



Trubs, but no trianas: filled and empty regions of angiosperm stem length-diameter-mechanics space

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Discrete plant habit categories such as ‘tree’, ‘shrub’, and ‘liana’ belie continuous variation in nature. To study the evolution of this continuous variation, we gathered data on stem length, diameter and tissue mechanical stiffness across a highly morphologically diverse highland xerophytic scrub on a lava flow in central Mexico. With stem allometric and mechanical data from 1216 segments from 50 species, we examined relationships between stem length–diameter proportions and tissue mechanical stiffness using linear mixed-effects models. Rather than a series of discrete clouds in stem length–diameter–tissue stiffness space, corresponding to traditional habit categories, the plants of this xerophytic scrub formed a single continuous one. Within this cloud, self-supporting plants had stems that became predictably longer and tissues that became stiffer for a given diameter increase, and there was no paucity of intermediates between trees and shrubs (‘trubs’). Non self-supporting plants had a steeper stem length–diameter slope and their tissues did not increase in stiffness with stem size. The area between self- and non self-supporting plants was sparsely occupied as stem size increased. We predict that this ‘empty’ space between lianas and trees is developmentally accessible but of low fitness, meaning that there should be few ‘trianas’ in nature. © 2015 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2015, 179, 361–373.

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INTRODUCTION

Studies covering hundreds of species show that different plant habits have largely predictable stem length–diameter allometries. Tree height scales with diameter over an ample range of life stages to the $\frac{2}{3}$ power (McMahon, 1973; Rich *et al.*, 1986; King, 1990, 1991, 1996; Farnsworth & Niklas, 1995; O’Brien *et al.*, 1995; Thomas, 1996; Sterck & Bongers, 1998; Muller-Landau *et al.*, 2006; Poorter, Bongers & Bongers, 2006; Feldpausch *et al.*, 2011; Olson & Rosell, 2013; Olson *et al.*, 2013). The scaling power of stem length–diameter allometry appears to vary to greater or lesser degrees across other self-supporting habits, such as mosses, herbs, palms, pteridophytes, pachycaul plants, and young or old trees (Niklas,

1993, 1994a,b, 1995a,b; Niklas & Buchman, 1994; Niklas, Cobb & Marler, 2006; Rosell *et al.*, 2012). Even liana stem length–diameter allometry scales predictably across species, although with much longer stems for a given diameter than in self-supporting plants (Olson *et al.*, 2014; Rosell & Olson, 2014). Stem length–diameter proportions across habits are often regarded as sufficiently predictable that palaeobotanists use them to estimate fossil plant height (Niklas, 1994c). Mapping the ways that stem length–diameter relationships vary across species is useful because it allows studying the ways that natural selection acts on the diversity of stem size and shape.

Mapping stem length–diameter relationships can be seen as part of a long tradition of studying organismal shape and size, but the simultaneous study of tissue mechanical properties has lagged behind. The size–shape tradition, prominent early examples being

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works of Cuvier and Dubois in the second half of the 19th century (Gayon, 2000), made shape and size the central aspects for studying organismal form and function (Alberch *et al.*, 1979; Sweet, 1980; Bookstein, 1989; Jungers, Falsetti & Wall, 1995; Klingenberg, 2010). However, all else being equal, any change in organismal shape or size will also change the mechanical behaviour of the structure in question (Thompson, 1924; McMahon, 1973; Bertram, 1989; Alfaro, Bolnick & Wainwright, 2004; Rosell *et al.*, 2012; Niklas, 2013). For example, given a cylinder of 1 cm in diameter and another of 100 cm, the thicker cylinder will be more resistant to bending than the thinner one, even if they are the same length and have identical mechanical properties of materials. Allometry is therefore ideally paired with biomechanics to study morphological and functional diversity of organismal structures.

In woody plants, simultaneous study of allometry and mechanics is ideal because small variation in combinations of stem proportions and tissue mechanical properties can have drastic effects on habit, producing a wide span from self-supporting trees and shrubs to semi-self-supporting shrubs to non self-supporting lianas or vines (see Rowe & Speck, 2005; Speck & Burgert, 2011). For example, lianas and vines have long stems for a given diameter with relatively low tissue stiffness. Trees and shrubs have stems that are moderately long for a given diameter with relatively high tissue stiffness. Much of the diversity in plant habit can therefore plausibly be seen as the interplay between stem length (L), stem diameter (D), and stem tissue Young's modulus (E_{stem}); we term this interplay hereafter as ' L - D - E_{stem} '. Young's modulus describes how resistant a given material is to bending; stems made of stiff materials have high E_{stem} values, whereas those of flexible ones have low values (Vogel, 2003; Gere & Goodno, 2009). Studies of individual species or small groups of species underscore the central importance of these variables in summarizing plant habit diversity (Bertram, 1989; Isnard, Speck & Rowe, 2003; Rowe, Isnard & Speck, 2004; Lahaye *et al.*, 2005; Ménard, McKey & Rowe, 2009; Rosell *et al.*, 2012), but almost nothing is known about the general patterns of how stem length, diameter, and tissue mechanics vary in relation to one another in woody plants generally.

To begin to explore the ways that plant stems vary across L - D - E_{stem} , we used allometric and biomechanical data from 1216 stem segments from 50 species of flowering plants from a habitally diverse community in central Mexico. We chose this community because it includes a remarkable combination of plant habits including small lianas, vines, scramblers, shrubs with low to high wood density, small to large forbs, trees, broomstick succulents (Rosell & Olson, 2007), stem

succulents with completely parenchymatized (fibre-free) xylem (Carlquist, 1966), leaf succulents, and small to large cacti (Fig. 1). This span of anatomical modes is greater than that occurring across many much larger temperate areas (e.g. Tutin *et al.*, 1993), and this community is important to study as a relatively small natural area threatened by urban sprawl. This diversity is therefore ideal to map quantitatively how plant habits vary across L - D - E_{stem} . This mapping allowed us to examine relationships between stem length–diameter proportions and tissue mechanics and the presence of unoccupied patches of L - D - E_{stem} space. For example, Scheffer *et al.* (2014) claimed to have identified an empty space between trees and shrubs. They asserted that heights intermediate between trees and shrubs (8–10 m) are rare, concluding that 'trubs' are selected against in nature (but see McGlone *et al.*, 2015; Qian & Ricklefs, 2015; Scheffer *et al.*, 2015). Our data permitted us to examine this issue with additional variables of great functional relevance. Our approach allowed us to test whether not just trees and shrubs, but also other traditional habit categories, have distinctive L - D - E_{stem} combinations.

Traditional habit categories overlapped considerably in our data. Rather than an area of sparse occupation between trees and shrubs, we found a distinct empty space between self- and non self-supporting plants at large stem sizes. We comment on the possible causes and significance of these patterns. We conclude by showing the capacity of our approach for mapping the limits in the stem morphological diversification and indicate methodological avenues for further research.

MATERIALS AND METHODS

Our study community, the Reserva Ecológica del Pedregal de San Ángel, is a xerophytic scrub on highland lava fields at 2000 m above sea level in the southern part of the Valley of Mexico (19°19'12.68"N, 99°11'26.99"W) with mostly thin, rocky soil, a mean annual precipitation of 835 mm, and a mean annual temperature of 15.5 °C (Castillo-Argüero *et al.*, 2004). To represent the habit diversity of our study community, we selected 50 species (Supporting Information Table S1), with vouchers deposited at Herbario Nacional de México (MEXU). Species sampling was guided by three criteria. The first was to span all woody plant habits in our community. Maximizing habit diversity helped map the extent of L - D - E_{stem} space occupation in our community and to test whether these traits recovered the traditional growth form categories or whether these categories represented a continuum in this space. The second was to include the most common species, and the third was to maximize the phylogenetic span of our collections.

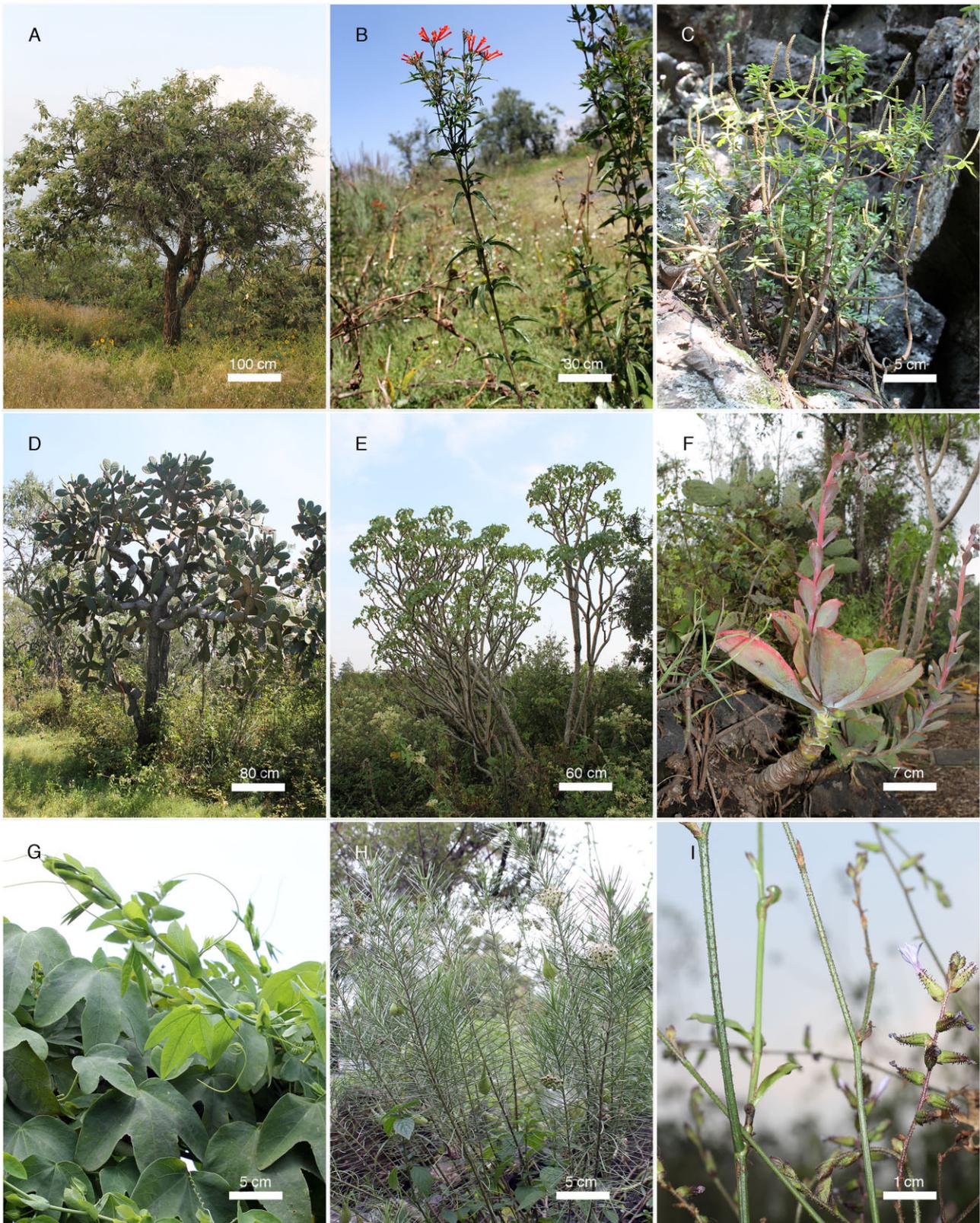


Figure 1. See caption on next page.

Figure 1. Stem form diversity, focusing on stem length, diameter, and mechanics in the Pedregal de San Ángel, central Mexico. A, *Buddleja cordata*, classed as a tree, has slender, flexible terminal stem tissues, and wide, stiff stem basal tissues. B, *Bouvardia ternifolia*, classed as a shrub, has one of the highest tissue stiffnesses in the community. C, *Peperomia galioides*, classed as a forb, has succulent stems, but has similar mechanical behaviour to self-supporting woody plants. D, *Opuntia tomentosa* has wider stems and more flexible tissues for a given length as compared to conventional trees. E, *Pittocaulon* (~*Senecio*) *praecox* is classed as a shrub, but in contrast to *B. ternifolia* it has longer, wider stems, and more flexible tissues. F, *Echeveria gibbiflora* has stubby and flexible stems in its main body and long, slender inflorescences. G, *Passiflora subpeltata*, classed as non self-supporting, has narrower, longer stems, and more flexible tissues than any self-supporting plant in the community. H, *Asclepias linaria* was classed as shrub. I, *Plumbago pulchella* has a scrambler habit with arching non self-supporting branches.

Including the common species gives a summary of the bulk of the plants in a given area, presumably reflecting the adaptive responses that result in high abundance in a given environment. Maximizing the phylogenetic span of a comparative study lends support to the idea that the patterns recovered are general ones.

We classified species in two ways. First, we classified each species as self- vs. non self-supporting (we use the term ‘climber’ interchangeably with non self-supporting). Non self-supporting stems were those that did not maintain their tips off the ground when the base of the sampled stem was held erect at ground level. Species not meeting this criterion were classified as self-supporting. The second classification assigned each of the self-supporting species to the classes tree, shrub, forb, and inflorescence following Calderón de Rzedowski & Rzedowski (2005; Supporting Information Table S1). When Calderón de Rzedowski & Rzedowski listed habit as a range, we classified the plant in the largest of the listed categories because our sampling emphasized the largest plants (e.g. if the range is listed from shrub to tree, we classed the species as tree). We sampled the inflorescences of *Agave salmiana* Otto ex Salm-Dyck, *Echeveria gibbiflora* DC., and *Manfreda scabra* (Ortega) McVaugh, classing them as ‘inflorescences.’ Finally, five self-supporting species were not assigned a habit category by Calderón de Rzedowski & Rzedowski, so we classed these species by following their habit descriptions of similar species of the same genera. Accordingly, *E. gibbiflora*, *Mammillaria magnimamma* Haw., *Reseda luteola* L., *Valeriana sorbifolia* Kunth, and *Zinnia peruviana* (L.) L. were classed as forbs.

For each species, we collected on average eight to ten branches from three to four large, healthy individuals. We selected straight branches that were < 5 cm in diameter due to the size limitations of our testing device, and then we cut an average of three straight segments per branch. We collected a total of 451 branches and 1216 segments (Table 1). For each segment, we measured the average basal diameter (D) using digital calipers and the distance between the base of the segment and the tip of the branch (L)

using a tape measure. To measure stem tissue Young’s modulus (E_{stem}), we mechanically tested each segment in three-point bending in an Instron 3345 testing machine using a 5 kN load cell and Series IX software (Instron Corporation, Canton, Massachusetts, USA). E_{stem} is calculated from the initial rate of flexural displacement of the segment under loading. High E_{stem} values represent stiff stem tissues whereas low values represent flexible ones. Each tested segment had a 1:20 diameter-length ratio to avoid shear (Vincent, 1992; Gere & Goodno, 2009). We measured E_{stem} using the diameter of each segment at its mid point and the formula for solid cylinders (Niklas, 1992).

For all analyses, we \log_{10} transformed L , D , and E_{stem} . We plotted L , D , and E_{stem} as a three-dimensional graph (Fig. 2). To examine differences in L vs. D scaling between self- and non self-supporting plants, we fit a linear model predicting L based on D , plus a categorical variable reflecting whether species were self- or non self-supporting, and an interaction term between D and the self-/non self-supporting variable. A significant interaction term indicated that the self- and non self-supporting habits differed in their L vs. D scaling relationship. In our model, segments of the same species were likely to be more similar to one another than to segments from other species. To take this similarity into account, we included a ‘species’ random variable that reflected species membership of segments in a mixed-effects model (Goldstein, 2003; Kutner *et al.*, 2005; Zuur *et al.*, 2009). Our mixed-effects model included D , the self-/non self-supporting variable and the $D \cdot$ self-/non self-supporting interaction as fixed terms. The ‘species’ random variable allowed nesting segments within species. We explored two alternative versions of our mixed model. In one of these, species were allowed to have random intercepts, i.e. species could have different mean L for a given D , but all species had the same mean scaling exponent. In a second version, species were allowed to have random intercepts as well as random slopes, i.e. species varied in their L - D scaling exponent. We used log-likelihood ratio tests and the Akaike and Bayesian information

Table 1. Descriptive data for each habit category

Habit category	Stem diameter (cm)			Stem length (cm)			Young's modulus (MPa)			Number of species	Number of segments
	Minimum	Median	Maximum	Minimum	Median	Maximum	Minimum	Median	Maximum		
All categories	0.07	0.74	10.52	5	85	820	1.73	3073	12 730	50	1216
Non self-supporting	0.10	0.46	1.55	10	158	820	31	2644	9 457	5	147
Self-supporting	0.07	0.81	10.52	5	80	558	2	3157	12 730	45	1069
Tree	0.18	1.43	10.52	9	107	542	54	2357	9 693	10	279
Shrub	0.12	0.82	5.75	9	83	558	22	4547	11 360	14	364
Forb	0.07	0.50	8.08	5	60	283	2	2787	12 730	19	375
Inflorescence	0.50	1.07	4.57	11	67	245	76	2783	5 845	4	51

criteria to compare the fit of these two alternative models (Zuur *et al.*, 2009). The model that best fit the data was that with random slopes (and thus random intercepts; Supporting Information Table S2). The final best-fitting model had the following form: $L = D + \text{self/non self} + D \cdot \text{self/non self} + S_r + e$, where S_r was the species random effect allowing species to have random intercepts and slopes, and e is the error term (Table 2). We hereafter abbreviate this model as $L \sim D \cdot \text{self/non self}$.

We analyzed in more detail the L vs. D scaling of our self-supporting species by comparing trees, shrubs, forbs, and inflorescences, according to the habit classification of Calderón de Rzedowski & Rzedowski (2005). To this end, following the same model fitting procedure for $L \sim D \cdot \text{self/non self}$, we fit a model predicting stem length based on stem diameter and the self-supporting habit categories expressed as $L = D + GF + D \cdot GF + S_r + e$, where GF (growth form) was a nominal variable with the levels tree, shrub, forb, or inflorescence and was represented in the model by three dummy variables with forb as the reference category. This model had a non-significant $D \cdot GF$ interaction ($F_{3,1043} = 2.00$, $P = 0.11$), meaning that the GF categories did not differ in their L - D scaling exponents, so we refit the model without the interaction (Table 3) and then tested whether intercepts differed significantly. We hereafter abbreviate this model as $L \sim D + GF$. Again, a structure with a random intercept and slope per species fit best (Supporting Information Table S2).

To examine relationship patterns between E_{stem} and stem L - D proportions, we calculated for each segment a length–diameter proportion index (L/D) by dividing the distance from the base of each segment to the tip of the stem by its diameter. Given that self- and non self-supporting habits represent different combinations of stem length–diameter proportions together with tissue mechanical properties, L/D provided a simple visualization of different mechanical strategies when plotted against E_{stem} . We followed the same model fitting procedure as for the model exploring L - D scaling. Accordingly, we fitted a model predicting E_{stem} based on L/D and the self-/non self-supporting habit categorical variable. This model took the form $E_{\text{stem}} = L/D + \text{self/non self} + L/D \cdot \text{self/non self} + S_r + e$. We used this model to test whether stems of self-supporting species increased in mechanical stiffness with increasing L/D . We hereafter abbreviate this model as $E_{\text{stem}} \sim L/D \cdot \text{self/non self}$. Again, a structure with a random intercept and slope per species fit best (Supporting Information Table S2). Finally, we fit a model that explored E_{stem} vs. L/D scaling across our GF categories, with the form $E_{\text{stem}} = L/D + GF + L/D \cdot GF + S_r + e$. This model had both a non-significant $L/D \cdot GF$ interaction ($F_{3,1016} = 0.91$,

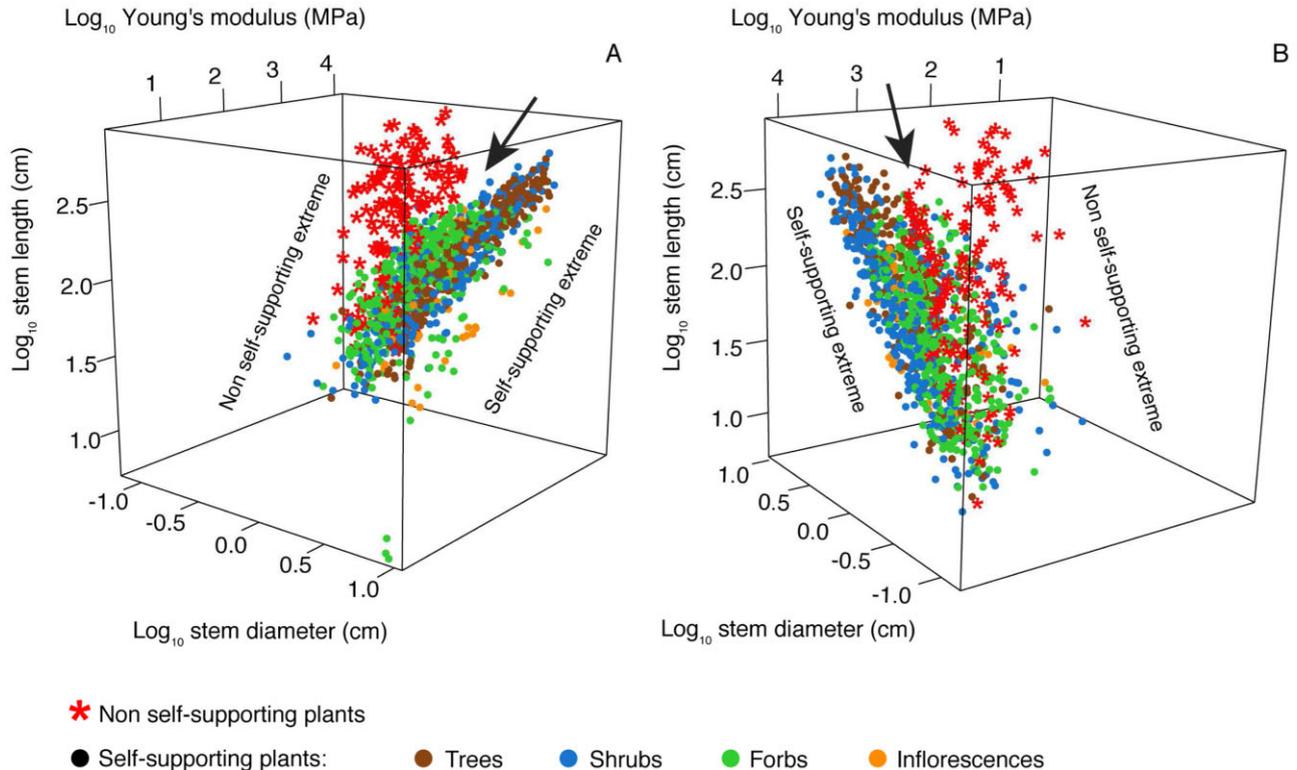


Figure 2. Two views of the stem length–diameter–mechanics space of the highland xerophytic scrub studied here. A, This view shows the diversity in stem length–diameter proportions across the community. There is a clear positive relationship between stem length and diameter across self-supporting plants. Non self-supporting plants have much longer stems for a given diameter than self-supporting plants. B, This view shows the wide span of stem tissue mechanical properties and their interplay with stem length–diameter relations. In self-supporting plants, longer, thicker stems have stiffer tissues. *Mammillaria magnimamma* occupies the corner corresponding to short, thick stems with low tissue stiffnesses. Non self-supporting plants have long, slender stems with low tissue stiffness. Black arrow in both views shows the empty region between large self- and non self-supporting stems (see Discussion section).

$P = 0.44$) and a non-significant GF term ($F_{3,1016} = 2.11$, $P = 0.10$), meaning that the GF categories did not differ in their scaling exponents and intercepts. That is, traditional habit categories did not differ in their E_{stem} vs. L/D scaling relationships. We base our discussion of $E_{stem} \sim L/D$ scaling on the model $E_{stem} \sim L/D \cdot self/non\ self$.

We calculated the coefficient of determination (R^2) for our final mixed models using the method of Nakagawa & Schielzeth (2013; Table 2). Their method uses a marginal coefficient of determination (R^2_m) that represents the variance explained by the fixed component and a conditional coefficient of determination (R^2_c) that represents the variance explained by the fixed and the random component.

Additionally, we tested for phylogenetic signal in our data. To do so, we built a community phylogenetic tree using the APG backbone (APG III, 2009). We then assigned branch lengths using the 'bladj' command of Phylocom v. 4.2 (Webb, Ackerly & Kembel, 2008) and divergence times of Wikström,

Savolainen & Chase (2001), removing singleton branches with the 'cleanphy' command. We then tested for the presence of significant phylogenetic signal in the residuals (Revell, 2010) of our three models using a randomization test based on phylogenetically independent contrasts and the descriptive statistics λ (Pagel, 1999) and K (Blomberg, Garland & Ives, 2003). All analyses were performed in R (R Development Core Team, 2013) using the packages 'rgl' (Adler & Murdoch, 2013), 'nlme' (Pinheiro *et al.*, 2013), 'MuMIn' (Barton, 2013), 'ape' (Paradis, Claude & Strimmer, 2004), and 'phytools' (Revell, 2012).

RESULTS

The ranges of L , D , and E_{stem} for each habit category are given in Table 1 and for each species in Supporting Information Table S1. A three dimensional graph of L - D - E_{stem} variation (Fig. 2) showed that habit categories formed a single continuum with two distinctive trends. First, self-supporting stems, consisting of

Table 2. Linear fit of the fixed components of $L \sim D \cdot self/non\ self$ and $E_{stem} \sim L/D \cdot self/non\ self$ models

	$L \sim D \cdot self/non\ self$	$E_{stem} \sim L/D \cdot self/non\ self$
n	1258	1216
R_m^2	0.58	0.17
R_c^2	0.94	0.81
Equality of slopes test	$F_{1,1205} = 9.32$, $P < 0.005$	$F_{1,1163} = 11.40$, $P < 0.001$
Non self-supporting		
Intercept	2.76 (2.42,3.11)	3.38 (2.42,4.35)
Slope	1.72 (1.41,2.04)	-0.05 (-0.47,0.37)
Self-supporting		
Intercept	1.96 (1.62,2.30)	1.93 (0.91,2.95)
Slope	1.20 (0.86,1.54)	0.74 (0.30,1.18)

Notes: Random effects are shown in Table S3. Equality of slopes test determined whether interaction terms of the models were significant. Values in parentheses are 95% confidence intervals. Abbreviations: n , sample size; R_c^2 , conditional determination coefficient; R_m^2 , marginal determination coefficient.

Table 3. Linear fit of the fixed components of $L \sim D + GF$ model

	$L \sim D + GF$
n	1096
R_m^2	0.65
R_c^2	0.95
Equality of slopes test	$F_{3,1043} = 2.00$, $P = 0.11$
Equality of intercepts test	$F_{3,1043} = 5.21$, $P < 0.005$
Tree	
Intercept	2.03 (1.79, 2.27)
Slope	1.23 (1.11, 1.35)
Shrub	
Intercept	1.87 (1.65, 2.09)
Slope	1.23 (1.11, 1.35)
Forb	
Intercept	2.10 (1.94, 2.26)
Slope	1.23 (1.11, 1.35)
Inflorescence	
Intercept	1.38 (0.97, 1.79)
Slope	1.23 (1.11, 1.35)

Random effects are shown in Table S3. Equality of slopes test determined whether interaction term of the model was significant. Because this interaction was not significant, we compared the intercepts across GF categories using the equality of intercepts test. Values in parentheses are 95% confidence intervals. Abbreviations: n , sample size; R_c^2 , conditional determination coefficient; R_m^2 , marginal determination coefficient.

trees, shrubs, forbs, and inflorescences, clearly had a lower slope and L - D intercept as compared to non self-supporting ones (Fig. 2A). Second, self-supporting stems tended to lie in high E_{stem} space, especially larger stems, whereas non self-supporting stems tend to fall in low E_{stem} space (Fig. 2B). These two trends were evident in the two mixed models: $L \sim D \cdot self/non\ self$ and $E_{stem} \sim L/D \cdot self/non\ self$ (Fig. 3A and Fig. 4, respectively).

Non self-supporting plants tended to have longer stems for a given diameter than self-supporting plants, with stems becoming markedly longer as diameter increased (Fig. 3A). Non self-supporting plants had a mean L - D scaling slope of 1.72 and self-supporting plants of 1.20. These slopes differed significantly as indicated by their mutually exclusive confidence intervals (Table 2). Across GF categories, there were no significant differences in scaling exponents (common slope of 1.23), but we observed a varying degree of overlap in the confidence intervals of intercepts (Table 3). Shrubs, trees, and forbs had similar intercepts (ranging from 1.87 to 2.10). In contrast, inflorescences had the lowest intercept (1.38) with relatively little overlap in confidence interval with those of the other growth forms (Fig. 3B).

Self- and non self-supporting species had different E_{stem} vs. L/D scaling relationships (Fig. 4). In self-supporting plants, E_{stem} tended to increase with higher L/D , i.e. stem tissues tended to be stiffer in larger stems (slope = 0.74). In strong contrast, E_{stem} did not vary in non self-supporting species with L/D (slope did not differ significantly from zero; Table 2). Therefore, the non self-supporting plants represented a different mechanical-allometric organization as compared to self-supporting plants.

Per-species random slopes and intercepts for our three models are given in Supporting Information Table S3. All models fitted the data well, with $R_c^2 = 0.94$ for $L \sim D \cdot self/non\ self$, $R_c^2 = 0.96$ for $L \sim D + GF$, and $R_c^2 = 0.81$ for $E_{stem} \sim L/D \cdot self/non\ self$. Phylogenetic signal was not significant in the residuals of $L \sim D \cdot self/non\ self$ as indicated by the randomization test based on phylogenetic independent contrasts ($P = 0.862$). This lack of signal was congruent with the small values of K (= 0.269) and λ (= 0.137). The same applied for $L \sim D + GF$ ($P = 0.965$) where $K = 0.236$ and $\lambda = 0.00007$ and for $E_{stem} \sim L/D \cdot self/non\ self$ ($P = 0.619$) where $K = 0.339$ and $\lambda = 0.00007$.

DISCUSSION

For the most part, traditional plant habit categories overlapped considerably in our data. This overlap means that it is possible to move between any two points via a series of intermediate forms. Within this

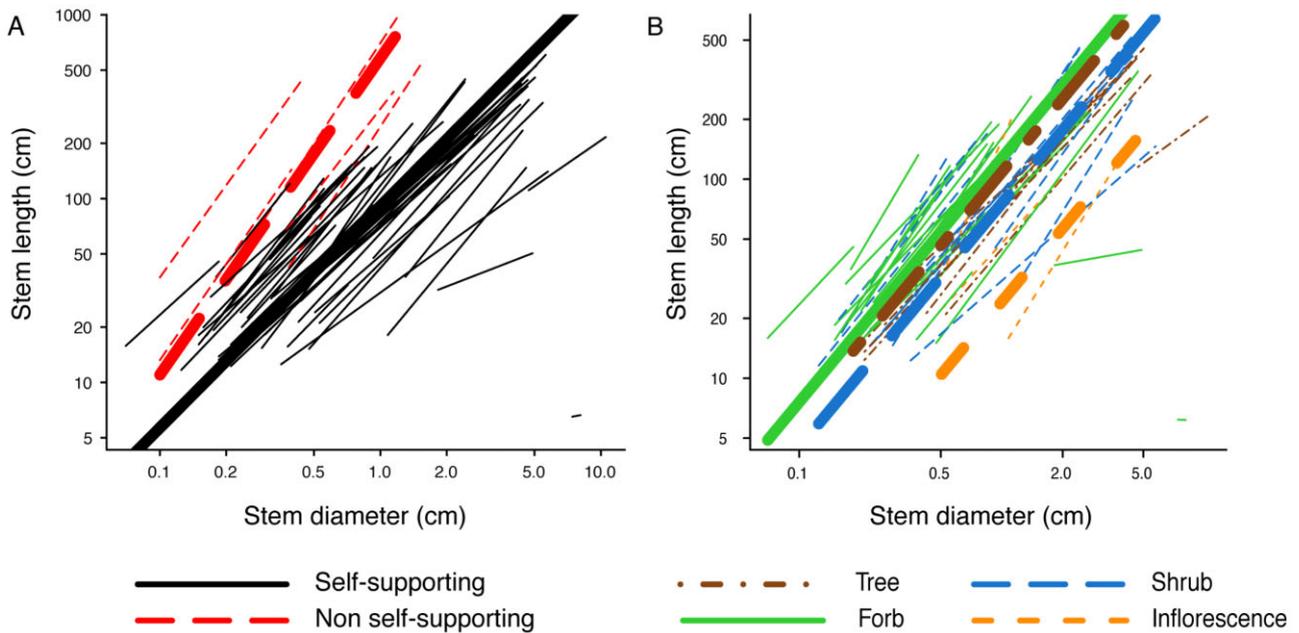


Figure 3. Self-/non self-supporting and traditional habit categories in L vs. D mixed models. A, Stem length predicted by stem diameter between self- and non self-supporting plants, showing that non self-supporting plants had longer stems for a given diameter than self-supporting plants. This figure corresponds to the $L \sim D \cdot self/non\ self$ model. Dashed red lines represent non self-supporting species and solid black lines represent self-supporting species. B, Stem length predicted by stem diameter between tree, shrub, forb, and inflorescence, showing that habits did not differ in their scaling exponents, but did differ in their intercepts. Forbs had the longest stems for a given diameter followed by trees, shrubs, and inflorescences. This figure corresponds to the $L \sim D + GF$ model. Solid green lines represent forbs and dashed blue lines represent shrubs, followed by orange lines representing inflorescences and then by brown lines representing trees. In A and B, thick lines represent the fixed component of the model, and thin lines represent species with random slopes and intercepts. Figure axes give non-transformed values whereas model lines are \log_{10} transformed. Model coefficients are presented in Tables 2 and 3.

continuous cloud, our data seemed to indicate areas that were more densely occupied than others, with some areas that corresponded to plausible morphologies being apparently empty in our community.

CONTINUOUS VS. CATEGORICAL DESCRIPTIONS OF PLANT HABIT DIVERSITY

The different habit categories did not form distinct clouds, but instead the entire range of stem forms formed a single continuous cloud, albeit an irregular one. This continuous cloud ranged from a non self-supporting region, made up of climbers and decumbent plants, to a self-supporting region, made up of a wide diversity of habits, including trees, shrubs, forbs, pachycaul species, and even inflorescences (see Calderón de Rzedowski & Rzedowski, 2005; Niklas *et al.*, 2006). The non self-supporting region included, at its farthest extreme from the self-supporting region, climbing plants with flexible tissues (low E_{stem}) and markedly long stems for a given diameter. The non self-supporting plants closest to the self-

supporting region were decumbent and prostrate plants, with stiffer tissues and shorter stems for a given diameter than tendrils and twining climbers. The self-supporting region included trees, shrubs, forbs, and inflorescences, with stiffer tissues and shorter stems for a given diameter compared to non self-supporting plants. The lower extreme of the self-supporting region was made up of succulent species that would be classified as pachycaul using the criteria of Niklas *et al.* (2006), though they were classed by Calderón de Rzedowski & Rzedowski (2005) as forbs. These plants had flexible tissues and short stems for a given diameter (see *Mammillaria magnimamma* highlighted in Fig. 2). In summary, the plants of our xerophytic community ranged from long-stemmed climbers, through conventional trees and shrubs, to squat pachycaul species in a single graded continuum across L - D - E_{stem} space.

In this continuum, the categories that overlapped most conspicuously were traditional self-supporting ones. These traditional categories did not differ in their E_{stem} vs. L/D scaling relationships (see model fitting

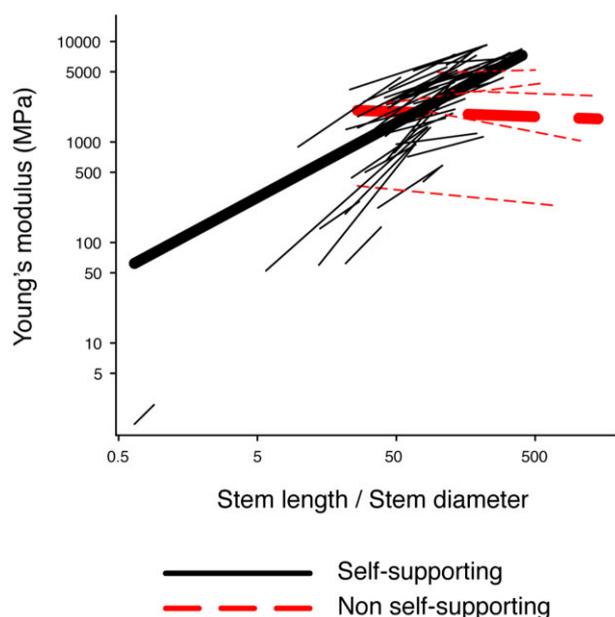


Figure 4. Self-/non self-supporting in E_{stem} vs. L/D mixed models. Stem Young's modulus increased with stem proportions in self-supporting plants. In contrast, there was no tendency for Young's modulus to increase with increasing size in non self-supporting plants. This figure corresponds to the $E_{stem} \sim L/D \cdot self/non\ self$ model. Dashed red lines represent non self-supporting species and solid black lines represent self-supporting species. Thick lines represent the fixed component of the model, and thin lines represent species, with random slopes and intercepts. Figure axes give non-transformed values whereas model lines are \log_{10} transformed. Model coefficients are presented in Table 2.

procedure in Materials and Methods). This extensive overlap means that all of our self-supporting plants fell in the same general L - D - E_{stem} space. In other words, we found no salient biological distinctions between trees, shrubs, forbs, and inflorescences in their combinations of stem proportions and tissue mechanical stiffness. All this means that, although plant habit categories may be appropriate for rapid communication in floras or forestry, no breakpoints separated the self-supporting categories in our community. Consequently, one question is whether L , D , and E_{stem} are appropriate for recovering biological distinctions across traditional plant habits (see, e.g., Küchler, 1988, or Gschwantner *et al.*, 2009). In traditional classifications, trees are often defined as woody plants with single stems little branched from the base that are > 10 cm in diameter at breast height. This definition makes a great deal of sense for foresters, who wish to know if there is wood to be harvested at any given location. However, it makes little sense in allometric-biomechanical terms, given that individuals with large single trunks, mul-

tiple trunks, small individuals with single or multiple trunks all apparently form a single biological continuum, in which no relevant breakpoints separate trees from shrubs or forbs.

Instead, our approach expressing L , D , and E_{stem} as a continuum offers a more biologically real and analytically powerful means of describing plant habit diversity. Our analyses showed that stem length, diameter, and tissue mechanical properties are inter-related in ways that are predictable and highly significant biologically. For example, the $E_{stem} \sim L/D \cdot self/non\ self$ model highlighted the tendency for self-supporting plants with low E_{stem} to have wide stem diameters for a given length. This tendency reached an extreme in pachycaul species (Niklas *et al.*, 2006), which have minimally stiff stem tissue and maximally thick stems for their diameters. We showed that across the continuum of self-supporting plants, as E_{stem} becomes stiffer, stems become predictably longer for a given diameter (see Rosell *et al.*, 2012). Replacing arbitrary habit categories for the continuous variables L , D , and E_{stem} thus gives access to quantification of trait relationships of central evolutionary importance. The relationship between organ size, proportions, and tissue mechanical properties that we documented here is essential not just for the study of plant morphological evolution, but of morphological evolution in general (Rosell *et al.*, 2012).

Our data also provide a means of addressing recent debate regarding the lack of 'trubs' in nature. 'Trubs' are intermediate in height between trees and shrubs, in the 8–10 m range or so, and some authors assert that they are rare in nature (Scheffer *et al.*, 2014, 2015; McGlone *et al.*, 2015; Qian & Ricklefs, 2015). Our data, which focused on the functionally crucial relationship between allometry and mechanics, did not reveal any difference between terminal branches of trees and shrubs (Bertram, 1989). We therefore found no reason to suspect that natural selection acts against self-supporting plants of intermediate size. We did, however, find evidence for a marked distinction between self- and non self-supporting plants as plants increased in size.

THE SELF-/NON SELF-GAP: NO TRIANAS

Our results identified a distinct lack of intermediate stem L - D relationships between self- and non self-supporting plants at longer stem sizes (see also Rosell & Olson, 2014). When predicting L by D and the self-/non self- categorical variable, we found significantly different slopes between self- and non self-supporting plants. At small sizes, self- and non self-supporting stem proportions were similar, but with increasing size, L - D proportions diverged.

Narrow climbers were not much longer than their self-supporting counterparts, but thick climbers were considerably longer than self-supporting stems of similar diameters. Bearing out the distinctness of self- vs. non self-supporting plants, their slope confidence intervals were different. Self- vs. non self-distinctiveness was underscored by the $E_{\text{stem}} \sim L/D \cdot \text{self}/\text{non self}$ model. This model suggested that self- and non self-supporting plants have markedly different stem proportion-tissue mechanics combinations. E_{stem} increased predictably with increasing stem proportions in our self-supporting species, but remained constant with size increase in non self-supporting species. This means that, although our community L - D - E_{stem} space was strictly continuous, the self-/non self-categorization does appear to identify biologically distinct length-diameter-mechanics combinations.

Our results imply that, at large sizes, selection favours plants being either strictly self-supporting or non self-supporting. This either-or condition is manifest in the empty space between self- and non self-supporting plants. Potentially explaining this empty space is a scenario in which, at least at large sizes, non self-supporting plants that ‘over-invest’ in support tissue are selected against in favour of those with maximally long stems for a given diameter with maximally flexible tissues (see also Niklas, 1994b). Similarly, self-supporting plants with stiff tissues but overly slender stems are probably vulnerable to buckling and selected against (Niklas, 1994b). It therefore seems likely that stems corresponding to the ‘empty’ space in our data are developmentally possible but would have lower fitness than either conventionally self-supporting or maximally long non self-supporting morphologies (Olson, 2012). In other words, we found plenty of ‘trubs’, but we found no ‘trianas’, intermediates between trees and lianas.

SOME METHODOLOGICAL CONSIDERATIONS

Although our approach is a significant advance over traditional habit categories, as implemented it had limitations to be overcome in subsequent studies. Although stems of all sizes and proportions could be included in our approach, we were limited to terminal stems because of the size limitations of our mechanical testing procedure, which used whole stem segments. As a result, the L - D - E_{stem} values did not represent the true extremes of length, diameter, and likely E_{stem} which would be expected to be represented by main trunks. Another limitation of our approach was that very flexible stems were outside the range of sensitivity of our mechanical load cell. As a consequence, we left out some flexible small herb and vine samples. However, our sampling strategy was entirely satisfactory for introducing our quantitative L - D - E_{stem}

approach and for illustrating the biological inferences that it can facilitate.

CONCLUSIONS

Categories such as ‘shrub’ or ‘forb’ serve as convenient terms for rapid communication, but they often artificially divide the continuous variation in nature. We show that much of the continuous functional diversity in plant stem morphology can be expressed by a simple stem length-diameter-tissue mechanics approach. The use of continuous variables avoids the loss of information that categorization causes (Roth & Mercer, 2000) and is biologically more accurate because rather than shoehorning all species, even intermediate ones, into one category or another, their attributes are simply depicted based on their L - D - E_{stem} values. Accordingly, our approach allowed us to identify an overlap between trees, shrubs, forbs, and inflorescences in L - D - E_{stem} space, not surprisingly given their functional similarity. We were also able to highlight a notably sparsely occupied area between self- and non self-supporting plants, especially at larger sizes. Based on our results, we predict that this area is developmentally accessible, but that the variants corresponding to it will have low fitness relative to either self-supporting plants of similar stem diameters or to maximally long climbers of similar diameters. Our approach therefore seems to offer a promising means of studying plant habit diversity with a small set of continuous variables within and between communities, and a useful tool for generating testable predictions regarding plant adaptation.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Table S1. Family, habit, and descriptive statistics for each species.

Table S2. Test and criteria to select the random components for each mixed-effects model.

Table S3. Random slopes and intercepts for each species in the random component of the three mixed-effects models.