ninety-five species of trees, shrubs and herbs native to arid New South Wales were selected for sampling. Species spanned 76 genera and 40 families, selected to maximise phylogenetic diversity and leaf trait variation evident throughout the sampling region (Table 1).

Sampling protocol

In this study we focused on the bivariate relationships of leaf traits that can protect against excessive radiation. 'Leaves' included both true leaves and photosynthetic plant organs that essentially function as a leaf when true leaves are absent, i.e., cladodes and phyllodes. Additionally, for species with compound leaves, the unit of measurement was the leaflet. The leaflet is deemed to act as
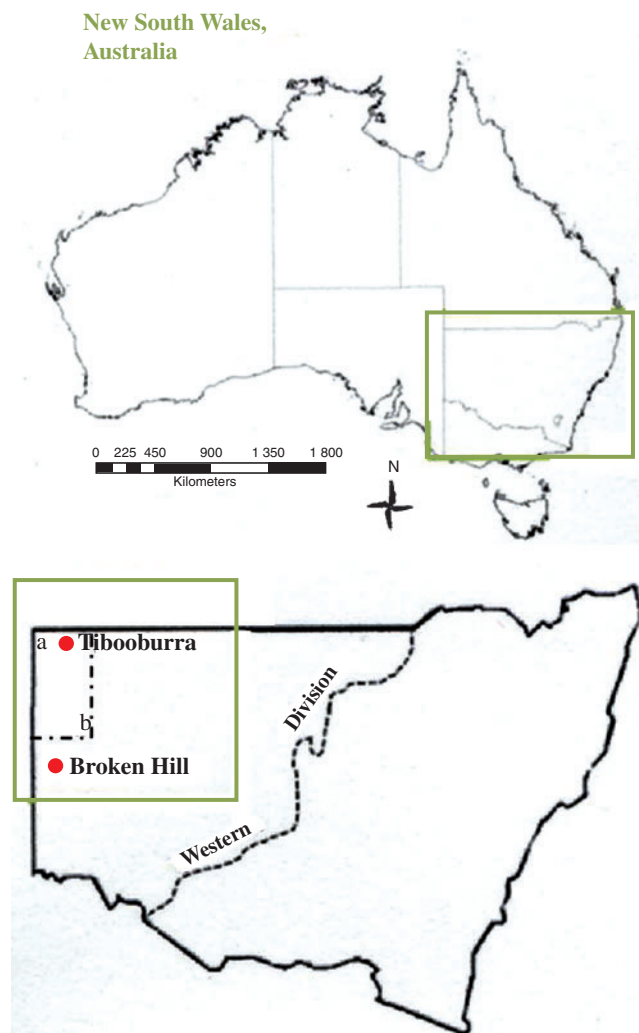
Fig. 1. Sampling region bounded by (*a*) Sturt National Park near Tibooburra to the north and (*b*) Mutawintji National Park near Broken Hill to the south and its approximate location within New South Wales, Australia.

a discrete unit influencing plant–habitat interactions (Niklas 1999), e.g. with respect to boundary layer convection, and is thus of greater relevance to this study in terms of thermodynamics than the entire compound leaf.

Nine leaf traits were measured for all sampled species: seven morphological and two spectral properties. These traits were: leaf length (mm), leaf width (mm), leaf thickness (mm), leaf area (mm^2), leaf mass per area (g m^{-2}), leaf water content (%), an inverse index of leaf pendulousness (see below), and reflectance of visible and near-infrared radiation (nm). All morphological leaf traits were measured on a minimum of three leaves from each of five plants (i.e. $\geq$15 leaves) for all 95 species. Sample leaves were collected from the outer north-facing canopy, where incident radiation is greatest in the southern hemisphere. Leaves were selected for sampling based on being healthy, fully expanded and of comparable age, i.e. having emerged in the most recent leaf flush (if perennial) or the middle of the current season (if annual or ephemeral). Where species had leaves too small to register a weight (i.e. <0.005 g), multiple leaves (5–20) were collected

and measured together and the mean obtained. In such cases, values for LMA and leaf water content were based on means. Leaf thickness was measured using digital callipers (accurate to 0.01 mm) placed away from major veins, midway along the leaf blade. If leaf thickness varied notably among leaves from the same species, up to 10 individual leaves per plant were measured to ensure that the variation was captured.

Reflectance of visible and near infrared wavelengths was measured in 0.5-nm increments between 300 and 1100 nm, as a percentage of incoming light for each species using a SpectraWiz fibre optic spectroradiometer probe and configured SL1 Tungsten Halogen light source (StellarNet Inc., Tampa, FL, USA). Prior to each leaf measurement the instrument was referenced to dark and light standards. Reflectance measurements were made on the upper surface of a representative leaf taken from the outer north-facing canopy for each species. As spectral variation can vary within a species seasonally (Ehleringer and Björkman 1978), all measurements for this study were made within a 3-week period during autumn.

Thickness, fresh weight and spectral measurements were obtained in the field. Leaf samples stored in zip-lock plastic bags with a damp sponge for at least 2 h after collection to fully hydrate before measurements of fresh weight. Leaves were then scanned on a flatbed scanner and stored for further measurements. Leaf area, leaf length, and leaf width were obtained from the scanned images using the graphic program ImageJ version 1.43r (United States National Institute of Health, Bethesda, MD, USA). Leaves were oven-dried for a minimum of 48 h at 60°C and LMA was calculated as a ratio using leaf dry mass and one-sided leaf area. Leaf water content was calculated as a percentage: (fresh weight – dry weight)/ fresh weight. All fresh and dry weights were measured using a precision analytical balance sensitive to 0.001 g.

Pendulousness was chosen as a means for estimating the extent to which leaves dangled more or less vertically. This measure was used in preference to leaf angle because the latter is often not consistent across the majority of a plant – even if leaf angle relative to the branch is maintained, branch angle may vary widely – whereas pendulous foliage generally pertains to a whole plant by virtue of gravity. The degree of pendulousness was determined by treating a leaf as the end-loading on a cantilevered beam, where the capacity to suspend that leaf horizontally depends on the strength of the supporting petiole relative to the length and/or weight of the leaf (Niklas 1992). Here, an inverse measure of leaf pendulousness was calculated using the ratio between the width of the petiole at the point where it connects to the leaf blade and the fresh weight of the attached leaf blade. Note this measure does not include the flexibility of the petiole, which would indicate the ability of the leaf to flutter (Niklas 1991). To test how robust the ratio was as a measure of pendulousness, each species was first subjectively categorised as having pendulous or non-pendulous foliage. That is (1) leaves that hung more or less vertically and/or (2) where the leaf blade was not held on a single plane and tended to droop downwards were categorised as pendulous (Fig. 2). A statistical comparison was then made between these two groups to determine the extent to which these categories correlated with leaf pendulousness. Pendulous species had significantly narrower petioles per fresh leaf mass

Table 1.   List of sample species arranged alphabetically according to family (*n* = 95)
Subfamilies of Fabaceae included here as these have only recently been combined as a single family

Species name	Family
Cheilanthes sieberi subsp. *sieberi*	Adiantaceae
Tetragonia tetragonioides	Aizoaceae
Gunniopsis quadrifida	Aizoaceae
Zaleya galericulata	Aizoaceae
Ptilotus obovatus var. *obovatus*	Amaranthaceae
Ptilotus nobilis var. *nobilis*	Amaranthaceae
Alternanthera denticulata	Amaranthaceae
Ptilotus polystachyus	Amaranthaceae
Ptilotus gaudichaudii var. *gaudichaudii*	Amaranthaceae
Calostemma purpureum	Amaryllidaceae
Sarcostemma australe	Apocynaceae
Pterocaulon sphacelatum	Asteraceae
Calotis cuneifolia	Asteraceae
Rhodanthe troedelii	Asteraceae
Leiocarpa tomentosa	Asteraceae
Cassinia laevis	Asteraceae
Senecio sp. (aff. *Cunnighammii*)	Asteraceae
Anemocarpa podolepidium	Asteraceae
Heliotropium europaeum	Boraginaceae
Trichodesma zeylanicum	Boraginaceae
Lepidium phlebopetalum	Brassicaceae
Arabidella trisecta	Brassicaceae
Harmsiodoxa brevipes	Brassicaceae
Wahlenbergia fluminalis	Campanulaceae
Polycarpaea arida	Caryophyllaceae
Casuarina pauper	Casuarinaceae
Chenopodium desertorum subsp. *anidiophyllum*	Chenopodiaceae
Enchylaena tomentosa	Chenopodiaceae
Maireana pyramidata	Chenopodiaceae
Atriplex holocarpa	Chenopodiaceae
Dissocarpus paradoxus	Chenopodiaceae
Chenopodium melanocarpum	Chenopodiaceae
Rhagodia spinescens	Chenopodiaceae
Salsola kali var. *kali*	Chenopodiaceae
Sclerolaena tricuspis	Chenopodiaceae
Einadia nutans	Chenopodiaceae
Atriplex nummularia	Chenopodiaceae
Convolvulus erubescens	Convolvulaceae
Callitris glaucophylla	Cyperaceae
Euphorbia parvicaruncula	Euphorbiaceae
Chamaesyce drummondii	Euphorbiaceae
Senna artemisioides subsp. *filifolia*	Fabaceae – Caesalpinioideae
Senna sturtii	Fabaceae – Caesalpinioideae
Indigofera australis	Fabaceae – Faboideae
Cullen australasicum	Fabaceae – Faboideae
Swainsona formosa	Fabaceae – Faboideae
Crotalaria eremaea	Fabaceae – Faboideae
Rhynchosia minima	Fabaceae – Faboideae
Acacia victoriae	Fabaceae – Mimosoideae
Acacia aneura	Fabaceae – Mimosoideae
Acacia tetragonophylla	Fabaceae – Mimosoideae
Acacia cana	Fabaceae – Mimosoideae
Erodium crinitum	Geraniaceae
Erodium cygnorum subsp. *glandulosum*	Geraniaceae
Scaevola spinescens	Goodeniaceae
Goodenia havilandii	Goodeniaceae
Haloragis aspera	Haloragaceae
Ajuga australis	Lamiaceae

(*Continued*)

Table 1.   (*continued*)

Species name	Family
Amyema maidenii	Loranthaceae
Amyema miraculosum subsp. *boormanii*	Loranthaceae
Abutilon leucopetalum	Malvaceae
Sida petrophila	Malvaceae
Sida corrugata	Malvaceae
Abutilon halophilum	Malvaceae
Hibiscus brachysiphonius	Malvaceae
Hibiscus sturtii var. *grandiflorus*	Malvaceae
Marsilea drummondii	Marsileaceae
Eremophila longifolia	Myoporaceae
Myoporum montanum	Myoporaceae
Eremophila duttonii	Myoporaceae
Eremophila latrobei	Myoporaceae
Eremophila gilesii	Myoporaceae
Eucalyptus camaldulensis subsp. *obtusa*	Myrtaceae
Corymbia terminalis	Myrtaceae
Eucalyptus coolabah subsp. *arida*	Myrtaceae
Boerhavia dominii	Nyctaginaceae
Pittosporum angustifolium	Pittosporaceae
Plantago debilis	Plantaginaceae
Dactyloctenium radulans	Poaceae
Astrebla pectinata	Poaceae
Portulaca oleraceae	Portulacaceae
Calandrinia ptychosperma	Portulacaceae
Grevillea striata	Proteaceae
Hakea eyreana	Proteaceae
Flindersia maculosa	Rutaceae
Santalum lanceolatum	Santalaceae
Dodonaea viscosa subsp. *spathulata*	Sapindaceae
Alectryon oleifolius subsp. *elongatus*	Sapindaceae
Atalaya hemiglauca	Sapindaceae
Stemodia florulenta	Scrophulariaceae
Solanum ellipticum	Solanaceae
Solanum chenopodinum	Solanaceae
Pimelea microcephala subsp. *microcephala*	Thymelaeaceae
Pimelea simplex subsp. *simplex*	Thymelaeaceae
Zygophyllum iodocarpum	Zygophyllaceae

(higher leaf pendulousness) than non-pendulous species [Student's t-test: $t(21.43) = 5.80$, $P < 0.05$, Table 2]. This result indicates that the ratio of petiole width to leaf fresh weight is an acceptable measure of leaf pendulousness for these desert species.

Statistical analyses

All data analyses were conducted using SPSS 15.0 (SPSS Inc., Chicago, IL, USA). Pearson pairwise correlations (r) were calculated among all variables. Additionally, correlations among variables were calculated for an individual family where five or more species were sampled. An independent-samples t-test was conducted to test for differences in leaf pendulousness between observed pendulous and non-pendulous species. Data normality was considered using the Kolmogorov–Smirnov test and non-normally distributed data were $\log_{10}$ (leaf width, leaf length, leaf thickness, leaf pendulousness, leaf mass per area), or arcsine square-root (reflectance of visible radiation, water content) transformed.

Results

Trait variation

For each leaf trait measured in this study, a large range in values was evident across the 95 species. A relatively wide variation in leaf size was observed: leaf area ranged from 8 to 3527 mm^2, leaf length from 4 to 367 mm, and leaf width from 1 to 62 mm (Table 2). Although not as dramatic as the 3-fold variation in leaf size, leaf thickness still varied by an order of magnitude. Not including one particularly large value representing the cladodes of *Sarcostemma australe* (at 5 mm thick), leaf thickness ranged from 0.15 to 2.2 mm (Table 2). Values for LMA also varied by an order of magnitude: from 27 to 639 g m^{-2} (Table 2). Water content varied considerably in this study, from a very dry 38% to highly succulent leaves at 98% water (Table 2). Lastly, spectral characteristics varied greatly among the 95 species measured, with the amount of visible light reflected from leaves ranging from 4 to 46% and near-infrared reflectance from 55 to 95%.

Significant bivariate relationships between each of the nine traits were numerous and therefore below are grouped into three

(*a*) *Flindersia maculosa* (Lindl.) Benth.

(*b*) *Eremophila longifolia* (R.Br.) F. Muell.

Fig. 2.   Examples of species subjectively classified as having pendulous foliage, (*a*) whereby the majority of leaves hang more or less vertically and are able to flutter with air movement and/or with the leaf blade not held on a single plain, and (*b*) with a large portion of the leaf drooping downwards, reducing exposure to overhead sunlight.

Table 2.   Summary statistics for each leaf trait

LA, leaf area; LL, leaf length; LMA, leaf mass per area; LP, an inverse index of leaf pendulousness (petiole width per leaf fresh weight); LT, leaf thickness; LW, leaf width; NIR, reflectance of near-infrared radiation; VIS, reflectance of visible radiation; WC, % leaf water content

Leaf trait	*n*	Mean	Minimum	Maximum
LL (mm)	95	52.40	4.36	366.61
LW (mm)	95	11.36	0.79	61.98
LA (mm^2)	95	568.18	8.29	3526.81
LP	95	23.57	1.26	192.50
Non-pendulous	84	26.05	1.26	192.50
Pendulous	11	4.64	1.78	9.95
LT (mm)	95	0.62	0.15	4.95
LMA (g m^{-2})	95	129.29	27.02	638.50
WC (%)	95	71.93	38.09	97.57
VIS (%)	95	16.95	3.73	45.64
NIR (%)	95	73.77	54.58	95.15

sections, which broadly correspond to how they are treated in the Discussion: relationships associated with (1) leaf pendulousness, the inverse index of pendulousness, (2) leaf size, LMA, thickness and water content and (3) leaf spectral reflectance. Non-significant correlations are presented separately below.

Pendulousness

The strongest relationship in this dataset was the negative correlation between leaf area and the inverse measure of pendulousness, LP (Table 3, Fig. 3*a*). Similar but slightly weaker relationships were found with leaf length, width and to a lesser extent LMA, all of which decreased with increasing LP (Table 3, Fig. 3*b–d*). Put another way, pendulous leaves were more likely to be larger (longer or wider) and/or have higher LMA than non-pendulous leaves.

At the family level, LP did not correlate with leaf area but was strongly negatively correlated with leaf length within

Asteraceae, Chenopodiaceae and Malvaceae and with leaf width in Asteraceae, Chenopodiaceae and Fabaceae (Table 4). That is, long and/or wide leaves were more likely to be pendulous in these families. Additionally, LP was negatively associated with water content in Myoporaceae (Table 4).

Leaf size, LMA, thickness, water content

As expected, leaf area was strongly correlated with leaf length and leaf width, which were also correlated with each other, though less strongly, indicating that large leaves tended to be so either because they were long or wide. LMA was weakly correlated with leaf length and quite strongly with leaf thickness, very strongly negatively correlated with water content and less negatively associated with leaf width (Table 3, Fig. 4*b*, *d*).

Many of the relationships that were statistically significant across the entire dataset were expressed also at the family level. In the four families best represented in the study, (Asteraceae, Chenopodiaceae, Fabaceae, and Malvaceae) LMA was strongly correlated with leaf thickness (Table 4). As with the total dataset, leaf width was inversely related to leaf thickness in Chenopodiaceae and Fabaceae; the latter family also mirrored the negative correlations between LMA and leaf width and water content (Table 4). In addition, although not evident in the pooled dataset, water content showed strong positive correlations with leaf thickness in Chenopodiaceae (Table 4).

Spectral reflectance

A strong positive relationship was found between reflectance of visible and near-infrared radiation (Table 3). Visible reflectance was also positively correlated with LMA and leaf thickness and negatively correlated with water content (Table 3, Fig. 5*b*, *d*, *f*). Similar, but weaker, relationships were found with near-infrared reflectance, which was positively correlated with LMA and negatively with water content (Table 3, Fig. 5*c*, *e*).

As with other trait relationships, many of those found across the dataset were found only within certain families,

Table 3. Pairwise comparisons for each leaf trait ($n = 95$)

Data given are r and P-value from tests for Pearson correlation between the trait pairs: LA, leaf area; LL, leaf length; LMA, leaf mass per area; LP, an inverse index of leaf pendulousness (petiole width per leaf fresh weight); LT, leaf thickness; LW, leaf width; NIR, reflectance of near-infrared radiation; VIS, reflectance of visible radiation; WC, % leaf water content. $\alpha = 0.05$. Note: bold, r-values ± 0.45; underlined, $P < 0.05$

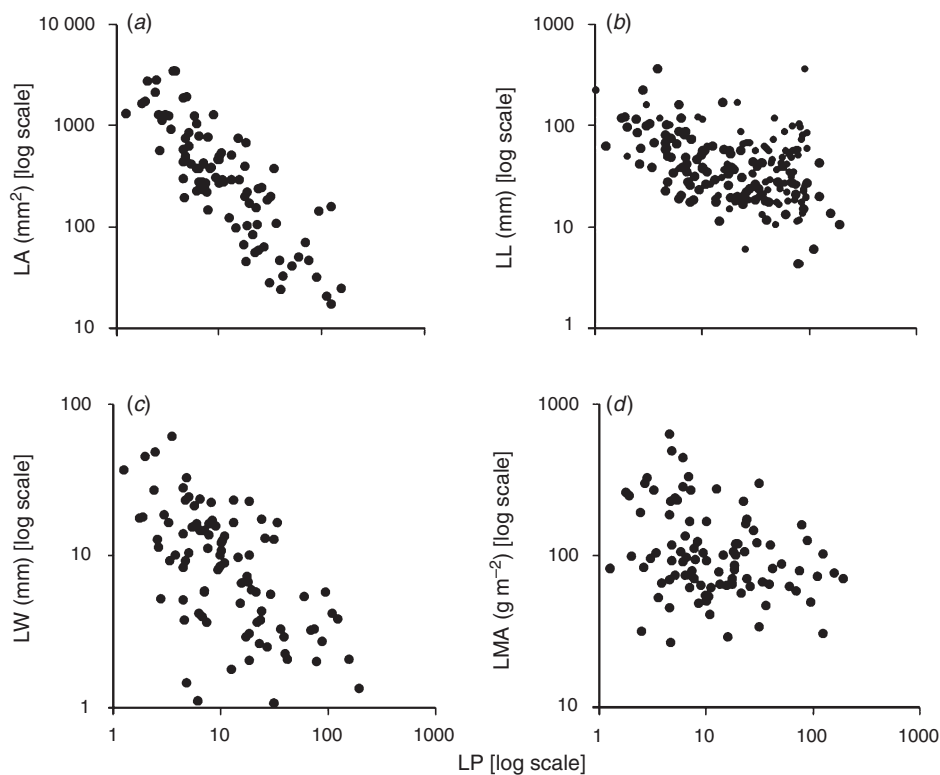| .	LL (mm)	LW (mm)	LA (mm^2)	LP	LT (mm)	LMA (g m^{-2})	WC (%)	VIS (%)	NIR (%)
LL (mm)	.								
LW (mm)	0.31	r							
	0.00	P							
LA (mm^2)	**0.76**	**0.76**	r						
	0.00	0.00	P						
LP	**−0.67**	**−0.58**	**−0.85**	r					
	0.00	0.00	0.00	P					
LT (mm)	0.11	−0.36	−0.13	−0.11	r				
	0.27	0.00	0.20	0.27	P				
LMA (g m^{-2})	0.22	−0.28	0.02	−0.29	**0.51**	r			
	0.03	0.01	0.82	0.01	0.00	P			
WC (%)	−0.19	0.17	−0.07	0.12	0.17	**−0.62**	r		
	0.06	0.11	0.51	0.25	0.09	0.00	P		
VIS (%)	−0.07	−0.12	−0.07	0.01	0.35	0.42	−0.25	r	
	0.52	0.26	0.50	0.92	0.00	0.00	0.01	P	
NIR (%)	−0.10	−0.24	−0.10	0.04	0.06	0.29	−0.38	**0.58**	r
	0.34	0.02	0.36	0.67	0.55	0.00	0.00	0.00	P

Fig. 3. Relationships between an inverse index of leaf pendulousness (LP) and (*a*) leaf area (LA), (*b*) leaf length (LL), (*c*) leaf width (LW) and (*d*) leaf mass per area (LMA).

in which these relationships were very strong. Myoporaceae was the only family in which visible and near-infrared reflectance were correlated with one another (Table 4). Visible reflectance was strongly correlated with leaf thickness in Asteraceae and with LMA in Myoporaceae, whereas near-infrared reflectance and LMA were correlated only in Malvaceae (Table 4). Water content was strongly negatively associated with near-infrared reflectance in Amaranthaceae, Asteraceae and Myoporaceae and with visible reflectance in Amaranthaceae (Table 4).

Table 4.   Significant pairwise comparisons for families where ≥5 species were sampled

Amaranthaceae ($n = 5$), Asteraceae ($n = 7$), Chenopodiaceae ($n = 11$), Fabaceae ($n = 11$), Malvaceae ($n = 6$), Myoporaceae ($n = 5$). Data given are r and P-value from tests for Pearson correlation between the trait pairs: LL, leaf length; LMA, leaf mass per area; LP, an inverse index of leaf pendulousness; LT, leaf thickness; LW, leaf width; NIR, reflectance of near-infrared radiation; VIS, reflectance of visible radiation; WC, % leaf water content. $\alpha = 0.05$, *$P < 0.05$; **$P < 0.01$. Note: bold, r-values were also significant when all data points were considered ($n = 95$ spp). The table has been divided into three sections to reflect the three key findings pertaining to pendulousness, LMA–WC–LT relationships and reflectance

Relationship	Amaranthaceae	Asteraceae	Chenopodiaceae	Fabaceae	Malvaceae	Myoporaceae
LP-LL	–	**−0.820***	**−0.637***	–	**−0.890***	–
		0.024	**0.035**		**0.017**	
LP-LW	–	**−0.792***	**−0.704***	**−0.624***	–	–
		0.034	**0.016**	**0.040**		
LP-WC	–	–	–	–	–	−0.879*
						0.050
LMA-WC	–	–	–	**−0.865****	–	–
				0.001		
LMA-LT	–	**0.807***	**0.661***	**0.907****	**0.960****	–
		0.028	**0.027**	**0.000**	**0.002**	
WC-LT	–	–	0.753**	–	–	–
			0.007			
LMA-LW	–	–	–	**−0.803****	–	–
			0.003			
LT-LW	–	–	**−0.842****	**−0.854****	–	–
			0.001	**0.001**		
VIS-WC	−0.921*	–	–	–	–	–
	0.026					
VIS-LT	–	0.899**	–	–	–	–
		0.006				
VIS-LMA	–	–	–	–	–	0.928*
						0.023
NIR-WC	−0.882*	−0.953**	–	–	–	−0.935*
	0.048	0.001				0.020
NIR-LMA	–	–	–	–	0.832*	–
					0.040	
NIR-VIS	–	–	–	–	–	0.907*
						0.033

Non-significant relationships

Several leaf trait pairs were not correlated across species (Table 3). No relationships were found between leaf pendulousness and leaf thickness, water content or spectral properties (reflectance of visible and near-infrared wavelengths). Nor were significant correlations found between leaf area and LMA, leaf thickness or water content. No relationship was found between leaf thickness and water content; however, separation of the data based on succulence (water content > 75%) indicates a bimodal relationship, where leaf thickness co-varies with water content for succulent leaves but replaces water content in non-succulent leaves. There were no significant relationships between spectral reflectance and any of the leaf size traits (area, length and width), nor between infrared reflectance and leaf thickness. As mentioned above, many patterns evident for the whole dataset were not significant within certain families (Table 4).

Discussion

This study investigated relationships between leaf traits that help to minimise extreme high temperature stress. In so doing, more commonly measured traits were included along with those that have not been incorporated in multi-trait datasets

pendulousness before: and spectral reflectance of VIS and near-infrared reflectance. Our aim was to determine if any variation in these traits within a biome varied in concert with, or potentially replaced, other traits in protecting leaves against heat stress. Such trait replacement could suggest alternative strategies for providing morphological protection against heat stress among desert species. Large variations in traits were found, as were several novel relationships between them. Below we discuss these relationships with a focus on three key aspects of leaf thermal protection: pendulous foliage, thermal mass/thickness, and spectral reflectance.

Pendulous foliage

Among our desert species, petiole width per leaf fresh weight (an inverse index of leaf pendulousness) decreased with increasing leaf size (length, width and area) and to a lesser extent LMA (Fig. 3), i.e. leaves that were pendulous tended to be large and often have high LMA. Such relationships have rarely been documented. There is evidence within a single South American species for plants growing in arid regions having more pendulous leaves with high LMA than those in more humid regions (Barchuk and Valiente-Banuet 2006). Research into the relationship between pendulous foliage and
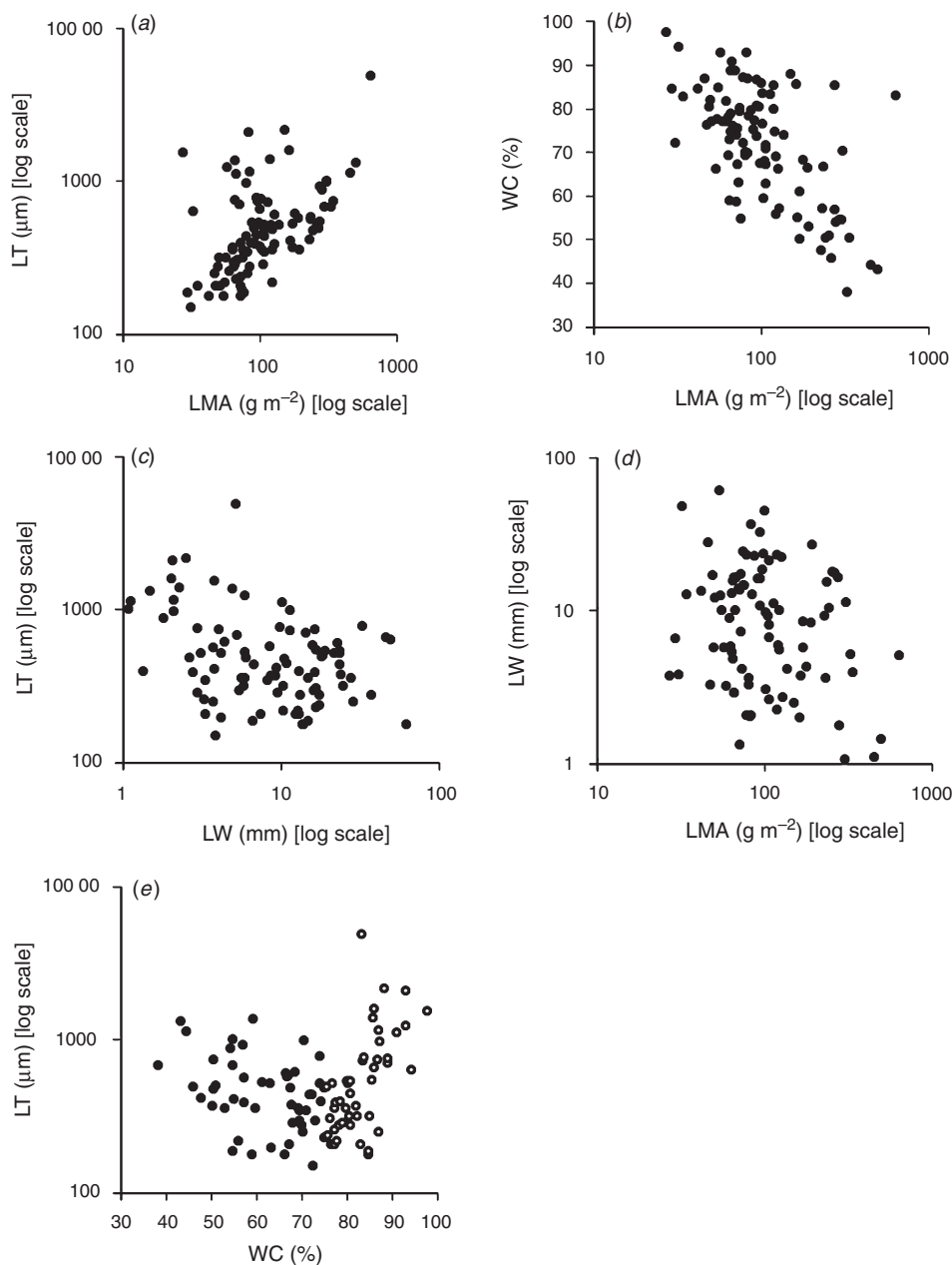
Fig. 4. Relationships between leaf mass per area (LMA) and (*a*) leaf thickness (LT) and (*b*) water content (WC), (*c*) leaf width (LW) and LT, (*d*) LMA and LW, and (*e*) WC and LT. (*e*) Two relationships with LT are highlighted according to whether species have succulent leaves (open circles; WC < 75%; $r = 0.603$, $P = 0.000$) or non-succulent leaves (closed circles; WC > 75%; $r = -0.369$, $P = 0.008$).

leaf size is scant but from an adaptive standpoint it could be imagined that pendulousness would not co-occur with large leaves. The production of large leaves in temperate environments is generally associated with increased light capture and displaying these leaves horizontally increases that possibility, given sufficient resources. To display a leaf horizontally requires a proportionately greater investment in supporting structures, particularly petioles, as leaf size (Niinemets and Kull 1999) and LMA (Royer *et al*. 2007) increases. For a large number of species across multiple biomes, the proportion of leaf dry mass represented by the

petiole scales with leaf fresh weight at just over 1 : 1 so that in large leaves the petiole fraction can be up to 50% compared with <1% for small leaves (Niinemets *et al*. 2007).

Increasing investment in support, however, not only requires the availability of resources to invest but also implies that supporting a leaf horizontally is beneficial in a given environment. Desert regions are generally nutrient-poor and solar radiation can be excessive so displaying leaves horizontally would both be costly and risky in terms of thermal damage. Indeed, there is evidence to suggest that the petiole-leaf weight scaling relationship is weaker in less
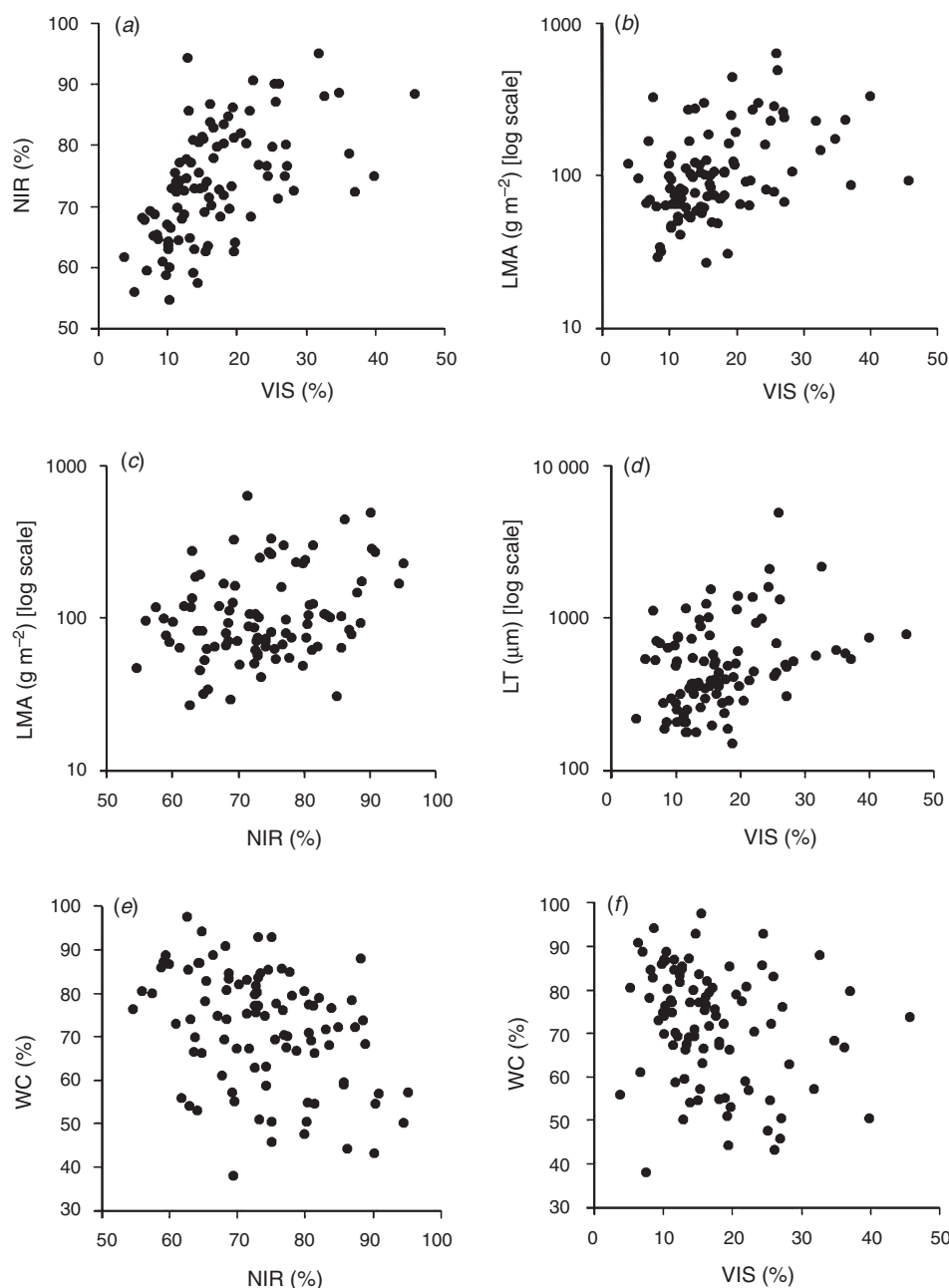
Fig. 5. Relationships between spectral reflectance of near-infrared radiation (NIR) and (*a*) reflectance of visible radiation (VIS), (*c*) leaf mass per area (LMA) and (*e*) water content (WC), and between VIS and (*b*) LMA, (*d*) leaf thickness (LT) and (*f*) WC.

favourable environments (Niinemets *et al.* 2007). Among the desert species in the present study, petiole dry mass fraction scaled at –0.1, with investment in petiole construction in the largest leaves representing <1% compared with ~2.5% for small leaves (results not shown), indicating that the production of large leaves is accompanied by a comparatively lower investment in structural support. Although this is in stark contrast to the relationships found across biomes, reducing investment in petiolar support with increasing size or LMA is intuitive within the context of a single desert biome. The

production of poorly supported – and therefore pendulous – leaves may represent a comparatively low cost way of displaying large, long-lived leaves able to avoid damaging leaf temperatures.

Leaf thermal mass – thickness, succulence and LMA

The temperature damping effect of high leaf thermal mass can be achieved through increasing thickness, either via dry matter density or succulence (Leigh *et al.* 2012). High leaf density

can result from numerous small cells per volume of leaf or from individual cells having high biomass (thick cell walls) and is hence often associated with high LMA (Castro-Diez *et al*. 2000). Succulence and LMA need not be mutually exclusive; some of the highest recorded measures of LMA are for desert succulents as a result of greater thickness (Poorter *et al*. 2009) and/or tough epidermis (Vendramini *et al*. 2002). However, hard-leafed succulents are by no means the rule and were not predominant in the present study, where high water content corresponded with low LMA (Fig. 4*b*). In turn, LMA was significantly correlated with leaf thickness (Fig. 4*a*), suggesting that these high LMA leaves have the potential to protect against rapid fluctuations in leaf temperature. While associating LMA with thermoregulation is perhaps a novel suggestion, it supports the well established assignment of LMA along the leaf economics spectrum, where high LMA leaves live longer. Particularly for Australian arid zone plants, which are subject to both nutrient-poverty and high temperature extremes, high LMA potentially serves a dual role in leaf survival: protecting against both structural and thermal damage.

In contrast to these patterns with LMA, the relationship between water content and leaf thickness was less clear in our dataset. Overall, water content was not significantly correlated with leaf thickness but closer scrutiny of this relationship suggests that it was bimodal, showing two contrasting patterns (Fig. 4*e*). For succulent leaves (i.e., water content >75%), leaf thickness increased with water content, whereas for non-succulent leaves, the opposite appeared to be the case. Phylogeny was partially driving the former relationship: a strong positive association between leaf thickness and water content was evident among the Chenopodiaceae species, all of which were deemed succulent using the above definition (Table 4). However, other families had both succulent and non-succulent representatives so phylogeny cannot be the only cause of this bimodal pattern. Either way, for these succulent species, a beneficial consequence of high water content would be the damping effect this afforded during transient periods of exposure to extreme high temperatures. For the non-succulent species, where leaf thickness and water content were inversely associated, increased structural material could provide a similar temperature-damping advantage to that afforded by increased water content in succulent leaves.

With respect to thick leaves, then, our desert species are protected if they have high LMA or water content; however, there is an apparent lack of alternative structural thermal protection for thin leaves. That is, we did not find negative correlations between leaf thickness and other thermal protective traits. In fact, one trait that might be expected to co-vary with leaf thickness is leaf width – with thin leaves being protected from high average temperatures through reduced leaf size – but leaf width was instead negatively correlated with thickness. There are various explanations for the presence of seemingly unprotected thin leaves. First, other traits that we did not measure, such as physiological thermal tolerance, afford protection to these thin leaves. Second, several different traits may replace thickness in thermal protection, which a bivariate correlation could not detect. Third, these thin leaves are in fact vulnerable to thermal damage and are therefore potentially short-lived. The latter possibility is currently being explored with respect to annual versus perennial species.

Spectral reflectance

While the leaves of many desert species are highly reflective, particularly to visible wavelengths, many are not. These less reflective leaves would be vulnerable to heat stress if they did not possess other morphological means for reducing high leaf temperatures, such as reduced size, pendulousness or increased thickness/thermal mass. In our study, this prediction of a trade-off with leaf reflectance was borne out with only one other thermal protective trait: water content. The negative association between reflectance (particularly of near-infrared wavelengths) and water content may have a functional cause – water absorbs strongly in the near infrared so high leaf water content would be expected to result in a high absorption signal (Woolley 1971). Irrespective of its proximal cause, this negative relationship suggests that the leaves of non-succulent species may be afforded protection against excess radiation through increased reflectance. This may especially be the case for families in which this negative correlation between water content and spectral reflectance was particularly strong: Amaranthaceae, Asteraceae and Myoporaceae.

Whereas the association between spectral reflectance and water content suggested a replacement of one trait for another, the opposite could be said for the co-occurrence of reflectance with other thermal protective traits. In particular, reflectance of near-infrared wavelengths increased with LMA and visible reflectance increased with both LMA and thickness. Again, the co-occurrence of these traits is intuitive when considered in terms of LMA's association with leaf longevity and survival. Across environmental gradients, both LMA (Poorter *et al*. 2009) and leaf reflectance (Ehleringer 1981; Skelton *et al*. 2012) increase in high temperature ecosystems. However, in spite of broad patterns across biomes, variation among reflectance and LMA clearly occurs *within* a desert biome, even if this variation may be more subtle. Our findings suggest that one end of the spectrum of co-variation represents structurally tough leaves that are protected both from herbivores and excess radiation while maintaining slow photosynthetic rates. At the other end are species capable of faster growth but whose leaves are short-lived due to higher rates of both structural and thermal damage.

Conclusion

Desert plants must withstand extreme high temperature stress and the traits that protect against thermal damage can vary markedly among species, even within a desert biome. Our aim was to determine if leaf thermal protective traits varied predictably in association with other traits, either as two traits co-varying or with one trait replacing another. Among the 95 desert species in this study, a small number of negative correlations between thermal protective traits suggested the replacement of one trait by an alternative mode of protection. Most notably, pendulous leaves were more likely to be large than non-pendulous leaves, suggesting that the pendulous habit is a means of arraying large leaves capable of capturing sunlight at low angles, making them less prone to thermal damage. Our results also showed that leaf water content, which increases the temperature damping effect via thermal mass, decreased with increasing LMA and spectral reflectance (of visible and near-infrared wavelengths), both of which could afford protection when water content was low.

Along with these bivariate relationships suggesting trait replacement, it is also plausible that several traits cluster to form distinct suites of protective traits, a possibility currently being investigated by the authors.

In contrast to the small number of trait replacement patterns, co-variation of thermal protective traits was found to occur repeatedly among the desert species in this study. Interestingly, a trait found to be common in many of these relationships was LMA, something not generally associated with thermal protection. As LMA increased, so too did certain other protective traits: high LMA leaves were more pendulous, thicker, narrower and more reflective than low LMA leaves. In theory then, these thermal protective traits fall along the leaf economics spectrum, with leaf longevity increasing by virtue of increased protection not only against structural damage but also against heat stress. This novel idea has arisen through the inclusion of new leaf traits, specifically pendulousness and spectral reflectance, in a multiple trait dataset based within a single biome. To determine the robustness of the above claim, thermal protective traits should be included in analyses within different ecosystems and across multiple biomes.

Acknowledgements

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