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Leaf cellulose density as the key determinant of inter- and intra-specific variation in leaf fracture toughness in a species-rich tropical forest

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Leaves as the main photosynthetic organ of plants must be well protected against various hazards to achieve their optimal lifespans. Yet, within-species variation and the material basis of leaf strength have been explored for very few species. Here, we present a large dataset of leaf fracture toughness from a species-rich humid tropical forest on Barro Colorado Island, Panama, reporting both among- and within-species variation in relation to light environment (sun-lit canopy versus shaded understorey) and ontogeny (seedlings versus adults). In this dataset encompassing 281 free-standing woody species and 428 species-light combinations, lamina fracture toughness varied *ca* 10 times. A central objective of our study was to identify generalizable patterns in the structural and material basis for interspecific variation in leaf lamina fracture toughness. The leaf lamina is a heterogeneous structure in which strong materials in cell walls, such as cellulose and lignin, contribute disproportionately to fracture toughness. We found significant increases in leaf fracture toughness from shade to sun and from seedling leaves to adult leaves. Both within and across species, leaf fracture toughness increased with total bulk density (dry biomass per unit volume) and cellulose mass concentration, but decreased with mass concentrations of lignin and hemicelluose. These bivariate relationships shift between light environments, but leaf cellulose density (cellulose mass per unit leaf volume) exhibits a common relationship with lamina fracture toughness between light environments and through ontogeny. Hence, leaf cellulose density is probably a universal predictor of leaf fracture toughness.

1. Introduction

In most angiosperm species including those in species-rich tropical forests, leaves have evolved thin laminar structures to achieve efficient light capture and gas exchange [1]. At the same time, leaves must have adequate stiffness in order to maintain optimal display angles and sufficient fracture toughness to resist breaking forces from herbivores and physical disturbance [2,3]. From these principles, a simple prediction can be made, which is supported by empirical data. The greater the fracture toughness, the greater the potential leaf lifespan, i.e. the maximum period over which a leaf can amortize the carbon cost of its construction and achieve a positive net carbon gain [4–7].

Biomechanical strength in plants is achieved primarily by cell walls consisting of strong fibre-rich composite materials [8–11]. Increasing cell wall fraction, however, not only increases the cost of leaf construction per unit light capturing surface but also reduces photosynthetic gas exchange efficiency [12]. Hence, there is a trade-off between efficiency of photosynthetic productivity and biomechanical strength. Available data suggest that tree leaves in species-rich tropical forests occur at various positions along this trade-off [7]. Leaf lifespan is expected to be longer in environments that constrain photosynthetic

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productivity [13,14]. Tree species whose regeneration is limited to high light levels in treefall gaps employ fast growth strategies with rapid turnover of short-lived leaves of limited mechanical strength. By contrast, juveniles of shade tolerant tree species employ conservative strategies to persist in light-limited forest understories and have tough leaves that live for several to greater than 10 years [4,5,14]. Whereas the magnitude and ecological significance of leaf-toughness variation among plant species are increasingly documented even at the global scale [15], the material and anatomical basis of leaf biomechanical strength remains underexplored [16–18].

A leaf is a composite structure consisting of tissues that vary in mechanical properties, including metabolically active mesophyll cells with thin cell walls, vascular tissues with thick secondary cell walls and epidermis which may have a thick cuticle layer [9,18,19]. It is, however, difficult to separate the mechanical properties that emerge in the leaf lamina as a whole. Thus, the mechanical strength of a leaf lamina is typically quantified as a bulk average per unit fracture length, per unit fractured surface, or per unit mass of fractured solid [20–22]. Work to shear (W_s) is defined as the work to propagate a crack per unit fracture length (J m^{-1}), which can be measured directly. W_s reflects both size (lamina thickness, T) and material strength. The latter is estimated as specific work-to-shear (W_{ss}) by dividing W_s by T, and it is appropriately interpreted as average fracture toughness per unit leaf cross-sectional area (J m^{-2}) [20,21,23]. Because a leaf includes space occupied by air and water, the average toughness per unit mass solids in a leaf (γ) can be estimated by dividing W_{ss} by the bulk density (ρ) [24]. In sum, these quantities are related to each other as follows:

$$W_s = T \cdot W_{ss} \tag{1.1}$$

and

$$W_{ss} = \rho \cdot \gamma. \tag{1.2}$$

Herbivore resistance, leaf lifespan and shade tolerance are strongly correlated with W_s and W_{ss}, but weakly correlated or unrelated to T in interspecific comparisons [6,11,24]. For these reasons, hereafter we examine only W_{ss} and traits that may provide a material basis for its variation. It is not possible to directly measure γ, but it can be estimated as W_{ss} divided by ρ (equation (1.2)). This estimate along with unknown measurement error together explain 60–70% of global variation in W_s [15].

We hypothesize that the abundance of strong materials in cell walls is the primary material basis for variation in γ, and consequently in W_{ss}. In particular, cellulose microfibrils cross-linked by hemicellulose and lignin are likely to be important [23,25,26]. Recent studies documented W_{ss}, ρ and fractions of cellulose, hemicellulose and lignin for leaves of 197 species from a shaded forest understorey [27], 13 species from small tree fall gaps and the shaded understorey [24] and 21 species from common gardens located in small gaps and the understorey [11]. For among-species comparisons within each light environment, the following generalizations can be made.

(1) W_{ss} and the cellulose mass fraction (henceforth %cellulose $= 100 \cdot$ g cellulose g^{-1} dry tissue) are positively related, but W_{ss} and %hemicellulose and %lignin are unrelated or negatively related.
(2) %cellulose and ρ are unrelated.
(3) W_{ss} is statistically predicted by ρ and %cellulose, each providing similar degrees of explanatory power.

These patterns are consistent with biomechanical theories of leaf fracture toughness [20], but it is surprising that %cellulose stands out as the only significant correlate among cell wall fibre fractions.

In terms of within-species variation of leaf traits, it is well known that adaptive and plastic responses to light environments result in variation in photosynthetic characteristics and leaf lifespan. Sun leaves of gap-grown plants or the upper forest canopy have higher photosynthetic capacity, but exhibit shorter lifespan compared with leaves developed in shade [11]. By contrast, plastic responses of leaf biomechanical strength and its material basis have hardly been examined, for which we predict the following patterns.

(4) The value of ρ is greater for sun leaves than for shade leaves.
(5) The cell wall fibre fraction is lower for sun leaves than for shade leaves.
(6) These offsetting changes in ρ and cell wall fibre fraction result in small differences in W_{ss} between sun and shade leaves within a species.

The fourth pattern has been documented many times [19]. The fifth and sixth patterns are suggested in a global compilation of leaf traits, which did not assess the contribution of intraspecific and interspecific variation to overall variation [15]. These patterns are also suggested in *Plantago major* [17] and 13 Australian rainforest trees [24]. Dominy *et al.* [28] report that sun-exposed canopy leaves are significantly tougher than shaded understorey leaves of 37 tree species in Panama, but they did not examine how bulk density and fibre fractions contribute to fracture toughness.

To further evaluate the generality of patterns (1)–(6) above, we conducted analyses of W_{ss}, ρ and cell wall fibre fractions for fully sun-exposed canopy leaves of 155 species and shaded understorey leaves of 273 species in a tropical moist forest, including 147 species in both environments. Our study is the first to report relationships among W_{ss}, ρ and cell wall fibre fractions for sun-exposed canopy leaves, as well as plastic responses to the full range of forest light environments. Given the patterns predicted in (3) and (6) above, we hypothesize that the product of ρ and %cellulose (i.e. leaf cellulose density = g cellulose per unit volume of leaf lamina) is a universal predictor of lamina fracture toughness within and across broad-leaved plant species. For brevity and ease of comprehension, hereafter we use 'lamina toughness' as a synonym for specific work-to-shear and lamina fracture toughness.

2. Material and methods

2.1. Study site and materials

We collected leaves during the 2007 rainy season from the 50-ha forest dynamics plot (9°10′ N, 79°51′ W) on Barro Colorado Island (BCI), Panama. Annual rainfall averages 2600 mm, with 90% falling during the eight-month rainy season. Temperatures average 27°C in April and 26°C otherwise. All free-standing woody plants larger than 1 cm in diameter at breast height (DBH) were mapped, measured for DBH and identified to species at 5-year intervals in the 50-ha plot [29]. The 2005 census included 206 387 individuals and 299 species. We targeted the six largest (by

DBH) and six smallest individuals of each species for leaf collection. The six smallest individuals were selected randomly for species with many equally small individuals. We used a shotgun and steel shot to collect fully sun-exposed leaves from the uppermost canopy of large trees. We collected the uppermost leaves from small, shaded, understorey individuals with hand pruners or a pole cutter. For each individual, we visually assessed the crown exposure index [30], and assessed the light environment of the sampled leaves. Those collected from tree crowns directly exposed to open sky are classified as sun leaves, whereas those collected from individuals shaded by other trees are classified as shade leaves.

In order to evaluate the effects of ontogeny, we used data for 2- to 4-year-old seedlings of 24 species reported previously for the same forest [11]. These seedlings had been grown from seeds for 2–4 years in treefall gaps (receiving 23–50% of full sun) and shaded understorey (0.8% of full sun). Lamina toughness and cell wall fibre fractions were measured with identical methods as described below.

2.1.1. Measurements of leaf lamina biomechanical traits and cell wall fibre fractions

We measured W_s with a shearing test using scissors mounted on a portable universal tester [20,31] and T with an analogue thickness gauge (Teclock SM112, Nagano Japan). We calculated lamina toughness (W_{ss}) as W_s/T for two or three leaves from each individual as described by Westbrook *et al*. [27]. The measurement of W_s and T avoided major veins, except in some species with dense parallel 2° veins (e.g. *Calophyllum*). All our study species were broad-leaved angiosperms, most of which had a network of tertiary and fine veins that were random relative to the cutting direction. The area and dry mass of all leaves were measured, and total bulk density (ρ) was determined as leaf dry mass divided by area and T. We pooled leaves of conspecific individuals for each light environment and determined mass fraction of neutral detergent fibre (NDF), acid detergent fibre (ADF) and lignin with a modified Van Soest method [32] with an ANKOM 200 fibre analyser (ANKOM Technology, Macedeon, NY, USA) [27]. NDF fraction is considered to represent the cell wall fraction per unit dry mass. Mass fraction of hemicellulose was estimated as NDF fraction minus ADF fraction, and the mass fraction of cellulose was estimated as ADF fraction minus lignin fraction. The density of each cell wall fibre component (gram fibre per unit volume) was estimated as a product of mass fraction and total bulk density. Hereafter, for brevity, *%fibre* (e.g. %cellulose) is used to indicate mass fraction (gram fibre per gram dry mass), whereas *leaf fibre density* (e.g. leaf cellulose density) is used to indicate gram fibre cm^{-3}.

2.1.2. Statistical analyses

We performed paired t-tests, analyses of covariance (ANCOVAs) and major axis regression analyses for nine variables. Paired t-tests evaluated differences between conspecific sun and shade leaves. Eight variables met the assumptions of homogeneity of variances (evaluated using the Fligner–Killeen test). The ninth, leaf lignin density, had several outliers, and analyses performed with and without the outliers gave qualitatively similar results. Thus, we report the results from the analyses including these outliers. Three ANCOVA tests evaluated relationships of lamina toughness (response variable) with light environment (grouping factor) and (i) total bulk density, %hemicellulose, %cellulose and %lignin (four covariates) or (ii) hemicellulose, cellulose and lignin density (three covariates) or (iii) cell-wall (NDF) density (single covariate, which is the sum of hemicellulose, cellulose and lignin density). We used the Bayesian information criterion (BIC), which penalizes models with additional parameters, to compare ANCOVA models. We used major axis regression to describe the best ANCOVA model.

We repeated the species-level analyses described above for phylogenetically independent contrasts (PICs). When phylogenetic relationships are well-resolved among study species as an evolutionary tree, for each bifurcation point in the tree, the direction and magnitude of trait-value change can be calculated as a PIC value. Then, it is possible to evaluate the observed patterns of PICs against a null model in which the trait values are assumed to change with time and evolutionary divergence in a random manner (i.e. Brownian motion). We calculated PIC values for a well-resolved phylogeny of the woody species of the BCI 50-ha plot based on DNA sequence information derived from three specific gene regions [33]. We assumed unit branch lengths, and PIC values for all study traits met the homogeneity of variance assumption except %hemicellulose. We used randomization tests and Blomberg's K statistic to assess phylogenetic signal for sun leaves, for shade leaves, and for the difference between conspecific sun and shade leaves. Values of K smaller or greater than one occur when phylogenetic signal is weaker or stronger than expected under the Brownian model, respectively [34]. The randomization test compares the observed variance in PIC values with 10 000 variances created by randomizing trait values across the tips of the phylogeny. If the observed variance is among the smallest 250 (largest 9750) randomized variances, there is significant phylogenetic conservatism (convergence) [35]. We performed analyses with the base, ape, picante and smatr libraries in R [35–37].

3. Results

3.1. Plastic responses of leaf fracture toughness and cell wall fibre contents to light

Lamina toughness, bulk density and lignin, hemicellulose and cellulose fractions were measured for 428 species–light combinations. This included 281 species from 56 families. Scatter plots of species mean values for sun versus shade leaves allow visual assessment of plastic responses across study species (figure 1). In these plots, points above and below the 1 : 1 line indicate trait value increase and decrease from sun to shade, respectively. Whether there is a generalizable pattern of plastic trait value shifts across species can be tested with paired t-tests of conspecific sun and shade mean values (table 1). These analyses show that sun leaves exhibited higher values than conspecific shade leaves for lamina toughness (figure 1*a*), ρ (figure 1*b*), leaf cellulose density (figure 1*c*), cell wall density (figure 1*d*), %lignin (figure 1*e*), lignin density (not shown) and hemicellulose density (not shown). By contrast, species mean trait values were significantly greater for shade leaves than for conspecific sun leaves for %hemicellulose (figure 1*f*), %cellulose (figure 1*g*) and %cell wall (figure 1*h*). Sun-to-shade differences tended to be strongly consistent across species for leaf density measures (e.g. figure 1*b*,*c*,*d*) and more variable, but still significant, for mass fraction measures (figure 1*e*,*f*,*g*,*h*). The results of PIC and species-level analyses differed strongly for the paired t-tests. The PIC values were indistinguishable for conspecific sun and shade leaves for all variables in paired t-tests (table 1).

3.2. Dependence of leaf fracture toughness on environment and cell wall fibre components

We performed three ANCOVAs to evaluate the relative contributions of cell wall fibre properties and plasticity in response to

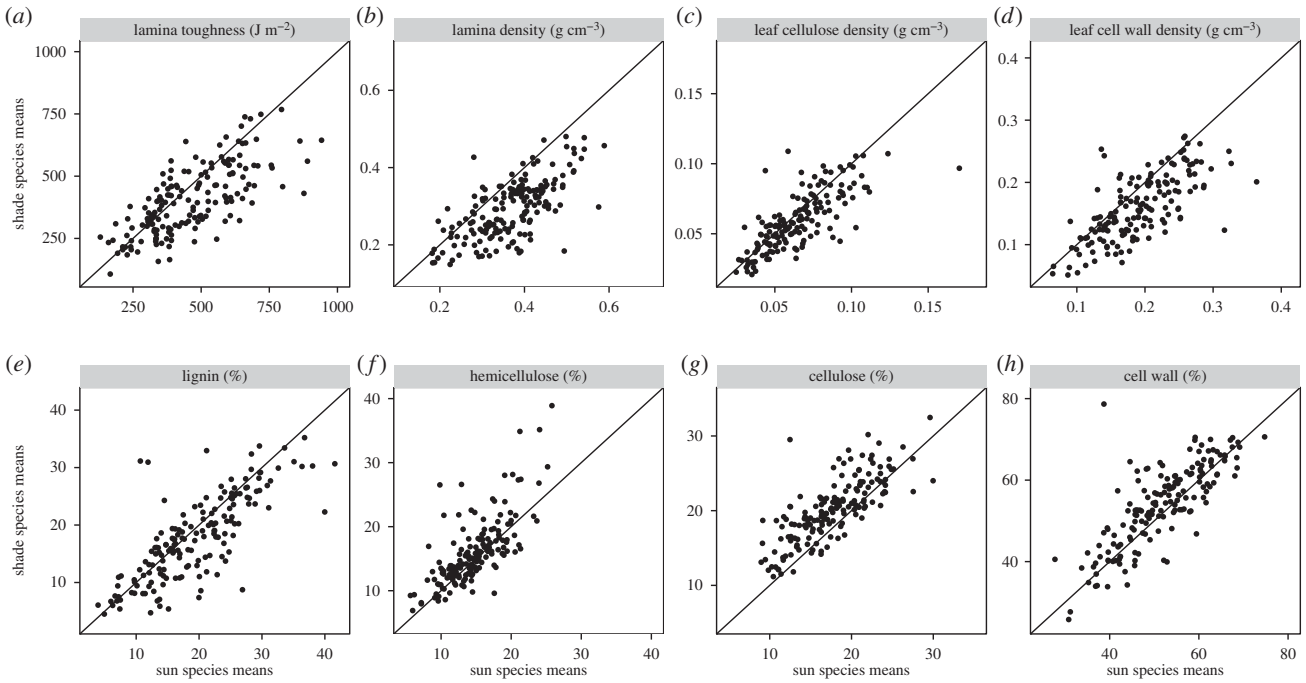

Figure 1. Scatter plots comparing species mean values of lamina fracture toughness (*a*), total bulk density (*b*), cellulose density (*c*), cell wall density (*d*), lignin fraction (*e*), hemicellulose fraction (*f*), cellulose fraction (*g*) and cell wall fraction (*h*) for sun-exposed, canopy leaves (horizontal axes) versus shaded, understorey leaves (vertical axes) of conspecific trees and treelets from Barro Colorado Island, Panama. The solid diagonal lines are 1 : 1 lines, representing equal values for sun and shade. Points below this line means plastic increase of trait values from shade to sun. The overall significance of this pattern is tested with paired *t*-test (table 1).

Table 1. Paired *t*-tests for species means and phylogenetically independent contrasts (PICs) for leaf traits comparing shade leaves to conspecific sun leaves. A negative *t*-value indicates that the species mean is greater for sun than for shade leaves. The paired *t*-tests for PICs evaluated the degree of evolutionary change of sun phenotype to that of shade phenotype at each of 124 bifurcation points of the evolutionary tree containing 125 study species. Even when there was a consistent difference between sun and shade leaf phenotypes of each species, PIC values often showed no signal in the magnitudes of evolutionary change for sun and shade leaves (i.e. parallel evolutionary trait-value shift for both phenotypes).

	species means			PIC	
trait	d.f.	*t*	*p*-value	*t*	*p*-value
lamina fracture toughness	166	−6.36	$<10^{-8}$	−0.77	0.44
total bulk density	173	−15.0	$<10^{-15}$	−1.45	0.15
cellulose density	167	−12.8	$<10^{-15}$	−0.88	0.38
cell wall density	157	−9.61	$<10^{-15}$	−1.75	0.08
lignin mass fraction	165	−4.09	$<10^{-4}$	−1.29	0.20
hemicellulose mass fraction	165	4.79	$<10^{-5}$	0.21	0.84
cellulose mass fraction	165	11.1	$<10^{-15}$	0.57	0.57
cell wall mass fraction	165	4.80	$<10^{-5}$	−0.60	0.55

light levels to intra- and interspecific variation in lamina toughness. The first ANCOVA evaluated variation in lamina toughness (response variable) as a function of light environment (grouping factor) and ρ, %hemicellulose, %cellulose and %lignin (four covariates). Insignificant interactions between light and ρ, %lignin and %hemicellulose were removed. There were strong positive relationships between lamina toughness and ρ and %cellulose (figure 2*a*) and weaker but significant negative relationships between lamina toughness and %hemicellulose and %lignin (table 2). Sun leaves tended to be tougher than shade leaves over the full range of %cellulose with the sun-shade difference increasing for larger %cellulose (figure 2*a*). Altogether, light, total bulk density, %cellulose, %hemicellulose, %lignin and the light × %cellulose interaction explained 59.4% (adjusted R squared) of the variation in lamina toughness ($F_{6,421} = 105$, $p < 10^{-15}$). A reduced model with just total bulk density and %cellulose explained 57.1% of the variation in lamina toughness ($F_{2,425} = 285$, $p < 10^{-15}$), indicating a relatively weak contribution of cell wall components other than cellulose to leaf fracture toughness.

The second ANCOVA evaluated relationships among lamina toughness (response variable), light environment (grouping factor) and leaf lignin, hemicellulose and cellulose densities (three covariates). All three interactions were insignificant and were removed. There was a strong

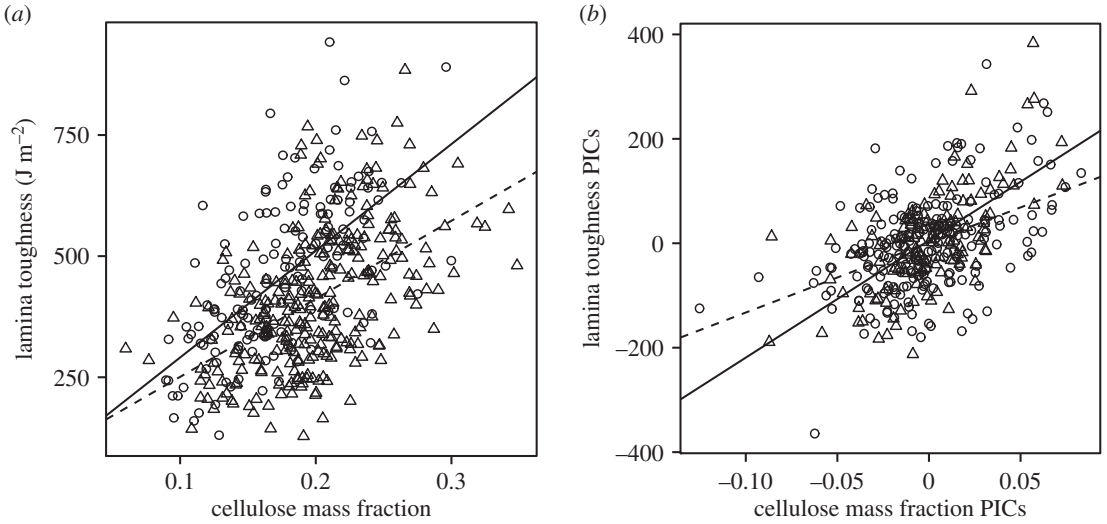

Figure 2. (*a*) Scatter plot for the relationship between lamina fracture toughness and cellulose mass fraction for sun-exposed (circles, solid regression line) and shaded (triangles, broken regression line) leaves of trees and treelets from Barro Colorado Island, Panama. Regression lines show strong positive effects of cellulose mass fraction on toughness both in sun and shade, but sun leaves are tougher than shade leaves at a given cellulose mass fraction with slightly steeper slope (table 2). (*b*) The same data analysed with phylogenetic independent contrasts (PICs).

Table 2. Two ANCOVA models to evaluate the dependence of leaf lamina fracture toughness on light environment (grouping factor—sun versus shade), total bulk density and cell wall fibre components. The coefficient estimated for each variable is shown along with *t*-values. Model 1 examined total bulk density and mass fractions of cellulose, hemicellulose and lignin as four covariates. Model 2 used densities (g fibre cm^{-3} lamina) of cellulose, hemicellulose and lignin as covariates. Because each fibre density is calculated by multiplying mass fraction with total bulk density, total bulk density was omitted in model 2. Insignificant interactions (n.s.) have been removed, resulting in 421 and 423 d.f. for models 1 and 2, respectively. The results of these analyses indicate the importance of bulk density and cellulose underpinning leaf lamina toughness, and weaker and counterintuitive contributions by hemicellulose and lignin.

	model 1 (covariates are mass fractions of fibre components)		model 2 (covariates are densities (g fibre cm^{-3} lamina) of fibre components)	
	coefficient	*t*-value	coefficient	*t*-value
intercept	−87.9	−2.5*	135	8.74***
light (sun)	93.1	2.3*	29.3	2.81**
total bulk density (ρ)	924	15.8***	—	—
cellulose	1590	12.1***	5670	19.7***
hemicellulose	−391	−3.7***	−790	−2.6**
lignin	−169	−2.4*	−446	−2.4*
light × cellulose interaction	622	2.9**	n.s.	n.s.

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

positive relationship between lamina toughness and leaf cellulose density and weaker but significant negative relationships between lamina toughness and hemicellulose and lignin density (table 2). Altogether, light and leaf hemicellulose, cellulose and lignin densities explained 59.3% (adjusted R squared) of the variation in lamina toughness ($F_{4,423} = 156$, $p < 10^{-15}$). A reduced model with just leaf cellulose density explained 58.2% of the variation in lamina toughness ($F_{1,426} = 596$, $p < 10^{-15}$). Thus, when lamina toughness was plotted against leaf cellulose density, there was one common regression slope for sun and shade leaves (figure 3*a*).

The third ANCOVA evaluated relationships among lamina toughness (response variable), light environment (grouping factor) and leaf cell wall (NDF) density (covariate). The interaction between light and cell wall density and the main effect of light were insignificant. This model explained 38.8% (adjusted *R* squared) of the variation in lamina toughness (not shown, $F_{1,426} = 272$, $p < 10^{-15}$), which was substantially weaker than the proportions explained by models 1 and 2 (table 2).

Comparisons of ANCOVA models with BIC suggest that the third model was clearly inferior ($\Delta BIC > 140$). Once insignificant effects were removed, the second ANCOVA model, which used three fibre densities as covariates, was superior to the first ANCOVA model, which used ρ and three fibre fractions as covariates ($\Delta BIC = 9.06$). The minimum BIC was for a model with two terms—an intercept and leaf cellulose density—and ΔBIC equalled 3.09 for the second best model. This indicates that variation in lamina toughness was best explained by a single linear relationship with leaf cellulose density irrespective of light environment (figure 3*a*).

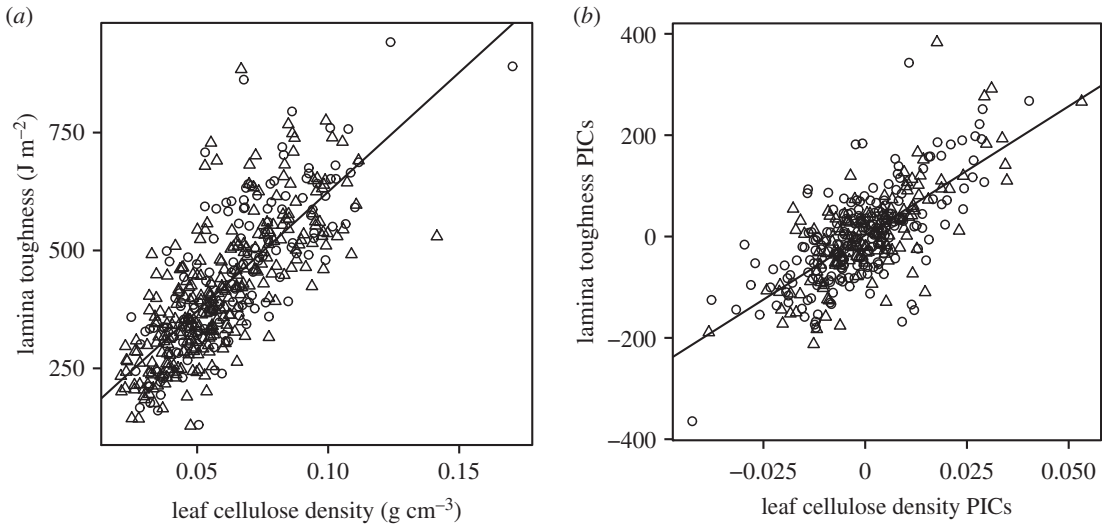

Figure 3. (*a*) Scatter plot for the relationship between lamina fracture toughness and cellulose density (mass per unit lamina volume) for sun-exposed (circles) and shaded (triangles) leaves of trees and treelets from Barro Colorado Island, Panama. Sun and shade leaves fall along one common regression line (table 2). (*b*) The same data analysed with phylogenetic independent contrasts (PICs).

We performed a major axis regression to describe this best model. The intercept was indistinguishable from zero (22.8 J m^{-2} with 95% CI of $\pm$26.7 J m^{-2}), i.e. no cellulose equates to negligible toughness. The slope 6626 (J m^{-2})/(g cellulose cm^{-3}) (95% CI range = 6231–7046) indicates a universal linear relationship of lamina toughness with leaf cellulose density across the tree species of BCI regardless of light environment.

3.3. Comparison with young seedlings

We performed a final ANCOVA to evaluate the relative contributions of leaf cellulose density and ontogenetic plasticity to intra- and interspecific variation in lamina toughness, adding data for 2- to 4-year-old seedlings of 24 species reported previously for the same forest [11]. We pooled sun and shade leaves because (i) sample size was small for shade seedlings with just seven species surviving for 2–4 years and (ii) a single relationship between lamina toughness and leaf cellulose density described sun and shade leaves for older, larger plants (figure 3*a*, table 2). We also excluded from ANCOVA one outlier (highest toughness value of *Aspidosperma cruenta* grown in shade). The relationship between lamina toughness and leaf cellulose density was highly significant and the interaction between ontogeny and leaf cellulose density was insignificant; however, intercepts differed significantly; comparing leaves with similar leaf cellulose density, older plants had tougher leaves than seedlings (figure 4, table 3).

3.4. Effect of phylogeny on leaf-toughness-related traits

There was significant phylogenetic signal for all eight traits for shade leaves and for seven of eight traits (except for %hemicellulose) for sun leaves in the randomization test ($p < 0.05$, data not shown). Blomberg's K values ranged from 0.194 to 0.353, which suggests relatively weak conservatism. By contrast, phylogenetic signal was absent for all eight traits for plasticity measured as the difference in PIC values between conspecific sun and shade leaves ($p = 0.06$ for hemicellulose, $p > 0.1$ for others).

The results of ANCOVAs using PICs and species means were broadly consistent. In the first ANCOVA, PIC values

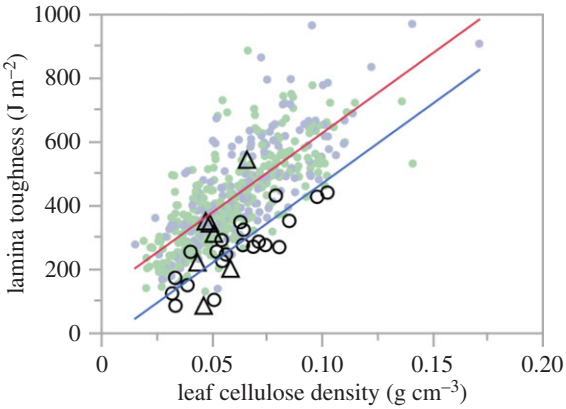

Figure 4. Fracture toughness of leaves of 2- to 4-year-old seedlings grown experimentally in treefall gaps (circles, 21 species) and deeply shaded understorey (triangles, seven species), along with the data for older plants shown as shadows (same data as figure 3*a*). Excluding one seedling data point (*Aspidosperma cruenta* grown in shade, with the highest toughness in the plot) from ANCOVA, seedlings and older plants exhibit common slopes, but seedling leaves are less tough than adult leaves for a given value of cellulose density. (Online version in colour.)

Table 3. ANCOVA analysis to evaluate the dependence of leaf lamina fracture toughness on ontogeny (older plants versus seedlings, see Material and methods) and cellulose density (covariate) for the data shown in figure 4. The insignificant interaction (t-value = 1.03, $p = 0.3$) was removed from the model. The coefficient estimated for each variable is shown along with t-values.

	model 1 (covariates are mass fractions of fibre components)	
	coefficient	t-value
intercept	43.6	2.9*
ontogeny	79.1	8.3*
cellulose density	5002	26.1***

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

Table 4. The results of ANCOVA using PICs to evaluate evolutionary patterns of the dependence of leaf lamina fracture toughness on light environment (grouping factor—sun versus shade), total bulk density (only in model 1), and cell wall fibre components. Models 1 and 2 are equivalent to models 1 and 2 for the analyses using species mean values in table 2, respectively. The coefficient estimated for each variable is shown along with *t*-values. Insignificant interactions and factors (n.s.) are omitted from model 2.

	model 1 (covariates are mass fractions of fibre components)		model 2 (covariates are densities (g fibre cm^{-3} lamina) of fibre components)	
	coefficient	*t*-value	coefficient	*t*-value
intercept	0.96	0.2	2.35	0.68
light (sun)	−4.3	0.6	n.s.	n.s.
total bulk density (ρ)	935	11.9***	—	—
cellulose	2370	11.7***	5536	19.2***
hemicellulose	−722	−5.9***	−1441	−4.06***
lignin	−234	−2.5*	n.s.	n.s.
light × cellulose interaction	−950	−3.8*	n.s.	n.s.

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

of ρ, %cellulose, %hemicellulose and %lignin, light and the light × %cellulose interaction explained 53.1% of the variation in PIC values of lamina toughness ($F_{6,343} = 66.8$, $p < 10^{-15}$) (table 4). In the second ANCOVA, PIC values of leaf cellulose density, hemicellulose density and lignin density explained 52.5% of the variation in PIC values of lamina toughness ($F_{1,346} = 129$, $p < 10^{-15}$), although the effect of leaf lignin density was not significant ($p = 0.081$). In the third ANCOVA, PIC values of leaf cell wall density explained 27.3% of the variation in PIC values of lamina toughness ($F_{1,348} = 132$, $p < 10^{-15}$). The model using PIC values of leaf cellulose density as the sole covariate was clearly preferable to the model that used PIC values of leaf cell wall density as the sole covariate ($\Delta BIC = 132$), and marginally preferable to the model 1 and model 2 shown in table 4 ($\Delta BIC < 2$). Thus, mass fraction-based and fibre density-based analyses using PICs both showed that cellulose was far more important than hemicellulose or lignin in determining lamina toughness, similar to the analyses using species mean values (figures 2 and 3, tables 2 and 4).

4. Discussion

4.1. Composition, structure and fracture toughness of sun and shade leaves

The light environment is extremely heterogeneous within humid tropical forests. Upper canopy leaves are exposed to strong tropical sun, whereas deeply shaded understorey leaves receive less than 1% sunlight. Many studies have described how morphology and physiology of tree leaves adjust plastically to this light heterogeneity. Yet, few studies have reported plastic responses in leaf toughness [28], which is critically important to achieve optimal lifespan [4–7]. Ours is the first community-wide analysis in a tropical forest to test how leaf toughness and cell wall fibre components respond to variation in light availability. Overall, within-species variation in leaf toughness and cell wall fibre components appear much smaller than among-species variation (figure 1), with several common trends across species in support of predictions 4–6 in the Introduction.

The cell wall fibre fractions of conspecific leaves differed between the sun-exposed canopy and the shaded understorey (figure 1). The key trend was lower total bulk density (dry mass per unit volume, figure 1*b*) offset by higher mass fraction of cellulose in shade leaves as compared to sun leaves (figure 1*g*). The greater cellulose mass fraction in shade leaves is probably the mechanistic basis of the notion that 'shade leaves punch above their weight' [24]. As a net result of these compensatory changes, differences in lamina toughness between sun and shade leaves were minimal in many species (i.e. many species fall along the 1 : 1 line in figure 1*a*). Compared with cellulose, the mass fraction of hemicellulose showed similar but weaker responses (figure 1*f*) and the mass fraction of lignin tended to show the opposite response (figure 1*e*). The sum of these three cell wall components that make up the bulk of cell wall showed little difference between canopy and understorey leaves (figure 1*h*).

It is not clear to what extent these changes in total quantities of cell wall components reflect anatomical changes. Our fibre analysis was done for ground samples from the entire leaf blade including veins. Relative representations of mesophyll (including metabolically active cells with relatively thin cell walls), vascular tissue (including xylem and fibre cells with thick secondary cell walls) and epidermis (with thick outer cuticle) are known to change plastically between light environments [17]. It is adaptive in sunny environments to upregulate photosynthetic capacity by packing more mesophyll tissue per unit leaf area (e.g. multi-layered palisade mesophyll) [12]. This results in increased leaf mass per area, which might require enhanced load-bearing capacity to maintain optimal leaf display. Furthermore, enhanced photosynthetic capacity must be accompanied by increased hydraulic conductance, which might require more investment in leaf venation. As a net result of these physiological and anatomical changes, we speculate that sun leaves might have a lower ratio of cellulose to cytoplasmic cell contents and an increase in lignin associated with secondary cell walls of xylem and other vascular tissues. To evaluate these possibilities, future studies need to address variation in cellulose, hemicellulose and lignin content among tissues within a leaf.

Interspecific variation was much larger than intraspecific variation for every leaf trait we examined (figures 1, 2*a*, 3*a* and 4). This is consistent with a strong role for life-history evolution in shaping leaf traits, all of which showed significant phylogenetic signals. By contrast, phylogenetic signal was insignificant for plasticity measured as the canopy–understorey difference in leaf traits. This means that phylogeny did not constrain the direction and magnitude of plastic responses, and all study species exhibited similar differences between sun-exposed canopy leaves and understorey leaves. For this reason, PICs were indistinguishable for canopy and understorey leaves (table 1).

One caveat of our study is that our sun leaves are from canopy adults and our shade leaves are from understorey juveniles. Hence, our results may be influenced by ontogenetic change. Many leaf traits that influence biomechanical properties, including lamina thickness and bulk density, change from small seedlings to understorey saplings to adults. Ontogenetic change is clearly evident even within the first year of seedling life as leaf bulk density increases from the first leaf to progressively larger values for leaves produced by 3- and 12-month-old seedlings [7]. A second caveat is that many environmental factors (e.g. humidity, vapour pressure deficit, wind and temperature) differ between the upper canopy and the shaded understorey. Factors other than light environment *per se* made unknown contributions to the observed differences between our understorey and canopy leaves.

4.2. Determinants of leaf fracture toughness

Leaf lamina fracture toughness varied widely among species (figure 1*a*), and was greater for sun than for shade leaves for many species (figures 1*a* and 2*a*). Across species, lamina toughness increased with total bulk density and cellulose fraction, and decreased with hemicellulose and lignin fractions (table 2). These results confirm predictions 1–3 in the Introduction. Total bulk density matters. As proportionally more space inside a leaf is occupied by solids instead of air or aqueous liquid, there is more material to be encountered by a fracturing force. But, what is critical is the bulk density of strong materials. Thus, we calculated individual fibre densities by multiplying each fibre mass fraction by total bulk density. Of the three main components of cell wall, cellulose stands out; cellulose density alone explained 58.2% of the variation in lamina toughness, subsuming the variation previously associated with canopy and understorey environments (figures 2*a* versus 3*a*).

Comparisons of alternative statistical models confirm that hemicellulose and lignin density did not improve the relationship between lamina toughness and cellulose density. The inclusion of these two additional variables result in an insignificant increase from 58.2% to 59.3% of the variation of lamina toughness explained. The sum of cellulose, hemicellulose and lignin (i.e. NDF) is an indicator of the total amount of cell wall in leaves [19,24], but leaf NDF density had much weaker explanatory power for lamina toughness than leaf cellulose density.

Cellulose microfibrils, being one of the densest biological materials with strongest resistance against tension, is the key among cell wall components in making leaves tough. Even though we considered only the bulk abundance of cellulose without considering structural organization, we could detect the overriding role of cellulose in explaining interspecific variations in leaf toughness. It was somewhat surprising that lignin and hemicellulose, which cross-link and bind cellulose microfibrils, had negative contributions to fracture toughness, similarly for leaves (table 2) and wood [38] analysed with the same cutting test. Detailed ultrastructual analyses might shed light on the reason why an increase of lignin, which enhances stiffness, somehow results in less work required for cracking. The analyses with PICs (table 4) support that these variations in cellulose density should be appropriately interpreted as the product of evolution [27].

4.3. Final remarks

Overall, the results of this study are consistent with previous studies and predictions based on them about the contribution of total bulk density and mass fractions of fibre on fracture toughness. These were directly measured quantities, but mass fractions (e.g. %cellulose, %lignin) are not statistically independent of each other, because a proportional increase in one fibre type may be the sole reason for a proportional decrease in another fibre type. By contrast, leaf densities of individual fibres are less constrained by each other in the space inside a leaf lamina containing much liquid and air. Hence, it is more appropriate to examine individual fibre densities in relation to mechanical properties, rather than their mass fractions in combination with total bulk density. Once we did so, a remarkably simple result emerged; leaf cellulose density alone had a linear relationship with leaf fracture toughness variation across and within species.

Sun and shade leaves fell along a common relationship between fracture toughness and cellulose density (figure 3*a*). Seedling leaves showed weaker fracture toughness per given cellulose density, but the fundamental relationship between fracture toughness and cellulose density was very similar for young seedlings and much older larger trees (figure 4). Thus, we conclude that leaf cellulose density is a generalizable predictor of leaf toughness in the tropical moist forests of the Barro Colorado Nature Monument, Panama. Additional studies will be necessary to evaluate the further generality of this conclusion that leaf density of cellulose, but not other cell wall fibres, is the key determinant of leaf fracture toughness.

Competing interests. We declare we have no competing interests.

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