

Ecological variation in leaf biomechanics and its scaling with tissue structure across three mediterranean-climate plant communities

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Summary

1. The mechanical resistance of leaves has key ecological implications but its basis has not been well understood, particularly at the tissue scale. We tested the hypotheses that leaf mechanical resistance should be a function of tissue density, increasing from the lamina to the midrib, and higher in drought-tolerant than drought-avoiding species.
2. In a common garden study, we quantified nine leaf biomechanical traits, including measurements of material and structural resistance, and in addition 17 morphological traits, at the tissue and whole-leaf scales, for 21 species from three semi-arid communities of California, USA.
3. The mechanical properties of leaves depended strongly on tissue density. Material resistance was significantly greater in the midrib than in the leaf lamina, and tissue resistances were significantly correlated among tissues, lower in deciduous coastal sage species and higher in evergreen drought-tolerant chaparral species. The proportion of the biomass invested in the midrib was lower in species bearing midribs and laminas of high material resistance.
4. Our results support the hypothesis of a hierarchical partitioning of leaf mechanical resistance among leaf tissues reflecting the investment of dry mass. Also, our data indicated a mechanical compensation in leaf design, where leaves with high material resistance and density deploy a relatively minor proportion of support tissue in the midrib. Finally, our results establish a quantitative basis for differences among communities in leaf biomechanics. Our results supported the classical characterization of the mediterranean-climate flora of California according to the dramatic increase in the mean leaf mechanical resistance from species of coastal sage to chaparral, with diverse leaf types in the Mojave Desert species.

Key-words: allometry, chaparral, coastal sage, Mojave Desert vegetation, plant economics, sclerophylly, stiffness, strength

Introduction

Plant ecologists have summarized a substantial amount of the variation within and across biomes in leaf size and morphology into a few essential trade-offs. For example, higher investments in leaf dry mass per area (LMA) have been related to leaf nutrient stoichiometry and leaf longevity (Reich, Walters & Ellsworth 1997; Wright *et al.* 2004; Niklas *et al.* 2007; Kattge *et al.* 2011). These differences in leaf construction costs are closely linked with variation in leaf mechanical traits across species and biomes (Read & Sanson 2003; Read, Sanson & Lamont 2005; Onoda *et al.* 2011). However, our knowledge of the underlying mechanisms that determine the strength and stiffness of leaves

remains scarce, particularly when considering the scale of tissues within the leaf, and leaves from different communities. Increasing our understanding of the basis of variation in leaf biomechanics may have important implications for plant carbon budgets, plant–animal interactions and nutrient cycling (Díaz *et al.* 2004; Balsamo *et al.* 2006; Read *et al.* 2006; Alvarez-Clare & Kitajima 2007; Peeters, Sanson & Read 2007; Kitajima & Poorter 2010).

There are compelling reasons to expect that overall leaf mechanics should depend on the construction costs of the different leaf tissues. In mechanical terms, a leaf acts as a cantilever beam, where any increase in mass and length will increase its susceptibility to bending (Niinemets & Fleck 2002a,b; Cooley, Reich & Rundel 2004; Niinemets, Portsmuth & Tobias 2007). Therefore, to optimize the surface for sunlight interception and, at the same time, to cope with

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mechanical forces such as wind (Anten *et al.* 2010), leaves have been selected to invest a significant fraction of their total dry biomass in mechanical support tissue (up to 70%) (Niinemets, Portsmuth & Tobias 2006; Niinemets *et al.* 2007). This investment is size dependent, as larger leaves tend to have larger LMA, with larger midribs (Milla & Reich 2007; Niklas *et al.* 2007; Sack *et al.* 2012). As previously noted, there is a strong relation between leaf tissue mechanical resistance and dry matter content or density of leaf tissues (Read & Sanson 2003; Read, Sanson & Lamont 2005; Kitajima & Poorter 2010; Onoda *et al.* 2011; Kitajima *et al.* 2012); therefore, we can expect that leaf mechanical resistance should be hierarchically partitioned according to differences in tissue density, with most in the midrib and other major veins (i.e. second- and third-order veins), less in minor veins (fourth order and higher), and least in leaf mesophyll tissue (Choong 1996), but this expectation needs testing, particularly because for many species, the leaf vein xylem and sclerenchyma are the only leaf tissues directly involved in support, while for other species, leaf lamina also provides direct mechanical support, for example, for sclerophyllous species with lignified epidermis and/or hypodermis (Edwards *et al.* 2000; Niinemets, Portsmuth & Tobias 2007; Wang *et al.* 2010).

The objective of this study was to determine the basis of the mechanical resistance of the leaf, and its relationship with the suite of traits that are involved in sclerophylly, particularly at the tissue level. We tested the relationship of the mechanical resistance of leaves to the density of support tissue using 21 species native to chaparral, coastal sage and Mojave Desert communities of Southern California, USA. Although not previously quantified, there are clear shifts in leaf biomechanics among these communities distributed across an aridity gradient, as implied in their very nomenclature. Indeed, since the classic works of Schimper (1903) and Cooper (1922), the Californian vegetation has been distinguished into types including chaparral, a vegetation characterized by sclerophylly, 'thick, stiff and hard, ordinarily flat and evergreen leaves' (Schimper 1903; Cooper 1922), and coastal sage, which includes species with deciduous and 'soft and white pubescent' leaves, also called malacophylls (Epling & Lewis 1942; Walter 1985). Species of these communities diverge in the typical mechanisms employed to tolerate drought (Jacobsen *et al.* 2008, 2009), with greatest cavitation resistance, less sprouting and more negative field water potentials in the chaparral and least in the coastal sage, and the Mojave Desert spanning a wider range of variation, reflecting in part the diverse water relations in this community in which water becomes highly available in occasional pulses (Pratt *et al.* 2007; Jacobsen *et al.* 2008). One might expect that species with traits that favour resistance to xylem cavitation to typically also favour sclerophylly, to support their greater drought tolerance (Balsamo *et al.* 2003; Bartlett, Scoffoni & Sack 2012).

Using these three plant communities that differ in their water use and drought tolerance (Jacobsen *et al.* 2008), we

tested three hypotheses relating to leaf mechanical resistance: first, within and across species the mechanical resistance of leaf tissues should increase with leaf size and density. Second, leaf mechanical resistance should differ across species due to variation in within-leaf mechanical traits, and midrib and lamina structural traits. Third, leaf mechanical traits should vary across species and communities, reflecting greater selection for leaf life span and drought tolerance with higher chronic aridity. We tested these hypotheses by quantifying nine biomechanical traits, including measurements of strength and stiffness, and 17 leaf morphological traits relating to the dry mass composition, for the whole leaf, the midribs and the leaf lamina.

Materials and methods

STUDY SITE AND SPECIES SAMPLING

Leaves were collected from adult plants in the Rancho Santa Ana Botanical Garden, Claremont, CA, USA (34°06' 49"N, 117°42'55" W), from January to April 2012. We selected seven common species from chaparral, seven from coastal sage and seven from Californian Mojave Desert plant communities (see Table S1, Supporting information). Coastal sage and chaparral communities experience similar levels of precipitation of approximately 400 mm per year, and Mojave Desert has much lower levels of precipitation (138 mm per year). Average summer and winter temperatures are 23 °C and 9 °C in chaparral, 26 °C and 10 °C in coastal sage and 26 °C and 4 °C in Mojave Deserts (Jacobsen *et al.* 2007). To sample for variation among species, and also for leaf size variation within species, for each of three individuals per species (range of basal diameters and heights 0.8–16.5 cm; and 0.4–5.5 m); we sampled four sun-exposed terminal branches that had at least nine mature leaves. Detached branches were immediately stored in water-filled buckets, covered with black plastic bags and transported to California State Polytechnic University, Pomona, to perform leaf tensile tests within the same day.

LEAF BIOMECHANICAL TRAITS

To determine leaf biomechanical traits, we conducted static tensile tests and calculated standard indices. We tested specimens in static tensile tests using screw side-action grips in an INSTRON 3345 mechanical testing machine with a 5 kN load cell (INSTRON Corporation, Canton, MA, USA), with the cross-head moving at a rate of 20 mm/min. We tested leaves in three treatments, and for each, we used three leaves from one branch per individual per species, selecting mature leaves in the best possible condition that clearly differed in size, resulting in nine leaves tested for each treatment for each species. Whole leaves were tested with their midrib aligned with the load applied (whole-leaf vertical treatment, 'leaf'). Additionally, the leaf lamina was sectioned to expose only the midrib and tested aligned with the load applied (midrib treatment, 'midrib'), or using rectangular section of leaf lamina of $\approx 3 \text{ cm} \times 0.5 \text{ cm}$ cut parallel to the midrib, halfway between midrib and margin (lamina treatment, 'lamina'). The lamina samples included secondary veins, running in perpendicular direction to the load applied. Grips were tight enough to generally avoid slippage, but when that occurred, the test was discarded and repeated. The original length of the sample (i.e. the vertical length of the sample between the upper and lower grips, L_0) was 10 mm when leaves were longer than 2 cm from base to apex, and when leaves were shorter than 2 cm L_0 was 5 mm for whole leaves, and 3.5 mm for midribs and laminae. We also measured the horizontal

length of the sample (leaf width, W) and the leaf thickness (T) of the segments used for mechanical tests. Tensile tests generally are performed using samples with a length 8–10 times their width, to allow the samples to narrow uniformly in the centre, thus avoiding an apparent increase in stiffness associated with wider sample shapes (Vincent 1990). However, this shape standardization could not be fulfilled due to the small leaf sizes of our study species.

Prior to testing each treatment, we measured the basal, central and apical diameters of the midrib and the lamina thickness in another set of leaves of comparable size and tapering values were entered into the BLUEHILL INSTRON software (INSTRON Corporation, Canton MA, USA), which plotted the stress-strain curve during each test. The linear portion of the curve, previous to the catastrophic failure of the tissue, was used to calculate the maximum slope (M_{slope} , a calculation of the tensile modulus of elasticity not normalized by sample dimensions; units, N/m) and maximum load at the moment of breakage (M_{load} ; units, N), two variables used to determine leaf tensile modulus of elasticity and strength.

The overall mechanical properties of biological composites depend on the material composition and the structural properties, that is the dimension and shape of the object (Aranwela, Sanson & Read 1999; Read & Sanson 2003; Vogel 2003; Onoda *et al.* 2011). We measured three properties of the material resistance of leaves based on tensile tests (Balsamo *et al.* 2003, 2006): the leaf tensile modulus of elasticity (TME; in N/m^2 , where MN/m^2 are equivalent to MPa), a measure of the intrinsic resistance of the leaf tissue to elastic deformation; the leaf tensile strength (TS; in N/m^2), the force required to fracture the leaf per unit of sectional area (Choong *et al.* 1992; Read & Stokes 2006; Sanson 2006); the flexural stiffness (FS, units of $\text{N} \times \text{m}^2$), calculated as the product of the TME and the second moment of area I (a property of the cross-sectional geometry of an object, with units of length to the fourth power, m^4). The FS measures the resistance to bending of a structure, where the larger the value, the smaller the deformation under a given load (Etnier 2001; Niinemets & Fleck 2002a; Vogel 2003; Gere & Goodno 2009). For whole leaves, midribs and leaf lamina sections, the TME was calculated as $(M_{\text{slope}} \times (L_o/W \times T))$, where W is the horizontal length of the sample clamped between the grips (leaf width), and T is the leaf lamina or midrib thickness. The TS was calculated as $(M_{\text{load}}/W \times T)$ (Gere & Goodno 2009). For the midrib, I was calculated using the formula for a tapering cylinder, as $I = (\pi/8) \times (r_1 r_2) \times (r_2 r_3)$, where r_1 , r_2 and r_3 are, respectively, the radii of the midrib at the base, central and terminal portions. For leaf lamina sections, I was calculated as a rectangle, $I = T \times ((b \times h^3)/12)$, where b is the base of the lamina section of 0.5 cm, h is the length of the section, that is 3 cm, and T is leaf thickness. For whole leaves, I was calculated as an ellipse, with $I = (\pi/4) \times LW^3$, where L is leaf length and W is leaf width (Niklas 1992; Gere & Goodno 2009).

LEAF MORPHOLOGICAL TRAITS

After the tensile tests were performed, leaves were scanned in both adaxial and abaxial orientations using a flatbed scanner (Epson Perfection 4490; Epson Corp., Nagano, Japan). Leaves were then sectioned in a glass petri dish over a white light transilluminator (Model TW-43, UVP, Upland, CA, USA) and under a dissection microscope (OM2300 ST, Omano, Zhejiang, China) to separate midrib from lamina tissue. We measured the basal, central and apical diameter of petioles and midribs ($D_{\text{petiole, b}}$, $D_{\text{petiole, c}}$, $D_{\text{petiole, a}}$; $D_{\text{midrib, b}}$, $D_{\text{midrib, c}}$, $D_{\text{midrib, a}}$, respectively) and lamina thickness (T) under the microscope to the nearest 0.01 mm using callipers (Fisher Scientific, Waltham, MA, USA). After sectioning, laminae and midribs were oven dried 3 days at 70 °C before measuring dry mass. Using IMAGE J software (<http://imagej.nih.gov/ij/>), we measured leaf petiole and midrib length, leaf

width and total area. We calculated the volume of leaf lamina as $V_{\text{lamina}} = (\text{Leaf total area} - \text{midrib area}) \times T$; in mm^3). We calculated the volume of the midrib (V_{midrib} ; in mm^3) as a truncated cone, using the midrib diameters and length (L_{midrib}): $V_{\text{midrib}} = (1/3 \pi) \times (L_{\text{midrib}}/2) \times ([D_{\text{midrib, b}}/2]^2 + [D_{\text{midrib, b}}/2] \times [D_{\text{midrib, c}}/2] + [D_{\text{midrib, c}}/2]^2)$. The analogous formula was used to calculate the volume of petioles (V_{petiole} ; in mm^3).

We calculated leaf density ($\rho_{\text{leaf}} = M_{\text{leaf}}/V_{\text{leaf}}$; in g/mm^3), leaf mass per area (LMA = M_{leaf}/LA ; in g/mm^2), leaf lamina density ($\rho_{\text{lamina}} = M_{\text{lamina}}/V_{\text{lamina}}$; in g/mm^3) and leaf lamina mass per area (LMA_{lamina} = $M_{\text{lamina}}/\text{Area}_{\text{lamina}}$; in g/mm^2).

We calculated the dry mass fraction of the leaf that was invested in support, F_L , as the ratio of the mass of the midrib (M_{midrib}) and the mass of the whole leaf (M_{leaf}) (Niinemets, Portsmuth & Tobias 2006). We also calculated the ratio $\rho_{\text{midrib}}/\rho_{\text{lamina}}$ as a second index of relative investment in structural support. Independently of the overall leaf density, species with high ratios should bear midribs of high density relative to their laminae, whereas species with low ratios should have denser laminae relative to their midribs (Table 1).

STATISTICAL ANALYSES

Prior to analyses, we ln-transformed TME and TS data to improve normality and heteroscedasticity. We assessed a priori-hypothesized relationships between traits using standard major axes (SMA; using SMATR 2.0; Warton *et al.* 2006). To present the intercorrelation patterns among all traits, we generated a correlation matrix. We avoided interpretation of non-hypothesized relationships, for which Bonferroni corrections would be necessary to establish statistical significance (Garcia 2003; Moran 2003). We summarized the patterns of correlation of the sets of leaf morphological and biomechanical traits using a principal component analysis (PCA), including the 20 species for which we had data for all variables, and excluding *Larrea tridentata*, which had leaves too small for the midrib tensile measurements.

To compare leaf traits across communities and species, we conducted nested ANOVA analyses, with species nested within communities as explanatory variables. To compare the variation in midrib, lamina and whole-leaf densities, we used a nested two-way repeated measures ANOVA, with species nested within communities as the first factor, treatment as the second factor and individuals as the repeated measurement. These analyses were performed in Minitab 15 (Minitab Inc., State College, PA, USA).

To assess within species relationships, we determined the proportion the proportion of species that had the correlations hypothesized to hold between traits. We tested overall significance of within-species correlations of traits using binomial proportion hypothesis tests (using Minitab 15). For the given hypothesized relationships, we also compared the mean values of the within species coefficients of determination vs. the across species coefficient of determination, using *t*-test (or Mann–Whitney rank-sum tests) hypothesis tests (Minitab 15, Minitab Inc., 2007).

Results

VARIATION IN LEAF BIOMECHANICS ACROSS SPECIES, AND ITS SOURCES WITHIN THE LEAF TISSUES

Leaf mechanical traits, structure and morphology varied strikingly across species and communities (Table 1; Figs 1–4). Midrib, lamina and leaf densities also differed strongly within individual leaves and across species and communities (nested two-way repeated measures ANOVA; $P < 0.001$ for all factors and interactions).

Table 1. Leaf traits measured in this study, including the minimum species mean, the mean of the species means, and the maximum species mean

Trait	Symbol	Units	Min – Mean – Max	Spp	Com
Leaf area	LA	mm ²	69 – 612 – 2753	***	***
Total leaf mass per area	LMA	g cm ⁻²	0.04 – 0.15 – 0.33	***	***
Lamina leaf mass per area	LamMA	g cm ⁻²	0.03 – 0.13 – 0.30	***	***
Fraction of tissue invested in support	F_L	g g ⁻¹	0.10 – 0.24 – 0.62	***	***
Density midrib	ρ_{midrib}	g cm ⁻³	0.17 – 0.42 – 0.97	***	***
Density lamina	ρ_{lamina}	g cm ⁻³	0.12 – 0.38 – 0.59	***	***
Total leaf density	ρ_{leaf}	g cm ⁻³	0.21 – 0.36 – 0.63	***	***
Ratio midrib/lamina density	$\rho_{\text{midrib}}/\rho_{\text{lamina}}$	—	0.11 – 0.37 – 0.58	***	***
Lamina thickness	T	mm	0.38 – 0.82 – 1.57	***	***
Midrib diameter, central	$D_{\text{midrib, c}}$	mm	0.09 – 0.23 – 0.49	***	***
Midrib diameter, basal	$D_{\text{midrib, b}}$	mm	0.29 – 0.64 – 1.26	***	***
Leaf volume	V_{leaf}	mm ³	23 – 276 – 1297	***	***
Midrib volume	V_{midrib}	mm ³	0.88 – 22 – 131	***	***
Petiole volume	V_{petiole}	mm ³	0 – 41 – 177	***	***
Lamina volume	V_{lamina}	mm ³	21 – 214 – 1034	***	***
Midrib and petiole dry mass	M_{support}	g	0.001 – 0.02 – 0.08	***	***
Lamina dry weight	M_{lamina}	g	0.006 – 0.09 – 0.56	***	***
Midrib length	L_{midrib}	mm	3.62 – 40.6 – 115	***	***
Petiole length	L_{petiole}	mm	0 – 6.2 – 23.6	***	***
Leaf width	W_{leaf}	mm	4.3 – 16.7 – 47.5	***	***
Tensile modulus of elasticity, lamina	$\text{TME}_{\text{lamina}}$	MPa	0.39 – 3.9 – 16.4	***	***
Tensile modulus of elasticity, midrib	$\text{TME}_{\text{midrib}}$	MPa	0.52 – 6.8 – 41.1	***	***
Tensile modulus of elasticity, whole leaf	TME_{leaf}	MPa	0.64 – 4.2 – 18.7	***	***
Tensile strength, lamina	$\text{TS}_{\text{lamina}}$	MPa	0.75 – 4.7 – 13.1	***	***
Tensile strength, midrib	$\text{TS}_{\text{midrib}}$	MPa	0.8 – 11.0 – 61.8	***	**
Tensile strength, whole leaf	TS_{leaf}	MPa	2.2 – 5.2 – 12.6	***	***
Flexural stiffness, lamina	$\text{FS}_{\text{lamina}}$	N m ²	1.3×10^{-6} – 1.7×10^{-5} – 1×10^{-4}	***	***
Flexural stiffness, midrib	$\text{FS}_{\text{midrib}}$	N m ²	0.0004 – 0.04 – 0.3	***	***
Flexural stiffness, whole leaf	FS_{leaf}	N m ²	0.003 – 0.72 – 5.2	***	***

Statistical significance of nested analyses of variance (with species, Spp, nested within communities, Com): *** P -value < 0.001, **0.001 < P -value < 0.01.

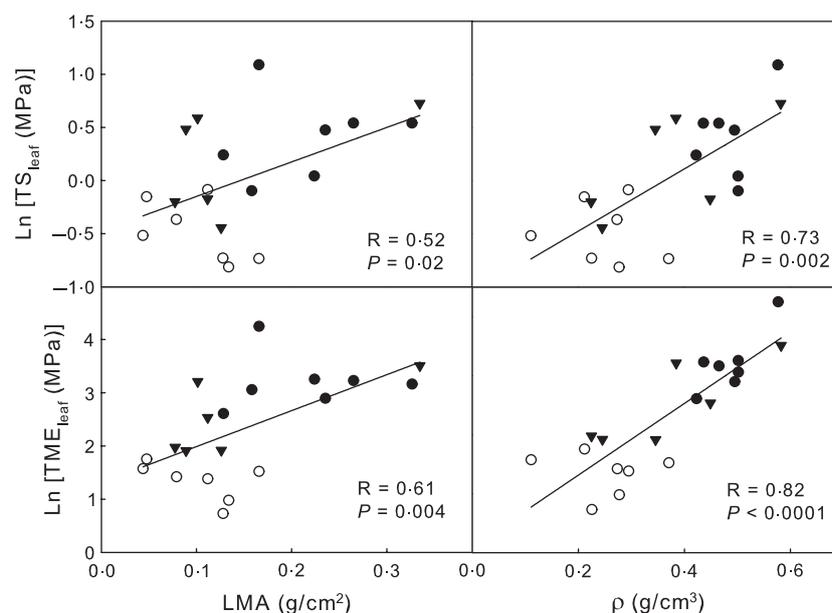


Fig. 1. Relationships of the whole-leaf tensile modulus of elasticity (TME_{leaf} , ln-transformed) and tensile strength (TS_{leaf} , ln-transformed) with leaf mass per unit area (LMA) and leaf density (ρ_{leaf}) for species of three Californian plant communities grown in a common garden. Open circles, coastal sage species; filled circles, chaparral species; triangles, desert species.

When tests were conducted on the whole leaf, the tensile modulus of elasticity (TME) and tensile strength (TS) were highly correlated ($R^2 = 0.66$, $P < 0.0001$), and the flexural stiffness (FS) was not correlated with TS ($R^2 = 0.04$, $P = 0.4$) and weakly correlated with TME ($R^2 = 0.19$, $P = 0.05$). Additionally, the material and structural properties of the whole leaf were highly correlated with those of component tissues. Thus, the whole-leaf TME, TS and FS were correlated with those of the midrib and lamina (Table 2).

The leaf mechanical properties correlated across species with leaf structure and composition. The whole-leaf TME and TS for the whole leaf were strongly related to LMA and ρ_{leaf} (Fig. 1, Table 3). Only the whole-leaf FS was significantly correlated with leaf area and midrib diameter (Table 3). Across species, the whole-leaf TME and TS were not related to leaf size, which was also independent of LMA in this data set (Table 3). The midrib TME, TS and FS were not correlated with midrib density, but the midrib FS was correlated with midrib diameter and LMA (Table 3). The leaf lamina TME, TS and FS were correlated with leaf lamina mass per area and leaf lamina density (Table 3).

Notably, the whole-leaf TME and TS correlated negatively with the fraction of dry mass that the leaf invested in support, whether quantified using the F_L or the $\rho_{\text{midrib}}/\rho_{\text{lamina}}$ ratio. This implies that species bearing leaves of intrinsically low strength and stiffness tended to partially compensate with higher proportions of support tissue in the midrib in comparison with leaves of high strength and stiffness (Fig. 2). Consequently, the whole-leaf FS, which represents TME normalized by the dimensions of the midrib, was unrelated to F_L ($R^2 = 0.15$, $P = 0.09$). We also tested the relationships between F_L and our two mechanical measurements of the leaf lamina (TME_{lamina} and TS_{lamina}). We found a significant negative correlation between TME_{lamina} and F_L ($R^2 = 0.54$, $P = 0.0002$), but no correlation between TS_{lamina} and F_L ($R^2 = 0.05$, $P = 0.37$). These findings imply that the larger the fraction of dry mass invested in midrib, the lower the stiffness of the lamina, but stiffness is unrelated to the maximum stress

Table 2. Pairwise Pearson correlations between whole-leaf tensile modulus of elasticity (TME_{leaf}), tensile strength (TS_{leaf}) or flexural stiffness (FS_{leaf}) vs. TME, TS or FS for midribs (TME_{midrib}, TS_{midrib} and FS_{midrib}) and leaf laminae (TME_{lamina}, TS_{lamina} and FS_{lamina})

Biomechanical traits		<i>R</i>	<i>P</i>
TME _{leaf}	TME _{lamina}	0.85	<0.0001
TME _{leaf}	TME _{midrib}	0.58	0.007
TME _{leaf}	FS _{leaf}	0.44	0.06
TS _{leaf}	TS _{lamina}	0.60	0.005
TS _{leaf}	TS _{midrib}	0.60	0.005
TS _{leaf}	FS _{leaf}	0.20	0.39
TME _{lamina}	FS _{lamina}	0.86	<0.0001
TME _{midrib}	FS _{midrib}	0.43	0.056
TS _{lamina}	FS _{lamina}	0.17	0.47
TS _{midrib}	FS _{midrib}	0.11	0.64

that a section of lamina can withstand. In the scleromorphic leaves, evidently, the lignified epidermis and hypodermis played a major role in whole-leaf mechanics, consistent with their higher values for leaf lamina TME and TS (Fig. 3).

VARIATION IN LEAF BIOMECHANICS WITHIN SPECIES, AND ITS SOURCES WITHIN THE LEAF TISSUES

We assessed the proportion of species that had within-species trait correlations as hypothesized and tested across species. Within species correlations were considered to be significant overall if they were found for four or more species (binomial proportion test; for proportions of 4/21, $P = 0.019$; for 3/21, $P = 0.085$). There were significant correlations between the density of the whole leaf and that of the midrib (within 14/21 species), and between the density of the whole leaf and that of the leaf lamina (21/21 species) ($R^2 = 0.14$ – 0.99 ; $P < 0.0001$ – 0.02).

Within 10/21 species, the whole-leaf tensile modulus of elasticity (TME) and tensile strength (TS) were correlated ($R^2 = 0.47$ – 0.88 ; $P < 0.0001$ – 0.04), and the whole-leaf flexural stiffness (FS) was correlated with the whole-leaf TME for 6/21 species and with the whole-leaf TS for 3/21 species ($R^2 = 0.46$ – 0.88 ; $P < 0.0001$ – 0.043). Further, within species, the leaf mechanical properties correlated with leaf structure and composition. The whole-leaf TME, TS and FS were related to LMA for 4/21 species but with ρ_{leaf} for only 3/21 species ($R^2 = 0.52$ – 0.86 ; $P < 0.0001$ – 0.043). The leaf lamina TME, TS and FS were correlated with leaf lamina mass per area and leaf lamina density for 4/21 species ($R^2 = 0.44$ – 0.97 ; $P < 0.0001$ to 0.048). The whole-leaf FS was significantly correlated with leaf area for 5/21 species ($R^2 = 0.59$ – 0.83 ; $P = 0.001$ – 0.016), and LMA and leaf size were related for 10/21 species ($R^2 = 0.44$ – 0.83 ; $P < 0.0001$ – 0.044).

We also compared the across species coefficient of determination (R^2) with the average R^2 for within-species relationships for our set of hypothesized relationships between leaf functional and biomechanical traits. In most cases, the values of R^2 differed significantly between the within and across species scales (Table 3).

VARIATION ACROSS VEGETATION TYPES IN BIOMECHANICAL TRAITS

Averaging values across communities, the TME showed a dramatic variation among chaparral to semi-desert and coastal sage vegetation types, with a high TME of 41.9 MPa for *Quercus berberidifolia*, a chaparral species, to the lowest TME of 0.78 MPa for *Salvia leucophylla*, a species from coastal sage vegetation. This pattern of variation among communities was most conspicuous for the midrib mechanical properties, then for the whole leaf, then for the lamina (Fig. 4). This same pattern was observed for TS (Fig. 4). The FS showed no significant trend across communities ($P > 0.05$).

Table 3. Results of tests of pairwise Pearson correlations between tensile modulus of elasticity and flexural stiffness of leaf ('leaf'), midrib ('midrib') and leaf lamina ('lamina') with leaf dimensional and compositional traits across species and the minimum, mean and maximum R^2 values and minimum and maximum P -values for within species tests. Refer to Table 1 for trait abbreviations. (%) is the percentage of species that had significant within-species relationships ($P < 0.05$)

	Across species		Within species	
	R^2	P	Min–Mean–Max R^2	Min–Max (%) P
<i>Correlations with leaf area</i>				
LMA	0.02	0.06	0.008–0.23–0.87	0.001–0.82 (47%)
ρ	0.01	0.75	0–0.2–0.8	0.003–0.98 (20%)*
$D_{\text{midrib, b}}$	0.53	<0.0001	0.002–0.32–0.93	0–0.91 (67%)*
L_{midrib}	0.67	<0.0001	0.05–0.64–0.97	0–0.56 (90%)
W_{leaf}	0.65	<0.0001	0.005–0.49–0.98	0–0.86 (62%)*
TME_{leaf}	0.01	0.69	0–0.22–0.64	0.009–0.97 (5%)*
FS_{leaf}	0.43	<0.0001	0.09–0.57–0.89	0–0.44 (75%)
<i>Correlations with leaf mass per area</i>				
TME_{leaf}	0.24	0.03	0–0.37–0.91	0–0.98 (35%)
FS_{leaf}	0.10	0.18	0.002–0.32–0.81	0.001–0.90 (35%)
TME_{midrib}	0.08	0.22	0.003–0.18–0.87	0–0.89 (15%)
TS_{midrib}	0.01	0.67	0–0.23–0.81	0.001–0.99 (25%)*
FS_{midrib}	0.56	<0.0001	0.001–0.26–0.79	0.001–0.94 (30%)*
<i>Correlations with TME_{leaf}</i>				
ρ	0.58	<0.0001	0–0.20–0.80	0.001–0.97 (20%)*
ρ_{lamina}	0.52	0.0003	0–0.26–0.84	0–0.99 (35%)*
ρ_{midrib}	0.21	0.04	0–0.23–0.88	0–0.96 (25%)
T	0.02	0.58	0.001–0.27–0.84	0–0.93 (20%)*
$D_{\text{midrib, b}}$	0.0001	0.95	0.005–0.25–0.97	0–0.86 (10%)*
<i>Correlations with FS_{leaf}</i>				
ρ	0.09	0.19	0.001–0.22–0.97	0–0.94 (15%)*
ρ_{lamina}	0.14	0.09	0.003–0.23–0.73	0.003–0.88 (30%)
ρ_{midrib}	0.01	0.61	0–0.26–0.88	0–0.98 (20%)
T	0.004	0.77	0–0.22–0.79	0.001–0.99 (25%)
$D_{\text{midrib, b}}$	0.36	0.004	0.002–0.29–0.84	0.001–0.91 (30%)
<i>Correlations with midrib density</i>				
TME_{midrib}	0.08	0.23	0.001–0.16–0.53	0.03–0.94 (15%)
TS_{midrib}	0.02	0.55	0–0.17–0.64	0.01–0.96 (15%)
FS_{midrib}	0.01	0.67	0.001–0.22–0.81	0.001–0.94 (25%)*
<i>Correlations with midrib diameter</i>				
TME_{midrib}	0.27	0.018	0–0.22–0.66	0.008–0.99 (20%)
TS_{midrib}	0.11	0.15	0–0.21–0.85	0–0.99 (15%)
FS_{midrib}	0.44	<0.0001	0.02–0.53–0.90	0–0.76 (60%)
<i>Correlations with lamina mass per area</i>				
TME_{lamina}	0.38	0.003	0.001–0.19–0.67	0.01–0.95 (20%)*
TS_{lamina}	0.004	0.79	0–0.17–0.64	0.01–0.99 (10%)*
FS_{lamina}	0.38	0.003	0.004–0.19–0.74	0.006–0.87 (15%)*
<i>Correlations with lamina density</i>				
TME_{lamina}	0.44	0.001	0.005–0.19–0.7	0.005–0.86 (15%)*
TS_{lamina}	0.04	0.31	0.003–0.16–0.47	0.04–0.89 (10%)
FS_{lamina}	0.44	0.002	0–0.19–0.78	0.003–0.99 (10%)*

*Indicates $P < 0.05$ of t -tests between the within species R^2 ($N = 21$) and the across species R^2 .

PATTERNS OF COVARIATION AMONG LEAF TRAITS

We summarized data from the 17 morphological and nine biomechanical traits across species using principal component analysis (PCA). As a result of strong covariation among leaf traits that were related to size, the first component in the PCA accounted for 41.2% of the variance (Fig. 5), and most variables related to leaf length, width and area were significantly correlated with the first component (Fig. 5, Table S2, Supporting information). The second axis, accounting for 32.2% of the variance, was

strongly influenced by leaf mass per area, leaf density, and leaf mechanical properties, as indicated by the significant correlation of this set of variables with the second component (Fig. 5, Table S2, Supporting information).

The PCA indicated that species from three Californian plant communities can be ordered according to their leaf properties, from species bearing leaves with low stiffness and strength, in the coastal sage, to species with strong and stiff leaves in the chaparral. The Desert species were spread across the range of variation in size and mechanical resistance, including species with small

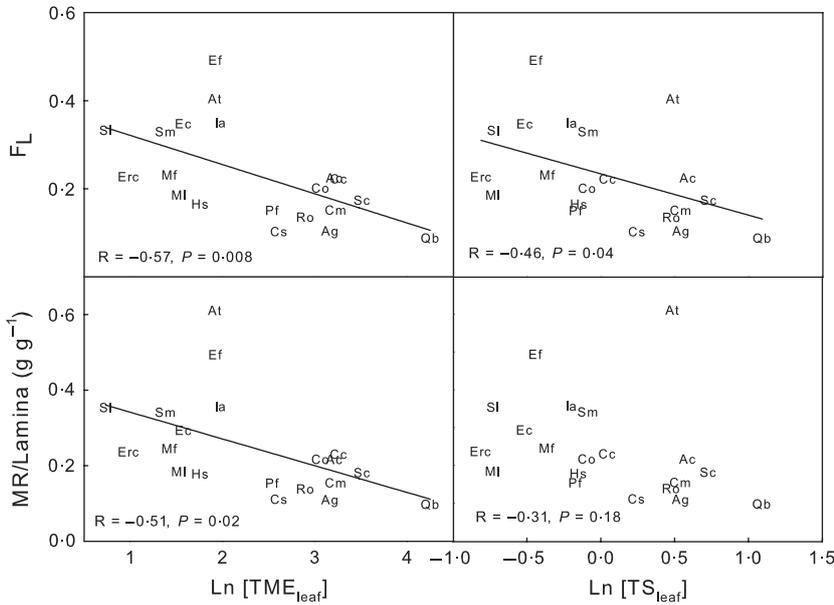


Fig. 2. Relationships between the fractions of leaf tissue invested in support (F_L), and the ratio of midrib over lamina tissue density (midrib/lamina) with the ln-transformed whole-leaf tensile modulus of elasticity ($\text{Ln} [\text{TME}_{\text{leaf}}]$) and whole-leaf tensile strength ($\text{Ln} [\text{TS}_{\text{leaf}}]$) for species of three Californian plant communities grown in a common garden. For species abbreviations, see Table S1 (Supporting information).

leaves of low mechanical resistance, such as *Artemisia tridentata* and *Isomeris arborea* and species with relatively large leaves of high mechanical resistance, such as *Simmondsia chinensis*. The chaparral species *Malosma laurina* and *Rhus ovata* (both Anacardiaceae) significantly deviated from the other species in their community in the first principal component because of their relatively larger leaf sizes (Fig. 5). Finally, we compared the scores of the first and second principal components across plant communities to determine how leaf traits varied across environments. Species did not differ in traits related to leaf area across plant communities (PC1, one-way ANOVA, $F = 2.135$, $P = 0.15$), but they differed significantly in the traits related to leaf mechanical properties (PC 2, one-way ANOVA, $F = 8.8545$, $P = 0.0023$).

Discussion

We quantified the strength and stiffness of leaves of dominant species originating from three contrasting environments in the California flora in a common garden and found three especially novel results highlighting the importance of leaf mechanical traits. First, we found that the mechanical properties of leaves depended strongly on size and tissue density according to hypothesized scaling relationships and that relationships within species were qualitatively distinct from those that hold across species. Second, mechanical resistance differed substantially among the component tissues of the leaf, being larger in the midrib than in the leaf lamina and that covariation in tissue dimensions partially compensated for differences in mechanical resistance of these

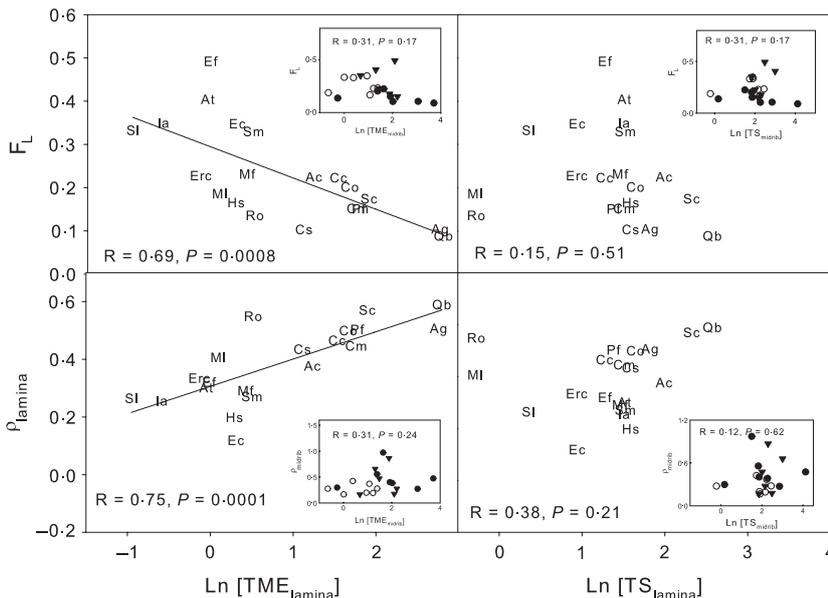


Fig. 3. Relationship between the fractions of leaf tissue invested in support (F_L) and tissue density (ρ) with with the ln-transformed tensile modulus of elasticity ($\text{Ln} [\text{TME}]$) and tensile strength ($\text{Ln} [\text{TS}]$) of leaf lamina (main panels) and midrib sections (insets) for species of three Californian plant communities grown in a common garden. For species abbreviations, see Table S1 (Supporting information). Open circles, coastal sage species; filled circles, chaparral species, triangles, desert species.

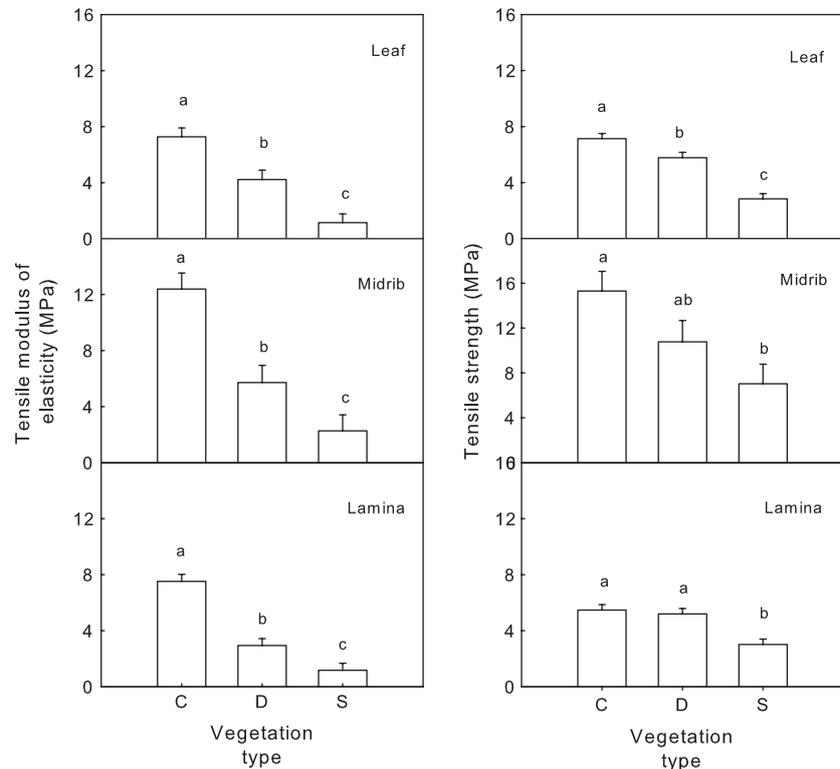


Fig. 4. Within-leaf variation in the tensile modulus of elasticity and tensile strength for whole leaves ('leaf'), for midrib ('midrib'), and leaf lamina ('lamina'), for 21 species of three Californian plant communities (Vegetation type) grown in a common garden. C, chaparral; D, desert; S, coastal sage.

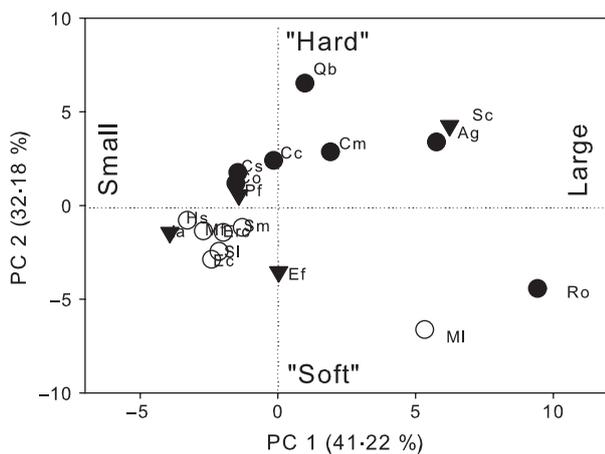


Fig. 5. Species from three Californian communities range from small 'soft' leaves in the coastal sage (open circles) to small 'hard' leaves in chaparral (filled circles). Desert species spanned across the range of variation in size and mechanical resistance (triangles) and Anacardiaceae (MI and Ro) were much larger in size, but had smaller values of mechanical resistance. The first principal component from a PCA synthesizing 17 morphological and nine biomechanical traits had $\lambda = 11.13$ and the second principal component had $\lambda = 8.69$.

tissues such that the flexural stiffness, that is, the leaf deformation in response to its own load, was less variable across species. Finally, leaf biomechanics are useful traits to characterize vegetation types, and placed on a quantitative basis the classical view of two California communities that differ in their leaf mechanics and drought tolerance.

SCALING RELATIONS BETWEEN LEAF MORPHOLOGY AND MECHANICS

Tensile experiments provided evidence of large variation across species and habitats in leaf mechanical properties and that these related strongly to leaf structure. Notably, using the tensile tests, we were able to determine the strength and stiffness of different tissues and different configurations of leaf architecture, which would have proven difficult using the punch and die test or the shearing test, which have been useful in calculating the shear resistance of leaves (Aranwala, Sanson & Read 1999; Onoda *et al.* 2011). Consistent with previous work, we found that the mechanical strength and stiffness of leaves was correlated with functional traits relating to the packing of cell material in the tissue, such as leaf mass per area and leaf density, and this relation was persistent in different tissues and across species and communities (Read & Sanson 2003; Read *et al.* 2006; Onoda *et al.* 2011). Our study also provides the first investigation of the mechanical strength and stiffness of leaves for different leaf tissues and spatial configurations, and the first quantitative determination of the variation in density of the midrib and leaf lamina, and its relation to biomechanics traits. We found that the construction of tissues was inter-related, such that a species with higher density in one tissue tended to have higher density in other tissues. Thus, our mechanical properties showed a conserved ranking of material resistance among tissues, such that species with higher material resistance in the midrib also invested significantly more in the support of the leaf lamina by increasing the strength and stiffness of the lamina

or minor veins. We found that the strongest differences across species and environments were in the mechanical resistance of the midrib, and thus related to the xylem and surrounding mechanical cells and not the leaf lamina.

Our measurements of mechanical properties confirmed and extended previous work which focused on mass allocation within leaves. A previous study reported a trade-off between the investment in the proportion of tissue in the midrib vs. the proportion of tissue invested in the other major and minor veins (Niinemets, Portsmouth & Tobias 2007). Consistent with this, we found a trade-off between the mechanical properties of the leaf tissues and the proportion of biomass invested in support. Species bearing leaves with low strength and stiffness deploy a higher proportion of the leaf dry mass in the midrib and petiole relative to species that bear leaves with high strength and stiffness. This finding indicates alternative designs for leaves to attain resistance to bending due to their own weight, that is, by investing in highly mechanical resistant but low volumes of midrib tissue or by investing in greater cross-sectional area, and deploying low-density material to construct the midrib. The achievement of adequate structural resistance in leaves (as quantified by the FS) is critical to maximize leaf light capture, as it allows the optimization of the inclination angles of leaves and thus the reduction of leaf self-shading by aggregation (Niinemets & Fleck 2002a; Niinemets, Portsmouth & Tobias 2006). We also found that species that deploy a small volume of highly resistant tissue in the midrib enhanced the mechanical resistance of the lamina, which can be achieved with lignification of the lamina and hypodermis (Edwards *et al.* 2000). The enhancement of the mechanical resistance of the lamina increases the overall safety of leaves against mechanical failure, though at the cost of larger investments in dry mass, and is congruent with the expectations of a trade-off between the fraction of tissue invested in the midrib vs. smaller veins and lamina providing similar flexural stiffness, and increased lamina protection would also contribute resistance to herbivory and extended leaf life span (Niinemets, Portsmouth & Tobias 2007).

The relationships of leaf biomechanics traits with leaf area and density were species specific. Indeed, we did not find interspecific relationships of leaf biomechanical properties with leaf size, but we found such relationships within species for more than half of the species studied. Our results suggest that leaf area and other size-related traits are decoupled from the material properties of the leaves (TME and TS) across species. However, the FS, a biomechanical trait that incorporates leaf dimensions, was strongly related to leaf size across species. These different relationships within and across species imply that, when the material resistance of the leaves is relatively fixed, as would occur within species, the larger leaves tend to have larger mechanical resistance, a larger amount of tissue invested in support. However, when the material resistance varies hugely across species, this would be independent of leaf size. Across species, we did not find evidence for a

significant positive relationship between the fraction of tissue invested in support (F_L) and leaf area reported in a previous study (Niinemets, Portsmouth & Tobias 2006). The lack of relationship across species may be due to the relatively small leaves in our study, ranging from 0.7 cm² in *Larrea tridentata* to 28 cm² in *Malosma laurina*, with only two species with leaf area greater than 10 cm².

VARIATION IN LEAF MECHANICS AMONG SPECIES AND VEGETATION TYPES

The Mediterranean flora is considered a key example of functional convergence in sclerophylly (Cody & Mooney 1978; Edwards, Read & Sanson 2000; Read & Sanson 2003). In our study, leaves showed a dramatically greater strength and stiffness in the chaparral than the coastal sage, with a sixfold increase on average from chaparral species to coastal sage species (from an average across treatments of 9.1 MPa in the chaparral vs. 1.5 MPa in the coastal sage), a finding consistent with obvious qualitative knowledge, and here placed on a quantitative basis. These properties are also consistent with what is known of the physiology of the vegetation types, where chaparral species are evergreens highly resistant to cavitation that have low transpiration rates and do not always resprout after fires (Jacobsen *et al.* 2007, 2008; Pratt *et al.* 2007). The high leaf mechanical resistance of chaparral species can be understood as an adaptation to nutrient limitation and/or herbivory (Turner *et al.* 1993; Salleo & Nardini 2000; Bartlett, Scoffoni & Sack 2012), with an indirect role in drought tolerance in seasonally dry ecosystems, including mediterranean climates, preventing excessive tissue shrinkage and dehydration (Bartlett, Scoffoni & Sack 2012). This would explain why a global meta-analysis found leaf mechanical resistance to be negatively correlated with water availability, although the percentage of variance explained was less than 30% (Onoda *et al.* 2011). Species from coastal sage and Mojave Desert, on the contrary, are generally facultatively drought-deciduous species that avoid very negative soil water potentials by producing leaves only in the wetter season of the year (Jacobsen *et al.* 2007). To control water loss through transpiration, species from Mojave Desert reduce total leaf area, and this dynamic response of leaves to water availability is reflected in a diversity of leaf biomechanical traits, with species including a mixture of sclerophylls and malacophylls (Jacobsen *et al.* 2008). Our results extend the classical works of Schimper (1903) and Cooper (1922), showing that the qualitative differences in leaf traits they observed can be assessed as quantitative leaf mechanical properties. This principle can be extended to other contrasting vegetation types in adjacent communities around the world. In conjunction with other traits that differ importantly among communities, such as stem hydraulics and mechanics (Jacobsen *et al.* 2008, 2009), such analyses can differentiate vegetation types in a functionally significant way, according to their contrasting resource exploitation.

Acknowledgements

We thank C. Vuong, C. Moctezuma, A. Escribano and C. García-Verdugo for assisting in data collection, L. McDade and the staff of the Rancho Santa Ana Botanical Garden for logistical support, A. Jacobsen and B. Pratt for valuable discussions about the plant communities and two anonymous reviewers for helpful advice. RMA gratefully acknowledges the support by UC-MEXUS-CONACYT postdoctoral program. The authors declare no conflict of interest.

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Received 1 August 2012; accepted 14 December 2012
Handling Editor: Niels Anten

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Mean values \pm SE for 17 functional and nine biomechanical traits for 21 species of three Californian plant communities.

Table S2. Pairwise Pearson correlations between leaf functional and biomechanical traits for 21 species of three Californian plant communities.