Current Biology 23, 722–726, April 22, 2013 ©2013 Elsevier Ltd All rights reserved http://dx.doi.org/10.1016/j.cub.2013.03.029

Report

Repeated Origin of Three-Dimensional Leaf Venation Releases Constraints on the Evolution of Succulence in Plants

R. Matthew Ogburn[1,2,*] and Erika J. Edwards[1]
[1]Department of Ecology and Evolutionary Biology,
Brown University, 80 Waterman Street, Box G-W, Providence,
RI 02912, USA

Summary

Succulent water storage is a prominent feature among plants adapted to arid zones, but we know little about how succulence evolves and how it is integrated into organs already tasked with multiple functions. Increased volume in succulent leaves, for example, may result in longer transport distances between veins and the cells that they supply, which in turn could negatively impact photosynthesis [1–4]. We quantified water storage [5] in a group of 83 closely related species to examine the evolutionary dynamics of succulence and leaf venation. In most leaves, vein density decreased with increasing succulence, resulting in significant increases in the path length of water from veins to evaporative surfaces. The most succulent leaves, however, had a distinct three-dimensional (3D) venation pattern, which evolved 11–12 times within this small lineage, likely via multiple developmental pathways. 3D venation "resets" internal leaf distances, maintaining moderate vein density in extremely succulent tissues and suggesting that the evolution of extreme succulence is constrained by the need to maintain an efficient leaf hydraulic system. The repeated evolution of 3D venation decouples leaf water storage from hydraulic path length, facilitating the evolutionary exploration of novel phenotypic space.

Results

Succulence and Vein Density

We used comparative phylogenetic methods to investigate relationships between leaf succulence and vein density in the Portulacineae + Molluginaceae lineage, an angiosperm clade prominent in semiarid to arid regions throughout the subtropics that shows considerable variation in leaf water storage [6] (see Table S2 available online). We inferred the phylogenetic relationships of 240 taxa of Portulacineae + Molluginaceae using multiple molecular markers and the Bayesian inference software BEAST v1.6.1 [7] (Table S1). To estimate succulence, we measured leaf saturated water content (SWC) [5] for 83 species representing the morphological and phylogenetic diversity of this group. Functional anatomical traits were then measured on a subset of 42 of these species (Table S2). We focused on vein density because the leaf venation network performs two critical functions: veins replenish leaf water lost through transpiration, and they also transport newly generated sugars from photosynthetic cells to other parts of the plant. High vein density reduces the resistance to water flow through the leaf by decreasing the distance between a vein and the leaf's internal evaporative surface, and an efficient hydraulic transport system is critical to photosynthesis [2–4]. We measured multiple anatomical traits that estimate the path length of water flow from vein to transpiring surface (Supplemental Experimental Procedures), as well as interveinal distance (IVD) as a more direct measure of vein density [1, 8]. In addition to the hydraulic path, we also investigated variation in the phloem-loading (i.e., photosynthetic sugar transport) path length as a function of leaf succulence. Because intercellular sugar transport occurs either mostly or entirely through the symplast [9], the greatest resistance to flow occurs at the cell-to-cell boundary. Therefore, we estimated the path length for sugar transport by counting the average number of cells from the subepidermal photosynthetic cell layer to the nearest vein.

Because vein density is modeled to scale inversely with leaf thickness [1], we predicted that increased succulence will lead to lower vein densities and increased resistance to both water and sugar transport throughout the leaf. Using phylogenetic regression models, we initially found that most of the predicted relationships between SWC and measures of vein density, the hydraulic path, and the sugar transport path were either marginally significant or nonsignificant (Figure 1, all points; Table 1, "Main Factor Only" column). However, during the anatomical surveys, we noted that the most highly succulent taxa tended to have venation systems ramifying in three dimensions (3D). This differs from most vascular plant leaves, which have venation systems restricted to a single plane (2D) [11]. We identified two distinct arrangements of veins in the 3D taxa (hereafter "type I" and "type II" 3D venation), suggesting that 3D venation can evolve via multiple developmental pathways (Figures 2A and S2). We note that type I 3D leaves are generally round in cross-section, whereas type II 3D leaves tend to be more oblong.

Factoring variation due to venation type (2D versus 3D) out of the regression models resulted in stronger correlations between SWC and IVD, as well as with all of the hydraulic path measures (Figures 1A and 1B, solid regression lines; Table 1, "Venation Type Factored Out" column). In other words, IVD and hydraulic path length both increase as a function of leaf succulence in taxa with 2D venation, but this relationship is disrupted in the most succulent species by 3D venation. In contrast, there was no significant relationship between leaf SWC and the number of cells intervening between veins and subepidermal cells, suggesting that increased succulence did not limit the movement of sugars into the vein system (Figures 1C and S1). This is further supported by positive correlations between leaf SWC and cell size, suggesting that increases in succulence and leaf thickness are achieved through changes in cell size rather than cell number (Figure S1) [8].

Evolution of Three-Dimensional Leaf Venation

To estimate the number and polarity of transitions between 2D, 3D type I, and 3D type II venation, we used stochastic mapping of ancestral trait values over a sample of 1,000 alternative phylogenies from the Bayesian posterior tree distribution. This approach revealed that 3D venation evolved independently

---

[2]Present address: Yale Institute for Biospheric Studies, Yale University, 21 Sachem Street, New Haven, CT 06511, USA
*Correspondence: matthew.ogburn@yale.edu

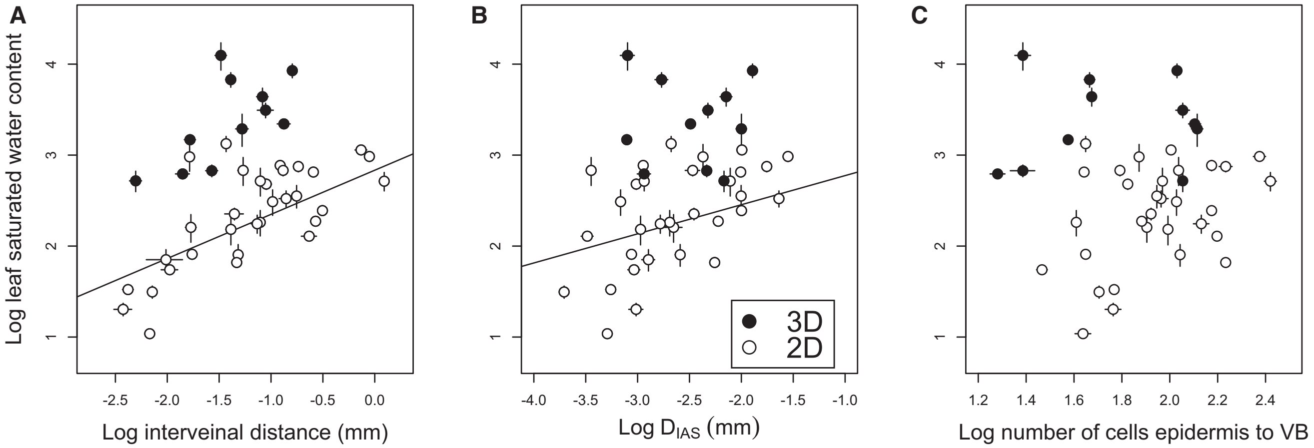

Figure 1. Relationship of Leaf Saturated Water Content to Functional Anatomical Traits

(A) Log leaf saturated water content (SWC) versus log interveinal distance (IVD), a measure of total venation density.

(B) Log leaf SWC versus log distance from vein to nearest intercellular airspace (D_{IAS}), a measure of hydraulic path length.

(C) Log leaf SWC versus log number of cells between veins and epidermis, a measure of the sugar-loading path. VB, vascular bundle.

White circles represent taxa with 2D venation; black circles represent taxa with 3D venation. Bars are ±1 SEM. Solid line in (A) and (B) indicates phylogenetic regression fit with variation attributed to venation type factored out. See also Figure S1.

multiple times in Portulacineae, with eight likely origins of type I 3D venation, three likely origins of type II 3D, and most likely no reversals to 2D and no transitions between the two 3D types (Figures 2B and S3). Increases in SWC were strongly correlated with the evolution of 3D venation (irrespective of 3D type) in the 83-taxon data set (phylogenetic ANOVA, p = 0.005) (Figure 2B) and the 42-taxon anatomical data set (phylogenetic ANOVA, p = 0.001).

We modeled the evolution of SWC on the posterior tree sample under a range of scenarios: a drift or fluctuating selection (Brownian motion) model, an Ornstein-Uhlenbeck (OU) selection model with evolution toward a single-trait optimum (OU1), and an OU model with different selective optima for 2D and 3D venation (OU2). The OU2 model fit the data significantly better than Brownian motion and OU1 models (Table 2), providing support for the hypothesis that evolution of 3D venation increases accessibility of the upper range of the SWC phenotype.

Table 1. Significance for Phylogenetic Generalized Least Squares Regression Models Fitting Leaf Saturated Water Content as a Function of Leaf Anatomical Traits

Model Main Factor	Frequency $p \leq 0.05$, Main Factor Only	Frequency $p \leq 0.05$, Venation Type Factored Out
IVD	0.549*	1.0*
D_{IAS}	0.218	0.701*
D_{CELL}	0.703*	1.0*
D_{EPI}	0.787*	0.921*
Leaf thickness	0.876*	0.068
Phloem path	0	0

All models fit with Brownian motion. “Main Factor Only” indicates single-predictor-variable models (e.g., SWC ~ IVD). “Venation Type Factored Out” indicates regression of residuals from the model (SWC ~ venation type) on the main factor. Because the regressions were performed on a posterior sample of Bayesian trees, the frequency of trees for which the model had a p value of 0.05 or less is shown, with a frequency of at least 0.5 taken as a conservative cutoff for significance [10]. Asterisks indicate $p \leq 0.05$ in at least 50% of posterior tree sample. IVD, interveinal distance; D_{IAS}, average distance from vein to intercellular air space; D_{CELL}, average distance from chlorenchyma cells to nearest vein; D_{EPI}, average distance from vein to epidermis. D_{IAS}, D_{CELL}, and D_{EPI} are all estimates of the extra-xylem hydraulic path (see Supplemental Experimental Procedures).

Discussion

Succulence and Vein Density

Leaves are the primary sites of photosynthesis in most plants, and capturing light and carbon for the production of sugars comes with the significant cost of losing water to transpiration. Balancing these trade-offs efficiently requires tight functional integration within the leaf. The evolution of a novel trait such as pronounced leaf succulence fundamentally alters geometry and may potentially disrupt this integration by resulting in greater resistances within the leaf to transport of water and photosynthate, or to diffusion of CO_2 [2–4, 12]. For example, recent modeling work has predicted that maximum leaf hydraulic flow saturates at interveinal distances roughly equal to the distance from vein to epidermis [1]. Increased leaf thickness therefore negates the hydraulic benefits of dense leaf venation and predicts that vein density should scale inversely with leaf succulence. It also predicts an upper limit to leaf thickness that would be set by the minimal functional vein density. We found support for these predictions in 2D-veined taxa; vein density was lower in more succulent leaves, resulting in longer hydraulic paths between veins and photosynthetic surfaces. However, the repeated evolution of 3D venation allowed for further increases in succulence while maintaining moderate hydraulic path lengths, providing evidence for a trade-off between water storage and hydraulic function as well as its solution.

The apparent sensitivity of succulent leaves to increasing hydraulic path length may be surprising, given that locally stored water should serve to buffer the transpiration stream, decreasing dependence on water uptake from soil as has been shown in other taxa with succulent leaves and stems [13–17]. If hydraulics are limiting in highly succulent 2D leaves to the extent that they drive the evolution of internal vascular rearrangement, this indicates that stored water may not be particularly important in succulent-leaved Portulacineae in supporting daily photosynthetic gas exchange, at least relative to the contribution of water obtained from the soil.

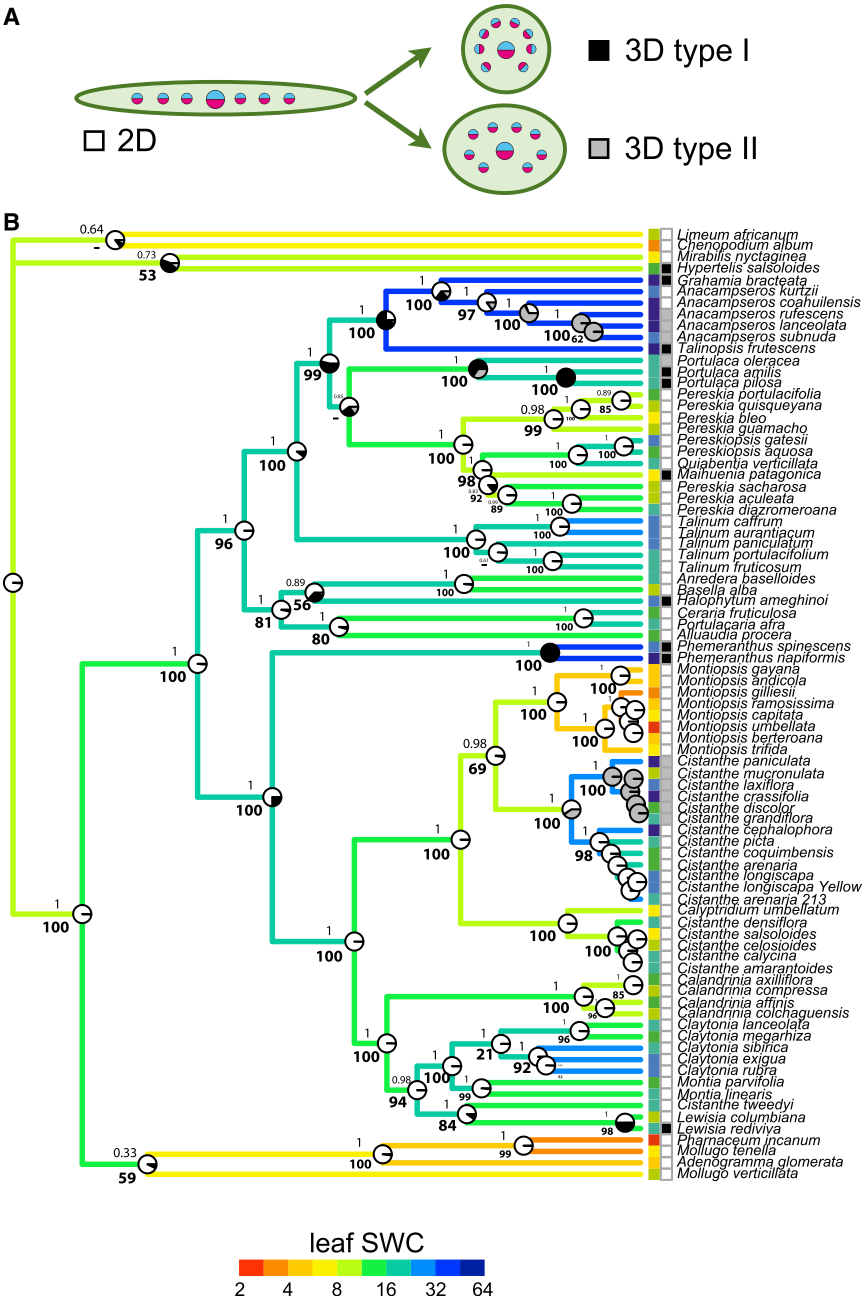

Figure 2. Evolution of Leaf Venation and Saturated Water Content

(A) Schematic drawings of leaf venation types in cross-section. Blue half-circles represent xylem; pink half-circles represent phloem. Note inversion of abaxial vascular bundles in 3D type I leaves versus consistent bundle orientation in 3D type II leaves. Arrows indicate direction of most frequently reconstructed evolutionary transitions. White, gray, and black boxes correspond to distribution of each venation type as shown by pie graphs in Figure 2B. See also Figure S2.

(B) Ultrametric tree of the 83-taxon data set from Portulacineae + Molluginaceae and outgroups showing mappings for leaf SWC and venation type. Branches are colored according to the reconstructed ancestral trait value of SWC at the subtending node. Posterior probabilities are indicated above nodes; maximum-likelihood bootstrap values are indicated below. Pie graphs at nodes show maximum-likelihood trait reconstructions for venation type. See also Figure S3.

3D venation has been reported previously from leaves in a range of angiosperm functional groups, including highly succulent (Portulacineae [18], *Aloe* [19], *Kalanchoe* [20]), halophytic (Amaranthaceae [21]), and sclerophyllous taxa (*Hakea* [22]), as well as grasses (*Ellisochloa* [23]). Gains of 3D venation in such an ecologically varied array of plants suggest that it is not associated with succulence per se but may rather be a general solution to any selective regime favoring thicker or rounded leaves in which hydraulic resistance may become limiting.

Geometry and Development of Succulence

It is possible that the association of 3D venation with high SWC is a byproduct of selection for a particular leaf geometry rather than a functional solution to hydraulic constraints. Maximum storage capacity will generally be achieved by minimizing the leaf surface area to volume ratio, so the most succulent leaves by definition will tend to be terete (i.e., round in cross-section) rather than planate. A terete "leaf" could be achieved by dispensing with true leaves entirely and replacing them with determinate stem segments ("phylloclades"). We can confirm the homology of the terete organs observed here with leaves, however, by their position on the shoot subtending axillary buds, as would be expected for true leaves. Alternatively, a terete leaf could develop by disrupting the abaxial identity of the leaf so that the entire surface is homologous to the adaxial side; this would result in a ring of vascular bundles, the lower half of which would be inverted with respect to their normal orientation. Such terete, adaxialized leaves, albeit with poorly organized vasculature, have been induced in mutant or transgenic *Arabidopsis* lines with overexpression of adaxial-identity-specifying *ARP* or *HD-ZIPIII* family genes [24, 25].

An adaxialization phenotype does match the pattern (type I) observed in most of the independent origins of 3D venation, in which leaves are terete and abaxial bundles show an inverted orientation relative to the leaf (Figures 2A and S2). However, at least three independent origins of 3D venation show a different pattern (type II), in which leaves are more oblong in cross-section, with veins that ramify in three dimensions without altering the orientation of xylem and phloem strands (Figures 2A and S2). These different routes argue against 3D venation as simply a developmental artifact of making a terete leaf and instead imply that high vein density is itself functionally advantageous. We also note that amphistomaty (i.e., stomata occurring equally on both sides of the leaf) is the general condition in the Portulacineae + Molluginaceae clade [26] and is widespread in plants inhabiting high-light environments [27].

Table 2. Comparison of Brownian Motion and Ornstein-Uhlenbeck Models for the Evolution of Leaf Saturated Water Content

Model	Log Likelihood	AICc	Rate	α	θ (2D/3D)
Brownian	−75.93	156.03	1.44	NA	2.60
OU1	−65.33	137.01	2.87	3.26	2.77
OU2	−61.07	130.71	3.50	4.95	2.61/3.31

Values shown are medians of parameters obtained from fitting all models over the posterior tree distribution. Brownian motion models a "random walk" of trait evolution across the phylogenetic tree. OU1 models selection toward a single-trait optimum for all taxa, and OU2 models distinct selective optima for taxa with 2D versus 3D venation. AICc, Akaike information criterion with sample size correction; α, strength of selection parameter; θ, selective optimum (in the case of Brownian motion, this is the trait mean across the tree); OU1, Ornstein-Uhlenbeck model with a single selective optimum; OU2, Ornstein-Uhlenbeck model with separate selective optima for 2D and 3D venation types, with internal nodes assigned a stochastically simulated value for each tree in the posterior sample.

If type I 3D venation does evolve through leaf adaxialization, amphistomaty may be an evolutionary prerequisite for viability of this phenotype.

The two cases in this study that potentially associate an origin of 3D venation with a reduction of leaf SWC are in *Maihuenia* and *Portulaca* (Figure 2B). *Maihuenia* are cushion-forming leafy cacti with small, terete leaves. Despite their position as sister to the diverse Cactoideae clade [28], they likely represent a highly specialized life form, and it is possible that their 3D venation is the signature of a lost ancestor with larger and more succulent leaves. *Portulaca* are distinguished among Portulacineae in having the C_4 photosynthetic pathway, and 3D venation may present distinct advantages in regard to the significant anatomical alterations associated with Kranz anatomy. A high leaf bundle sheath:mesophyll ratio has been inferred as a precursor trait for the evolution of C_4 photosynthesis [29–31], and the evolution of 3D venation in *Portulaca* may have been co-opted for this divergent ecological strategy. It is noteworthy that diverse varieties of 3D venation have been reported in other C_4 taxa (e.g., Amaranthaceae [21], *Eleocharis* [32]).

Concluding Remarks

Multiple transitions to 3D venation appear to represent a solution to a generalized transport problem imposed by evolutionary changes in organ geometry, namely, the shift from nearly planate to three-dimensional leaves as a function of increasing water storage. Such basic biophysical constraints are not unique to plants; similar transitions can be found in the multiple repeated shifts between asconoid, syconoid, and leuconoid forms as a function of size in calcareous sponges (Calcarea) [33]. Previous work in highly succulent plants has hypothesized that the evolution of extraxylary vascular bundles in stems of cacti [34–36] and *Adenia* [37] facilitated the incredible diversity in growth form in these lineages, in particular allowing the evolution of massive forms such as barrel and columnar cacti. Succulent stems have relatively large volumes and would be expected to be vulnerable to the negative effects of higher transport resistances with large increases in size. We found strong evidence for a similar pattern in leaves, despite their smaller size. The evolution of novel arrangements in transport tissues appears to be a general solution in all of these systems, allowing for the evolutionary exploration of otherwise inaccessible phenotypic space.

Accession Numbers

Gene sequences generated for this study have been uploaded to the NCBI trace archive with GenBank accession numbers JX456275–JX456346. Primary data have been deposited at Dryad with the DOI http://dx.doi.org/10.5061/dryad.qd19h.

Supplemental Information

Supplemental Information includes three figures, two tables, and Supplemental Experimental Procedures and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2013.03.029.

Acknowledgments

We thank R. Kiesling, I. Peralta, R. Nyffeler, U. Eggli, M. Arakaki, P. Giraldo Ramírez, M. Kalin Arroyo, A. Patiño Acevedo, M.A. Ogburn, and the staff at the Universidad de Concepción Herbarium, Chile (CONC) and the South African National Biodiversity Institute Compton Herbarium (NBG) for support in the field. J. Schmitt, E. Brainerd, C. Dunn, P.-A. Christin, D. Chatelet, E. Spriggs, A. Leslie, and two anonymous reviewers provided insightful comments that substantially improved the manuscript. This work was supported in part by National Science Foundation grant DEB-1026611 to E.J.E.

Received: December 7, 2012
Revised: February 6, 2013
Accepted: March 12, 2013
Published: April 11, 2013

References

1. Noblin, X., Mahadevan, L., Coomaraswamy, I.A., Weitz, D.A., Holbrook, N.M., and Zwieniecki, M.A. (2008). Optimal vein density in artificial and real leaves. Proc. Natl. Acad. Sci. USA *105*, 9140–9144.
2. Sack, L., and Frole, K. (2006). Leaf structural diversity is related to hydraulic capacity in tropical rain forest trees. Ecology *87*, 483–491.
3. Brodribb, T.J., Feild, T.S., and Jordan, G.J. (2007). Leaf maximum photosynthetic rate and venation are linked by hydraulics. Plant Physiol. *144*, 1890–1898.
4. Boyce, C.K., Brodribb, T.J., Feild, T.S., and Zwieniecki, M.A. (2009). Angiosperm leaf vein evolution was physiologically and environmentally transformative. Proc. Biol. Sci. *276*, 1771–1776.
5. Ogburn, R.M., and Edwards, E.J. (2012). Quantifying succulence: a rapid, physiologically meaningful metric of plant water storage. Plant Cell Environ. *35*, 1533–1542.
6. Nyffeler, R., Eggi, U., Ogburn, M., and Edwards, E. (2008). Variations on a theme: repeated evolution of succulent life forms in the Portulacineae (Caryophyllales). Haseltonia *14*, 26–36.
7. Drummond, A.J., and Rambaut, A. (2007). BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evol. Biol. *7*, 214.
8. Gibson, A.C. (1982). The anatomy of succulence. In Crassulacean Acid Metabolism: Proceedings of the Fifth Annual Symposium in Botany, January 14–16, 1982, University of California, Riverside, I.P. Ting and M. Gibbs, eds. (Rockville, MD, USA: American Society of Plant Physiologists), pp. 1–17.
9. Turgeon, R. (2010). The role of phloem loading reconsidered. Plant Physiol. *152*, 1817–1823.
10. Pagel, M., Venditti, C., and Meade, A. (2006). Large punctuational contribution of speciation to evolutionary divergence at the molecular level. Science *314*, 119–121.
11. Beerling, D.J., Osborne, C.P., and Chaloner, W.G. (2001). Evolution of leaf-form in land plants linked to atmospheric CO_2 decline in the Late Palaeozoic era. Nature *410*, 352–354.
12. Maxwell, K., von Caemmerer, S., and Evans, J.R. (1997). Is a low internal conductance to CO_2 diffusion a consequence of succulence in plants with crassulacean acid metabolism? Aust. J. Plant Physiol. *24*, 777–786.
13. Smith, J.A.C., Schulte, P.J., and Nobel, P.S. (1987). Water flow and water storage in *Agave deserti*: osmotic implications of crassulacean acid metabolism. Plant Cell Environ. *10*, 639–648.
14. Schulte, P.J., Smith, J.A.C., and Nobel, P.S. (1989). Water storage and osmotic pressure influences on the water relations of a dicotyledonous desert succulent. Plant Cell Environ. *12*, 831–842.
15. Nobel, P.S. (1976). Water relations and photosynthesis of a desert CAM plant, *Agave deserti*. Plant Physiol. *58*, 576–582.

16. Nobel, P.S. (1977). Water relations and photosynthesis of a barrel cactus, *Ferocactus acanthodes*, in the Colorado Desert. Oecologia *27*, 117–133.
17. Sinclair, R. (1983). Water relations of tropical epiphytes: II. Performance during droughting. J. Exp. Bot. *34*, 1664–1675.
18. Hershkovitz, M.A. (1991). Leaf morphology of *Cistanthe* Spach (Portulacaceae). Ann. Mo. Bot. Gard. *78*, 1022–1060.
19. Cutler, D.F. (2004). *Aloe* leaf anatomy. In Aloes: The Genus *Aloe*, T. Reynolds, ed. (Boca Raton, FL, USA: CRC Press), pp. 361–366.
20. Balsamo, R.A., and Uribe, E.G. (1988). Leaf anatomy and ultrastructure of the Crassulacean-acid-metabolism plant *Kalanchoe daigremontana*. Planta *173*, 183–189.
21. Kadereit, G., Borsch, T., Weising, K., and Freitag, H. (2003). Phylogeny of Amaranthaceae and Chenopodiaceae and the evolution of C_4 photosynthesis. Int. J. Plant Sci. *164*, 959–986.
22. Groom, P.K., Lamont, B.B., and Kupsky, L. (1994). Contrasting morphology and ecophysiology of co-occurring broad and terete leaves in *Hakea trifurcata* (Proteaceae). Aust. J. Bot. *42*, 307–320.
23. Peterson, P.M., Romaschenko, K., Barker, N.P., and Linder, P.H. (2011). Centropodieae and *Ellisochloa*, a new tribe and genus in Chloridoideae (Poaceae). Taxon *60*, 1113–1122.
24. McConnell, J.R., and Barton, M.K. (1998). Leaf polarity and meristem formation in *Arabidopsis*. Development *125*, 2935–2942.
25. Lin, W.-C., Shuai, B., and Springer, P.S. (2003). The Arabidopsis *LATERAL ORGAN BOUNDARIES*-domain gene *ASYMMETRIC LEAVES2* functions in the repression of *KNOX* gene expression and in adaxial-abaxial patterning. Plant Cell *15*, 2241–2252.
26. Ogburn, R.M., and Edwards, E.J. (2009). Anatomical variation in Cactaceae and relatives: Trait lability and evolutionary innovation. Am. J. Bot. *96*, 391–408.
27. Smith, W.K., Vogelmann, T.C., DeLucia, E.H., Bell, D.T., and Shepherd, K.A. (1997). Leaf form and photosynthesis. Bioscience *47*, 785–793.
28. Arakaki, M., Christin, P.-A., Nyffeler, R., Lendel, A., Eggli, U., Ogburn, R.M., Spriggs, E., Moore, M.J., and Edwards, E.J. (2011). Contemporaneous and recent radiations of the world's major succulent plant lineages. Proc. Natl. Acad. Sci. USA *108*, 8379–8384.
29. Sage, R.F. (2002). Are crassulacean acid metabolism and C_4 photosynthesis incompatible? Funct. Plant Biol. *29*, 775–785.
30. Christin, P.-A., Osborne, C.P., Chatelet, D.S., Columbus, J.T., Besnard, G., Hodkinson, T.R., Garrison, L.M., Vorontsova, M.S., and Edwards, E.J. (2013). Anatomical enablers and the evolution of C_4 photosynthesis in grasses. Proc. Natl. Acad. Sci. USA *110*, 1381–1386.
31. Edwards, E.J., and Ogburn, R.M. (2012). Angiosperm responses to a low-CO_2 world: CAM and C_4 photosynthesis as parallel evolutionary trajectories. Int. J. Plant Sci. *173*, 724–733.
32. Ueno, O. (1998). Induction of kranz anatomy and C_4-like biochemical characteristics in a submerged amphibious plant by abscisic acid. Plant Cell *10*, 571–584.
33. Manuel, M., Borchiellini, C., Alivon, E., Le Parco, Y., Vacelet, J., and Boury-Esnault, N. (2003). Phylogeny and evolution of calcareous sponges: monophyly of calcinea and calcaronea, high level of morphological homoplasy, and the primitive nature of axial symmetry. Syst. Biol. *52*, 311–333.
34. Mauseth, J.D., and Sajeva, M. (1992). Cortical bundles in the persistent, photosynthetic stems of cacti. Ann. Bot. *70*, 317–324.
35. Mauseth, J.D. (1993). Medullary bundles and the evolution of cacti. Am. J. Bot. *80*, 928–932.
36. Mauseth, J.D. (2004). The structure of photosynthetic, succulent stems in plants other than cacti. Int. J. Plant Sci. *165*, 1–9.
37. Hearn, D.J. (2009). Developmental patterns in anatomy are shared among separate evolutionary origins of stem succulent and storage root-bearing growth habits in *Adenia* (Passifloraceae). Am. J. Bot. *96*, 1941–1956.