Light-induced plasticity in leaf hydraulics, venation, anatomy, and gas exchange in ecologically diverse Hawaiian lobeliads

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Summary

• Leaf hydraulic conductance (K_{leaf}) quantifies the capacity of a leaf to transport liquid water and is a major constraint on light-saturated stomatal conductance (g_s) and photosynthetic rate (A_{max}). Few studies have tested the plasticity of K_{leaf} and anatomy across growth light environments. These provided conflicting results.

• The Hawaiian lobeliads are an excellent system to examine plasticity, given the striking diversity in the light regimes they occupy, and their correspondingly wide range of A_{max}, allowing maximal carbon gain for success in given environments. We measured K_{leaf}, A_{max}, g_s and leaf anatomical and structural traits, focusing on six species of lobeliads grown in a common garden under two irradiances (300/800 µmol photons $m^{-2}\,s^{-1}$). We tested hypotheses for light-induced plasticity in each trait based on expectations from optimality.

• K_{leaf}, A_{max}, and g_s differed strongly among species. Sun/shade plasticity was observed in K_{leaf}, A_{max}, and numerous traits relating to lamina and xylem anatomy, venation, and composition, but g_s was not plastic with growth irradiance. Species native to higher irradiance showed greater hydraulic plasticity.

• Our results demonstrate that a wide set of leaf hydraulic, stomatal, photosynthetic, anatomical, and structural traits tend to shift together during plasticity and adaptation to diverse light regimes, optimizing performance from low to high irradiance.

Introduction

Leaf hydraulic conductance (K_{leaf}), the efficiency of liquid water transport through the leaf, is an important constraint on rates of transpiration, photosynthesis, and growth (Sack & Holbrook, 2006). Water first moves through the petiole and then the vein xylem, and then traverses the bundle sheath and mesophyll before evaporating and diffusing through stomata. Because water moves through both xylem and living cells, K_{leaf} responds to many environmental factors, including leaf water status, temperature, and irradiance (Sack *et al.*, 2004; Sack & Holbrook, 2006; Sellin & Kupper, 2007; Scoffoni *et al.*, 2008, 2012). Several studies have examined the basis of K_{leaf} and its dynamics in the structure and anatomy of the leaf, such as venation and mesophyll internal anatomy, and their correlation with stomatal anatomy and rates of gas exchange across species (e.g. Sack & Frole, 2006; Sack & Holbrook, 2006; Brodribb *et al.*, 2007; Sack & Scoffoni, 2013). In fact, the coordination of hydraulics and gas exchange is often so strong that it can be shown even among four to six species (Aasamaa & Sober, 2001; Nardini *et al.*, 2012a). However, little is known of plasticity in leaf hydraulics with growth irradiance

and its coordination with anatomical and compositional variables. The aim of our study was to determine the sun/shade plasticity in leaf hydraulics, and its coordination with structure and function, focusing on a model adaptive radiation, the Hawaiian lobeliads.

The endemic Hawaiian lobeliads (Campanulaceae) include six genera and 141 species, representing 12% of the entire native Hawaiian flora, and arose from a single ancestor *c.* 13 million yr ago (Givnish *et al.*, 2009; Gustafson *et al.*, 2014). Thus, lobeliads are a spectacular example of adaptive radiation, given their exceptional rapid diversification into a variety of ecological niches, including dry forests, mesic forests, rainforests, open bogs, and sea cliffs (Losos & Miles, 2002; Ackerly, 2009). In particular, lobeliads have radiated into a wide range of light regimes, from full sun to shaded understories, diversifying in photosynthetic traits such as maximum photosynthetic rate (A_{max}), with greater rates found in higher light environments, providing species with an advantage in net carbon gain (Givnish *et al.*, 2004; Montgomery & Givnish, 2008; Givnish & Montgomery, 2014; Gustafson *et al.*, 2014). Further, the plasticity of leaf photosynthetic physiology and anatomy induced by light regime also varies among

species and contributes to the establishment of Hawaiian lobeliads in strikingly different light environments (Givnish & Montgomery, 2014). This work was additionally motivated toward determining detailed information of plastic responses that can ultimately contribute to improved characterization of species niches, and thus inform species conservation (Cooke *et al.*, 2013; Valladares *et al.*, 2014).

Surprisingly, little is known about the plasticity of K_{leaf} to growth irradiance. On the one hand, many studies of a wide range of species have found K_{leaf} to be higher in sun leaves than shade leaves within the canopy; K_{leaf} was higher in sun leaves for all 11 species in the nine previous studies, ranging from 18% higher in *Betula papyrifera* to 238% in *Prunus dulcis* (Table 1). On the other hand, the very few results for the acclimation of whole plants to growth irradiance have been conflicting (Table 1). Only two studies to our knowledge have examined shifts in K_{leaf} for plants of a given species after acclimation to sun vs shade, though the nature of such shifts would give a strong insight into the correspondence of these traits to ecological specialization. One study found higher values in high irradiance, corresponding to higher vein length per area (VLA) (Murphy *et al.*, 2012; Table 1), while the other found no significant differences in K_{leaf} across growth irradiances (Raimondo *et al.*, 2009). We hypothesized that for Hawaiian lobeliads, K_{leaf} would be higher for plants grown under higher irradiance. Moreover, several studies have indicated that a number of anatomical traits are 'drivers' of K_{leaf}, such as major and minor VLA (Sack & Frole, 2006; Brodribb *et al.*, 2007; McKown *et al.*, 2010), mesophyll surface area per leaf area (Sack & Scoffoni, 2013), and theoretical midrib conductance corresponding to width and/or number of conduits (Sack & Frole, 2006; McKown *et al.*, 2010; Sommerville *et al.*, 2012). Here we tested these different potential drivers for the first time within a rapidly evolved lineage.

The hydraulic and photosynthetic systems are fully integrated within the leaf, and the generally higher K_{leaf} for sun than shade leaves within tree crowns is consistent with a matching of hydraulic supply with hydraulic demand (g_s) (Sack *et al.*, 2003a; Sellin & Kupper, 2007; Brodribb & Jordan, 2011). Indeed, the positive correlations of stomatal and xylem traits reported in studies across diverse species and across species within lineages, and across species adapted to sun vs shade also indicate matching of hydraulic supply and demand (Nardini *et al.*, 2005; Sack *et al.*, 2005; Edwards, 2006; Dunbar-Co *et al.*, 2009; Zhang *et al.*, 2012; Brodribb *et al.*, 2013). However, the coordination of K_{leaf} and g_s has not been tested across plants of a given species grown at different light intensities. We hypothesized that for species of Hawaiian lobeliads (Table 2), individuals grown under higher irradiances would achieve higher light-saturated carbon dioxide (CO_2) assimilation rates (A_{max}) and stomatal conductance (g_s), consistent with many classic and recent studies (e.g. Bjorkman & Holmgren, 1963; Givnish, 1988; Murphy *et al.*, 2012), and that K_{leaf} should also increase, matching g_s or even resulting in a higher $K_{leaf} : g_s$ ratio under high irradiance. Such a disproportionate increase of hydraulic supply would match the greater evaporative demand arising from the higher vapor pressure deficit (VPD) experienced under sunnier conditions. Indeed, for sun leaves of given species acclimating to light intensities while transpiring on detached shoots in the lab, $K_{leaf} : g_s$ rapidly achieves higher values under high than under low irradiance (Guyot *et al.*, 2012). In fact, even for plants grown under high light, a high $K_{leaf} : g_s$ arises during acclimation to high VPD (Murphy *et al.*, 2014), and across species, a higher $K_{leaf} : g_s$ provides an advantage under high VPD, drought, and/or heat load, buffering gas exchange against declines in leaf water potential during leaf dehydration (Brodribb & Jordan, 2008).

Table 1 Results of previous studies of the plasticity of leaf hydraulic conductance (K_{leaf}) in response to irradiance, indicating species, light treatment applied, and values of K_{leaf} under low and high irradiances

Species	Study	K_{leaf} low light (mmol m^{-2} s^{-1} MPa^{-1})	K_{leaf} high light (mmol m^{-2} s^{-1} MPa^{-1})	% increase from low to high irradiance
Comparing plants grown in low and high irradiance				
Olea europaea cv Leccino	Raimondo *et al.* (2009)	4.7	4.7	0
Toona ciliata	Murphy *et al.* (2012)	5.5	12.2	122
Comparing sun and shade leaves within canopies				
Acer rubrum	Sack *et al.* (2003a)	8.5	10.1	18
Betula papyrifera	Sack *et al.* (2003a)	14.3	12.1	18
Retanilla patagonica	Iogna *et al.* (2011)	11.5	15	30
Quercus rubra	Sack *et al.* (2003a)	9.96	15.2	53
Betula pendula	Ounapuu & Sellin (2013)	2.4	3.75	56
	Sellin *et al.* (2008)	6.32	7.96	26
Acer saccharum	Sack *et al.* (2003a)	4.06	7.04	73
Quercus ilex	Nardini *et al.* (2012b)	4.1	7.9	93
Tilia cordata	Sellin & Kupper (2007)	1.2	3.1	158
Nothofagus cunninghamii	Brodribb & Jordan (2011)	3.24	8.55	164
Colliguaja integerrima	Iogna *et al.* (2011)	5.5	17.5	218
Prunus dulcis	Egea *et al.* (2012)	4	13.5	238

All measurements cited here were made based on sampling shoots and measuring under laboratory conditions, except for two studies (Sellin *et al.*, 2008; Egea *et al.*, 2012).

No studies to our knowledge have tested for simultaneous plasticity in a wide set of leaf hydraulic and associated traits to growth irradiance. While many studies have focused on sun/shade plasticity within canopies (as discussed earlier; see Table 1), previous studies have indicated that in general sun/shade plasticity of leaf traits within crowns may be more limited, and less variable across species, than the sun/shade plasticity of leaf traits that arises when plants are grown in different conditions (Walters & Reich, 1999; Sack *et al.*, 2003b, 2006; Niinemets *et al.*, 2014). We evaluated for the first time the simultaneous light-induced shifts in leaf hydraulic, gas exchange, structural, and anatomical traits for related species that occupy different light regimes in their native habitats. We hypothesized that, overall, Hawaiian lobeliads would show shifts in many traits consistent with the previous literature on the sun/shade plasticity of individual traits for diverse species (e.g. Givnish, 1988; Dunbar-Co *et al.*, 2009; Pasquet-Kok *et al.*, 2010; Pivovaroff *et al.*, 2014; see shifts predicted for individual traits in Tables 3, 4 and 5). Plants grown in high light should have higher A_{max} and K_{leaf}, and a leaf anatomical structure that enables more effective transport of direct light, more effective transport, more water and sugars, and better ability to withstand dehydration stress. Plants grown in high light should thus have higher VLA, greater leaf thickness (and thickness of the different tissues within the leaf), higher leaf mass per area (LMA) and leaf density (LD), higher nitrogen and carbon concentrations, and, with larger midribs, smaller cell sizes and greater percentage intercellular air spaces, and more numerous and wider conduits in the midrib. The opposite traits for leaves of plants grown in shade would be expected to confer a reduced construction cost. We further hypothesized that the degree of plasticity in these traits across species would correlate with the light regime experienced in their natural habitat, with species of brighter environments showing greater plasticity. Such a pattern would be consistent with the general trend across diverse species for fast-growing competitive species of high-resource environments to have greater plasticity (Strauss-Debenedetti & Bazzaz, 1991; Valladares *et al.*, 2002). Further, a greater plasticity in hydraulic traits and associated traits would enable species native to higher irradiance to better withstand the higher natural variation in evaporative demand.

The adaptive evolution of species' traits across sun/shade gradients is in many cases analogous to the sun/shade plasticity expressed by given species (Givnish, 1988; Valladares & Niinemets, 2014). However, very few studies have considered the evolution of fine-scale physiological and anatomical traits across light gradients within plant adaptive radiations. In the Hawaiian lobeliads, photosynthetic traits are correlated with the light regime experienced by each species in their natural habitat (Givnish *et al.*, 2004), and qualitatively similar patterns are seen in *Schiedea* and *Sonchus*, which diversified across a range of native light regimes, from shaded understories to exposed cliffs (Kapralov & Filatov, 2006; Santiago & Kim, 2009; Kapralov *et al.*, 2013). For the first time, to our knowledge, we determined the variation of K_{leaf} within a rapidly diversifying lineage, and, additionally, the variation in leaf anatomy and gas exchange across six species of lobeliads. We hypothesized that traits affecting carbon gain – and hence water loss – would shift in the same way in species adapted to shady or sunny conditions as described earlier for sun/shade plasticity (see shifts predicted for individual traits in Tables 3–5). In this paper, we use the term 'adapted' to signify that a species possesses traits that, in principle, would contribute to plant performance and fitness under given conditions, according to current theory and previous studies (Givnish, 1986).

We thus applied an integrated approach to examine plasticity in physiological, anatomical, and structural traits in relation to light regime, addressing four questions: is K_{leaf} higher in plants grown in sun than in shade, and how does it relate to anatomy? How does sun/shade plasticity affect the coordination of K_{leaf} with gas exchange? To what extent do species differ in the sun/shade plasticity of leaf hydraulics and associated physiological and anatomical traits? How do leaf hydraulics, gas exchange, anatomy and composition relate to native light habitat within a rapidly evolving lineage? Our study is particularly novel in extending the study of K_{leaf} variation to growth irradiance, especially for related species within a rapidly diversifying lineage, resolving the controversy as to why contrasting results had been

Table 2 Study species of Hawaiian lobeliads, habitat, elevation, rainfall, light regimes and geographic locations

Species	Habitat	Elevation (m)	Annual rainfall (mm)	Light regimes	Localities (islands)
Clermontia clermontioides	Mesic and wet forest	670–1825	1000–2500	Forest, gaps, forest edges	TNC Kaʻū Preserve, TNC Kona Heme Preserve (Hawaiʻi)
Clermontia parviflora	Wet forest	120–1460	2500–5000	Gaps, forest edges	Olaʻa forest, Hawaii Volcanoes National Park (Hawaiʻi)
Cyanea leptostegia	Diverse mesic forest	970–1300	1500–2500	Open forest, subcanopy	Forest off Mohihi Road above YMCA camp, Canyon Trail, Kōkeʻe (Kauaʻi)
Delissea rhytidosperma	Diverse mesic forest	300–1000	1000–2500	Open forest, understory	Limahuli Preserve (Kauaʻi)
Lobelia niihauensis	Forest, seeps in dry regions	125–725	500–2000	Open cliffs, ridges, broken crests	Limahuli Living Collection (Kauaʻi)
Lobelia yuccoides	Diverse mesic and wet forest	750–1200	1500–3000	Open cliffs, ridges, broken crests	Kalalau Valley rim and forest near Hongwanji camp, Kōkeʻe (Kauaʻi)

Table 3 Leaf hydraulic and gas exchange traits for six ecologically diverse species of Hawaiian lobeliads, with expected plastic and adaptive responses to irradiance (+ or − in parentheses; see the Introduction), and results of the ANOVA testing the effects of species differences, growth irradiance, and their interaction

Trait	Symbol	Unit	Leaf water potential (covariate)	Species	Growth irradiance	Species × growth irradiance	Error
Leaf hydraulics							
Leaf hydraulic conductance (+)	K_{leaf}	mmol m^{-2} s^{-1} MPa^{-1}		**0.532, 12.0, (5) ****	**1.61, 7.05, (1) ** (26% increase)**	0.186, 4.12, (5)^{ns}	0.128, 76.9, (135)
Leaf hydraulic conductance (+)	K_{leaf}	mmol m^{-2} s^{-1} MPa^{-1}	**6.60, 33.1, (1) ****	**0.587, 12.6, (5) *****	**0.567, 2.53, (1) ****	**0.184, 4.08, (5) *†**	**0.080, 47.6, (134)**
Leaf gas exchange							
CO$_2$ assimilation rate (+)	A_{max}	μmol m^{-2} s^{-1}		**0.013, 34.4, (5) *****	**0.016, 8.68, (1) ** (8% increase)**	0.002, 5.16, (5)^{ns}	0.002, 51.8, (47)
Stomatal conductance (+)	g_s	mol m^{-2} s^{-1}		**0.080, 29.2, (5)****	0005, 0.382, (1)^{ns}	0.014, 4.93, (5)^{ns}	0.019, 65.5, (47)

For ANOVA results, mean square values and % variance are shown with degrees of freedom in parentheses, and for growth irradiance, the % increase or decrease of the trait value from low to high irradiance is presented, averaged across species means. Values in bold indicate a significant impact of growth irradiance (i.e. significant plasticity). ns, $P > 0.05$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; †, loss of significance when accounting for multiple tests using the sequential Bonferroni analysis (Supporting Information Table S1).

previously observed, and, equally, testing with high resolution the integration of leaf hydraulic plasticity with gas exchange, leaf anatomy, and composition.

Materials and Methods

Species and plant cultivation

Six species of Hawaiian lobeliads were selected to span a range of light and moisture regimes occupied by the lobeliad lineage: *Clermontia clermontioides* (Gaudich) A. Heller, *Clermontia parviflora* Gaudich, *Cyanea leptostegia* A. Gray, *Delissea rhytidosperma* H. Mann, *Lobelia niihauensis* H. St John and *Lobelia yuccoides* Hillebr (Fig. 1; Table 2). Two of these species are federally listed as endangered (*D. rhytidosperma* and *L. niihauensis*; US Fish and Wildlife Service; Gustafson *et al.*, 2014), three are rare (*D. rhytidopserma*, *L. niihauensis*, and *L. yuccoides*; Wagner *et al.*, 1999a; Gustafson *et al.*, 2014) and one is vulnerable (*C. leptostegia*; Wagner *et al.*, 1999a; Gustafson *et al.*, 2014). These species were grown in a common-garden glasshouse at the Hawai'i Agricultural Experiment Station of the University of Hawai'i in Volcano, HI, USA (Big Island), in the heart of the elevational range of the lobeliad lineage (*c.* 1190 m). Investigation of genotype-level plasticity was not feasible, given the impossibility of obtaining clones of wild lobeliad species and thus we focused on species-level plasticity (Richards *et al.*, 2006; Funk, 2008). Plants of the six species were grown under low and high irradiance (daily average of *c.* 300 vs 800 μmol photons m^{-2} s^{-1}). The glasshouse consisted of a hoop structure with a clear plastic cover, open to ambient air along the lower walls (0–1 m off the ground) and at both ends of the structure. The glasshouse was divided into two irradiance treatments that provided 6% transmission of full sunlight (low irradiance treatment) and 35% of full sunlight (high irradiance treatment), selected to provide a wide range of irradiance that would be experienced by plants of forest and exposed species in the wild (Givnish *et al.*, 2004). Irradiance intensities were achieved using a combination of neutral density shade cloth and the plastic cover. Given the constraints on available plant material as a result of the difficulty of collection and species' rarity, we focused in this study on the effect of light quantity (and not light quality), although a low red : far red ratio is associated with natural (green) shade; previous studies have shown that while light-demanding herbs show strong responses to low red : far red ratio, especially in their internode length (e.g. Dudley & Schmitt, 1995), the effects of low red : far red ratio on leaf hydraulics, photosynthesis and relative growth rate were relatively subtle, or even negligible, for many woody species, in comparison to the effects of strong differences in light quantity (e.g. for *Betula pendula* (Sellin *et al.*, 2011); for 13 species of tropical tree seedlings, see 'Methods ' in Kitajima & Poorter (1994), and for four species of temperate woody seedlings, see Sack & Grubb (2002)). The glasshouse air temperatures and relative humidity were recorded with EL-USB-2 + Hobo data loggers (Lascar Electronics, Erie, PA, USA). In the low-irradiance treatment the mean daily minimum, maximum and

Table 4 Gross leaf structure and nutrient and isotope composition traits for six ecologically diverse species of Hawaiian lobeliads, with expected plastic and adaptive responses to irradiance (+ or – in parentheses; see the Introduction), and results of the ANOVA testing the effects of species differences, growth irradiance, and their interaction

Trait	Symbol	Unit	ANOVA results			
			Species	Growth irradiance	Species × growth irradiance	Error
Gross leaf structure						
Leaf area (–)	LA	cm^2	**0.673, 66.3, (5) *****	**0.832, 17.0, (1) *** (67% decrease)**	**0.054, 5.50, (5) ****	0.011, 11.2, (50)
Leaf thickness (+)	T_{leaf}	mm	**0.113, 48.6, (5) *****	**0.150, 11.8, (1) *** (23% increase)**	**0.043, 17.8, (5) *****	0.005, 21.8, (50)
Leaf mass per area (+)	LMA	g m^{-2}	**0.246, 33.0, (5) *****	**1.50, 39.2, (1) *** (124% increase)**	**0.102, 13.6, (5) *****	0.011, 14.2, (50)
Leaf density (+)	LD	g cm^{-3}	**0484, 61.3, (5) *****	**0.698, 18.1, (1) *** (63% increase)**	0.022, 2.80, (5)^{ns}	0.014, 17.8, (50)
Midrib diameter (–)	–	mm	**0.230, 65.9, (5) *****	0.006, 0.346, (1)^{ns}	**0.082, 23.5, (5) *****	0.007, 10.3, (24)
Nutrient and isotope composition						
Carbon isotope ratio (+)	$\delta^{13}C$	‰	**0.004, 37.3, (5) *****	**0.013, 23.1, (1) *** (7% increase)**	0.001, 4.65, (5)^{ns}	0.0004, 34.9, (48)
Carbon concentration per mass (+)	C_{mass}	%	**0.016, 14.6, (5) *,†**	0.0004, 0.079, (1)^{ns}	**0.038, 35.1, (5) *****	0.006, 50.2, (48)
Nitrogen concentration per mass (+)	N_{mass}	%	**0.119, 35.9, (5) *****	0.075, 4.51, (1)^{ns}	0.017, 4.98, (5)^{ns}	0.019, 54.6, (48)
Nitrogen concentration per area (+)	N_{area}	g m^{-2}	$P = 0.09$			
Photosynthesis per nitrogen concentration per area (+)	A_{max}/N_{area}	µmol g^{-1} s^{-1}	**$P = 0.03$**	(89% increase)		
Nitrogen : carbon ratio (+)	N : C	–	**0.091, 39.4, (5) *****	**0.064, 5.54, (1) * (13% decrease)**	0.024, 10.5, (5)^{ns}	0.011, 44.6, (48)

For ANOVA results, mean square values and % variance are shown with degrees of freedom in parentheses, and for growth irradiance, the % increase or decrease of the trait value from low to high irradiance is presented, averaged across species means. For variables calculated from mean species values, paired t-tests were conducted between species means for low vs high irradiance, and only P-values are shown. Values in bold indicate a significant impact of growth irradiance (i.e. significant plasticity). ns, $P > 0.05$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; †, loss of significance when accounting for multiple tests using the sequential Bonferroni analysis (Supporting Information Table S1).

Table 5 Leaf venation and anatomical traits for six ecologically diverse species of Hawaiian lobeliads, with expected plastic and adaptive responses to irradiance (+, −, or = in parentheses; see the Introduction), and results of the ANOVA testing the effects of species differences, growth irradiance, and their interaction

Trait	Symbol	Unit	ANOVA results			
			Species	Growth irradiance	Species × growth irradiance	Error
Leaf venation architecture						
Major vein length per area (+)	Major VLA	mm mm^{-2}	**0.117, 68.1, (5)***	**0.070, 8.12, (1)*** (19% increase)**	**0.004, 12.0, (5)**	0.004, 11.7, (24)
Minor vein length per area (+)	Minor VLA	mm mm^{-2}	**0.020, 49.3, (5)***	**0.013, 6.53, (1)*† (9% increase)**	0.004, 9.12, (5)ns	0.003, 35.0, (24)
Total vein length per area (+)	VLA	mm mm^{-2}	**0.029, 60.0, (5)***	**0.020, 8.25, (1)** (10% increase)**	0.005, 10.4, (5)ns	0.002, 21.4, (24)
Number of free ending veins per area (=)	FEVs	no. mm^{-2}	**0.43, 88.2, (5)***	0.024, 0.99, (1)ns	0.014, 2.96, (5)ns	0.008, 7.84, (24)
Epidermal and mesophyll cross-sectional anatomy						
Upper cuticle thickness (+)	$T_{cut,ad}$	μm	**0.071, 20.5, (5)**	**0.578, 33.3, (1)*** (79% increase)**	**0.078, 22.5, (5)**	0.017, 23.7, (24)
Lower cuticle thickness (+)	$T_{cut,ab}$	μm	**0.112, 30.1, (5)**	**0.420, 22.0, (1)** (64% increase)**	0.048, 12.5, (5)ns	0.028, 35.3, (24)
Upper epidermal thickness (+)	$T_{ep,ad}$	μm	0.008, 23.2, (5)ns	0.002, 0.971, (1)ns	0.007, 20.7, (5)ns	0.004, 55.2, (24)
Lower epidermal thickness (+)	$T_{ep,ab}$	μm	**0.036, 56.0, (5)***	0.003, 0.997, (1)ns	**0.011, 16.5, (5)* †**	0.004, 26.5, (24)
Thickness spongy mesophyll (+)	T_{spo}	μm	**0.137, 60.5, (5)***	0.018, 1.56, (1)ns	0.016, 6.93, (5)ns	0.016, 6.93, (24)
Thickness palisade mesophyll (+)	T_{pal}	μm	**0.048, 33.4, (5)**	**0.187, 26.2, (1)*** (40% increase)**	0.015, 10.3, (5)ns	0.015, 10.3, (24)
Number of spongy cell layers (+)		–	0.025, 20.4, (50)ns	**0.112, 18.4, (1)* (30% increase)**	0.006, 5.29, (5)ns	0.014, 5.29, (24)
Number of palisade cell layers (+)		–	**0.106, 42.5, (5)***	**0.339, 27.3, (1)*** (50% increase)**	0.027, 10.7, (5)ns	0.010, 19.6, (24)
Air space spongy mesophyll (−)	%Air$_{spo}$	%	**0.112, 60.4, (5)***	0.010, 1.11, (1)ns	**0.027, 14.4, (5)* †**	0.009, 24.2, (24)
Air space palisade mesophyll (−)	%Air$_{pal}$	%	0.132, 19.2, (5)ns	0.156, 4.51, (1)ns	0.089, 14.7, (5)ns	0.089, 61.6, (24)
Upper epidermis cell area (−)	$A_{ep,ad}$	μm^2	**0.076, 40.5, (5)**	0.018, 1.87, (1)ns	0.036, 18.9, (5)ns	0.015, 38.8, (24)
Lower epidermis cell area (−)	$A_{ep,ab}$	μm^2	**0.120, 55.7, (5)***	0.015, 1.34, (1)ns	0.016, 7.53, (5)ns	0.016, 35.4, (24)
Palisade cell area (−)	A_{pal}	μm^2	**0.133, 57.2, (5)***	0.004, 0.367, (1)ns	0.030, 12.7, (5)ns	0.014, 29.7, (24)
Spongy cell area (−)	A_{spo}	μm^2	**0.016, 44.8, (5)**	0.008, 4.38, (1)ns	0.004, 12.0, (5)ns	0.003, 38.8, (24)
Bundle sheath cell area (−)	A_{bs}	μm^2	0.051, 22.0, (5)ns	0.062, 5.29, (1)ns	0.019, 9.67, (5)ns	0.027, 63.0, (22)
Bundle sheath surface area per leaf area (+)	$A_{mes\,bs}/A$	–	**0.042, 38.4, (5)***	**0.068, 12.3, (1)* (21% increase)**	0.002, 1.40, (5)ns	0.011, 47.9, (24)
Spongy surface area per leaf area (+)	$A_{mes\,spo}/A$	–	**0.031, 28.2, (5)***	**0.077, 14.0, (1)** (26% increase)**	0.022, 20.1, (5)ns	0.009, 37.6, (24)
Palisade surface area per leaf area (+)	$A_{mes\,pal}/A$	–	**0.182, 70.7, (5)***	**0.242, 18.8, (1)*** (48% increase)**	0.005, 1.94, (5)ns	0.005, 8.55, (24)
Mesophyll surface area per leaf area (+)	$A_{mes,corr}/A$	–	**0.072, 55.3, (5)***	**0.192, 24.8, (1)*** (37% increase)**	0.007, 5.34, (5)ns	0.004, 14.5, (24)
Midrib xylem cross-sectional anatomy						
Theoretical conductance per leaf area (+)	$K_{t,\,area}$	mmol m^{-2} s^{-1} MPa^{-1}	**0.440, 46.7, (5)***	0.001, 0.013, (1)ns	**0.267, 28.3, (5)**	0.049, 24.9, (24)
Theoretical conductance per leaf length and area (+)	$K_{t,\,length/area}$	mmol m^{-1} s^{-1} MPa^{-1}	**0.632, 50.2, (5)***	0.032, 0.505, (1)ns	**0.304, 24.1, (5)**	0.066, 25.2, (24)
Number of conduits in the midrib (+)		–	0.040, 25.2, (5)ns	0.062, 7.91, (1)ns	0.029, 18.2, (5)ns	0.016, 48.7, (24)
Maximum conduit diameter (+)		μm	**0.029, 43.2, (5)***	0.017, 4.97, (1)ns	**0.014, 21.2, (5)** †**	0.004, 30.6, (24)
Mean conduit diameter (+)		μm	**0.027, 36.5, (5)**	**0.053, 14.4, (1)** (15% decrease)**	**0.016, 22.1, (5)***	0.004, 27.0, (24)

For ANOVA results, mean square values and % variance are shown with degrees of freedom in parentheses, and for growth irradiance, the % increase or decrease of the trait value from low to high irradiance is presented, averaged across species means. ns, $P > 0.05$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; †, loss of significance when accounting for multiple tests using the sequential Bonferroni analysis and the false detection rate method (Supporting Information Table S1). Significant values appear in bold.
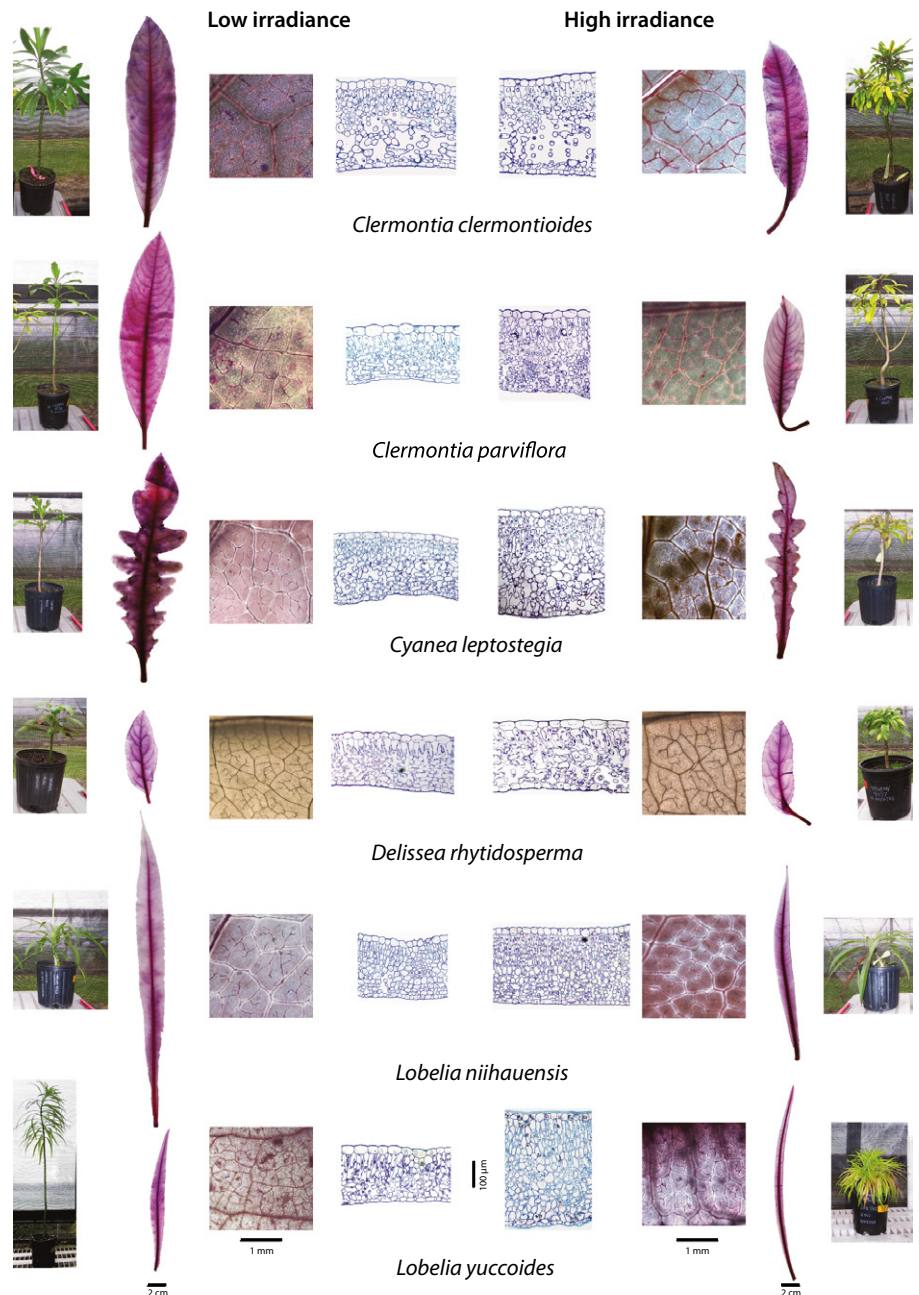
Fig. 1 Plasticity of leaf anatomy and structure in response to growth irradiance for six ecologically diverse species of Hawaiian lobeliads. Photograph of a representative plant, chemically cleared leaf, micrograph of the minor vein system, and lamina cross-section for each species grown under low irradiance (300 μmol photons m^{-2} s^{-1}; left images) and high irradiance (800 μmol photons m^{-2} s^{-1}; right images). Note the leaf chlorosis of the first three species under high irradiance.

average temperatures ($\pm$ SE) were 11.4 ± 0.08, 26.8 ± 0.11, and $17.3 \pm 0.04°C$, respectively, and the relative humidities were 62.5 ± 1.1, 87.9 ± 0.3, and $78.3 \pm 0.16\%$, respectively; in the high irradiance treatment, these values were 11.3 ± 0.15, 27.7 ± 1.43, and $17.3 \pm 0.40°C$, and 56.7 ± 4.2, 90.1 ± 1.9, and $78.9 \pm 3.0\%$, respectively. Plants were checked every day and all plants were watered to maintain the soil moist across treatments. Plant germination and growth are described in Supporting Information Methods S1.

Physiological and anatomical measurements were made when species had grown in the assigned light treatment for 6–12 months and were 10–85 cm tall (*c.* 1.5–2 yr old). Measurements were made on mature leaves that had developed and matured under the assigned light treatment. All plants were

randomly selected for light treatments and for placement on benches within each light treatment.

Measurements of K_{leaf}

K_{leaf} was measured in May 2010, using the evaporative flux method (Sack *et al.*, 2002; Brodribb & Holbrook, 2007; Sack & Scoffoni, 2012), which is described in more detail in Methods S2. Measurements were made for two to three leaves per individual and for five to six individuals per species (10–16 leaves per species).

To obtain maximum K_{leaf} values, we plotted the K_{leaf} values obtained against the Ψ_{leaf} at the end of the measurement, which in some cases reached relatively low values (down to -1.4 MPa), and fitted linear functions to the data. For the eight out of 12

cases of species grown in high or low light in which the K_{leaf} –Ψ_{leaf} regressions were significant ($P < 0.05$), we calculated maximum K_{leaf} as the y-intercept of the function fitted through the points (Brodribb et al., 2007); for the remaining four cases we averaged all K_{leaf} values.

Measurements of photosynthetic rate and g_s

We measured light-saturated photosynthetic rate (A_{max}) and g_s in the glasshouse using a portable gas exchange system equipped with a red/blue LED light source and a CO_2 mixing system (LI-6400; LI-Cor, Lincoln, NE, USA). All measurements were made between 08:00 and 13:00 h. We found that photosynthetic rate showed a peak within this period for these species (Givnish et al., 2004), as is generally the case for many woody species. We alternated measurements among species and treatments to best sample the average performance during this time period. A_{max} was sampled on the newest fully expanded leaf on four to five individuals of each species within both irradiance treatments. Leaves were clamped into the cuvette and exposed to saturating light (1000 µmol m^{-2} s^{-1}). Indeed, although a previous study showed that saturation irradiance varied across these species, there was no sign of decline in photosynthetic rate in any species at 1000 µmol m^{-2} s^{-1} (Givnish et al., 2004). We maintained relative humidity, leaf temperature, and cuvette CO_2 concentration at near ambient conditions (c. 75% relative humidity, 20–25°C, and 400 ppm, respectively). Gas exchange was logged when the photosynthetic rate had been stable for at least 60 s. We calculated the ratio of hydraulic supply over demand ($K_{leaf} : g_s$) and that of hydraulic supply over photosynthetic capacity ($K_{leaf} : A_{max}$); because different individuals were used for hydraulic and gas exchange measurements, we obtained standard errors for the quotients using propagation of error.

Measurements of leaf structure and composition

Leaf area (LA; cm^2) was measured for eight to 12 leaves from four to six individuals per species on images made using a flatbed scanner, using ImageJ (http://imagej.nih.gov/ij/; Abramoff et al., 2004). Fresh leaf thickness was measured on those same leaves before sampling from the plant using digital calipers (± 0.01 mm; Fowler, Chicago, IL, USA). After leaves were scanned for leaf area, they were placed in an oven at 70°C for 3 d and their dry mass was measured using an analytical balance (± 0.01 mg; XS205; Mettler, Toledo, OH, USA). LMA (g m^{-2}) was determined as dry mass/turgid leaf area and LD (g cm^{-3}) as LMA/leaf thickness).

To determine leaf chemical and isotopic composition, five leaves from five individuals per species were oven-dried at 70°C for 72 h, and ground, weighed, and sealed in tin capsules, according to standard protocols of the UC Davis Stable Isotope Facility (http://stableisotopefacility.ucdavis.edu/). Leaves were analyzed for carbon isotope ratio ($\delta^{13}C$), carbon per mass (C_{mass}), and nitrogen per mass (N_{mass}) using an elemental analyzer interfaced to a continuous flow isotope ratio mass spectrometer (IRMS; Sercon Ltd, Crewe, UK). Carbon to nitrogen ratios (C : N) were calculated for individual leaves as C_{mass}/N_{mass} and averaged to the species, and nitrogen per area (N_{area}) was calculated for each species as $N_{mass} \times$ LMA. Maximum CO_2 assimilation rate per nitrogen mass per unit area was calculated as A_{max}/N_{area}.

Measurements of leaf venation

To determine vein traits, we chemically cleared one leaf from each of three individuals per species in 5% NaOH and bleach following standard procedures (Scoffoni et al., 2013). Cleared leaves were imaged using a flatbed scanner (Epson Perfection 4490 Photo Scanner, Long Beach, CA, USA; 1200 pixels inch^{-1}), and then using a light microscope (Leica Lietz DMRB; Leica Microsystems, Buffalo Grove, IL, USA) at the top, middle and bottom thirds of the leaves using a $\times 5$ objective with a camera (SPOT Imaging Solutions; Diagnostic Instruments Inc.; Sterling Heights, MI, USA), resulting in $\times 287$ total image magnification (Sack et al., 2014). Leaf area (cm^2), perimeter (cm), length (cm) and width (cm), major VLA (mm mm^{-2}), minor VLA (mm mm^{-2}), total VLA (mm mm^{-2}), number of secondary veins and numbers of free vein endings per area (FEV; number per mm^{-2}), and midrib diameters were measured using ImageJ (http://imagej.nih.gov/ij/; Abramoff et al., 2004) following previously described standard procedures (Scoffoni & Sack, 2013).

Measurements of leaf cross-sectional anatomy

For measurements of leaf cross-sectional anatomy, one leaf from each of three individuals per species was sampled. From each leaf center, a 1×0.5 cm rectangle was cut and gradually infiltrated with mixtures of increasing strength low-viscosity acrylic resin (L. R. White; London Resin Co., London, UK) in ethanol, under vacuum over the course of 1 wk. Once fully infiltrated, the samples were embedded in resin in gelatin capsules in an oven at 55°C overnight. Using glass knives (cut using an LKB 7800 KnifeMaker; LKB Produkter; Bromma, Sweden), samples were sectioned in the transverse plane at 1 µm thickness in a rotary microtome (Leica Ultracut E; Reichter-Jung, Buffalo Grove, IL, USA). Sections were then placed on slides and stained with 0.01% toluidine blue in 1% sodium borate and imaged using a $\times 5$, $\times 10$, $\times 20$, and $\times 40$ objective using a light microscope (Leica Lietz DMRB; Leica Microsystems) with a camera utilizing SPOT advanced imaging software (SPOT Imaging Solutions; Diagnostic Instruments Inc., Sterling Heights, MI, USA) for a total image magnification of $\times 287$ to $\times 2300$.

We measured cell and tissue dimensions using ImageJ. Tissue thicknesses were measured in the middle of the left, center, and right thirds of the cross-sections and averaged. For the upper and lower cuticles and epidermises, and for the palisade and spongy mesophyll, cell cross-sectional areas were averaged for three cells per tissue per leaf cross-section.

Palisade and spongy mesophyll surface areas per leaf area ($A_{mes,p}/A$; $A_{mes,spo}/A$) were estimated from cross-sectional anatomy (Chatelet et al., 2013; Sack et al., 2013a), with a novel correction for the mesophyll volume taken up by minor veins and bundle sheath cells. The calculation is as follows:

$$A_{\mathrm{mes,x}}/A =$$

$$\frac{\mathrm{SA_x} \times (T_{\mathrm{xt}} - (\mathrm{ASF_{xt}} \times T_{\mathrm{xt}}) - (0.5 \times (\mathrm{CSA_{bs}} \times minorVLA)))}{\mathrm{VC_x}}$$

Eqn 1

where $\mathrm{SA_x}$ is the palisade or spongy cell surface area, T_{xt} is the thickness of the palisade or spongy mesophyll tissue, $\mathrm{ASF_{xt}}$ is the airspace fraction of the palisade or spongy mesophyll tissue, $\mathrm{CSA_{bs}}$ is the bundle sheath cross-sectional area, and $\mathrm{VC_x}$ is the palisade or spongy mesophyll cell volume. This equation treats the minor veins as if they were distributed half in the palisade tissue and half in the spongy tissue, as was observed, and thus half their volume was subtracted from each tissue. All component traits were calculated according to published detailed protocols (Sack *et al.*, 2013a).

We calculated the bundle sheath surface area per area ($A_{\mathrm{mes,bs}}/A$) as the mean perimeter of bundle sheath cell × minor VLA. The total mesophyll surface area per area ($A_{\mathrm{mes,bs}}/A$) was then calculated as the sum of $A_{\mathrm{mes,p}}/A$, $A_{\mathrm{mes,spo}}/A$, and $A_{\mathrm{mes,bs}}/A$.

We did not calculate the mean maximum mesophyll pathway (D_{m}) as used by Brodribb *et al.* (2007) for several reasons. First, D_{m} is calculated based on measurements of inter-veinal distance (IVD) and the distance from vein to epidermis (LE), both of which are difficult to accurately measure from cross-sections. Indeed, the distance between veins can be greatly variable in reticulate venation (except when looking at grass leaves, which have a highly regular pattern), so that the distance measured in a cross-section between two veins would depend on the angle and location at which the section was made in the leaf. Secondly, LE could only be compared across species if measured for a given vein order. However, in cross-sections, it is hard to determine minor vein order. Finally, D_{m} is essentially driven by VLA (Brodribb *et al.*, 2007; Sack *et al.*, 2013b), and LE has been shown to be positively correlated to K_{leaf} across species, rather than negatively, as it would contribute to a negative correlation of D_{m} with K_{leaf} (Aasamaa & Sober, 2001; Sack *et al.*, 2003a; Brodribb & Jordan, 2011). Thus, recent papers have focused on VLA rather than D_{m} to investigate correlations with physiology across species and between sun and shade leaves for given species (Brodribb & Feild, 2010; Brodribb *et al.*, 2010; Brodribb & Jordan, 2011).

To characterize the xylem anatomy, we measured major and minor axis diameters of all the xylem conduits in the midrib of each leaf sampled for anatomy. The total number of conduits and maximum conduit diameter were averaged across the midribs of the three sections. Additionally, we determined the theoretical conductivity of xylem conduits in the midrib of each leaf using Poiseuille's equation modified for ellipses (Lewis & Boose, 1995; Cochard *et al.*, 2004):

$$K_t = \sum \frac{\pi a^3 b^3}{64\eta(a^2 + b^2)}$$

Eqn 2

where a and b are the major and minor axes of xylem conduit and η is water viscosity at 25°C. We then calculate the theoretical hydraulic conductance normalized by leaf area as K_t/LA.

Statistics

We tested differences in traits among species, irradiance growth treatments, and their interaction, using two-way ANOVAs (Minitab Release 16). All data were log-transformed before analyses to improve normality and heteroscedasticity (Sokal & Rohlf, 1995). To test species differences in K_{leaf}, we performed an additional analysis, accounting for differences in the leaf water potential during measurement, because K_{leaf} is dynamic with leaf water status even at high water potentials and our method determined K_{leaf} across a range of water potentials (Scoffoni *et al.*, 2012, 2014). Thus, for K_{leaf}, we repeated the ANOVA described earlier, adding leaf water potential as a covariate, effectively comparing species in their K_{leaf} at a given leaf water potential. For those variables calculated from mean species values (N_{area} and $A_{\mathrm{max}}/N_{\mathrm{area}}$), paired t-tests were conducted to determine the significant differences between values at low vs high irradiance.

Given the large number of traits in our analyses, to account for multiple significance testing, we applied the sequential Bonferroni correction to all ANOVA results (Rice, 1989; Benjamini & Hochberg, 1995); results were considered nonsignificant when indicated to be so by this method (Table S1). To quantify and compare with one another the plastic responses of individual traits to growth conditions, we calculated the relative distance plasticity index (RDPI; Valladares *et al.*, 2006) for all six species as $\frac{x_i' - x_i}{x_i' + x_i}$ where x_i and x_i' are the mean trait values of individuals grown under low and high irradiance, respectively.

Multiple regressions were used to predict K_{leaf} from major VLA, minor VLA, A_{mes}/A, and theoretical midrib conductivity across species and growth irradiances combined (Minitab Release 16). We selected the multiple regression that exhibited the highest r^2 and in which the influence of given traits was in the realistic direction based on previous studies (see the Introduction).

Results

Variation across species in leaf physiological, structural, and anatomical traits

We found substantial variation across the six lobeliad species in hydraulic conductivity, g_s, and photosynthetic physiology, as well as leaf venation, tissue anatomy, and chemical composition. Averaging trait values for each species across the two growth irradiances, K_{leaf} varied by 4.5-fold, A_{max} by 1.4-fold and g_s by 2.1-fold (ANOVA, $P < 0.01$; Fig. 2; Table 3). Species varied by 3.2- to 11-fold in LA, LMA, and leaf thickness and density. The species also varied significantly in all nutrient and isotope compositional traits (Table 4). Species varied by 1.6- to sixfold in vein diameters and venation lengths per area for each vein order (Table 5). Species varied by 1.4- to 5.1-fold in leaf tissue anatomical traits across species, except for the upper epidermis thickness, the number of spongy cell layers, the percentage intercellular airspace in the palisade mesophyll, and the bundle sheath cell area, which were statistically similar across species (Table 5). Species varied by two- to 13-fold in midrib cross-sectional anatomical traits, but were statistically similar in conduit numbers (Table 5).
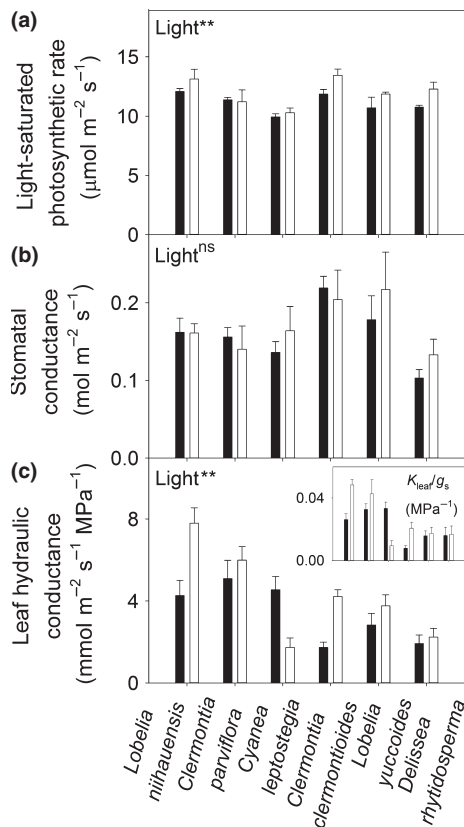
Fig. 2 Plastic response of physiological traits in response to growth irradiance for six ecologically diverse species of Hawaiian lobeliads. Mean ± SE values for light-saturated CO_2 assimilation rate (A_{max}) (a), stomatal conductance (g_s) (b), and leaf hydraulic conductance (K_{leaf}) (c) under low irradiance (closed bars) and high irradiance (open bars). The inset in (c) shows hydraulic supply over demand (K_{leaf}/g_s) under low irradiance (closed bars) and high irradiance (open bars). All traits showed significant variation across species ($P < 0.01$, ANOVA, Table 2: ^{ns}, $P > 0.05$; **, $P < 0.01$).

Plasticity across growth irradiances in leaf hydraulic and gas exchange traits

On average, K_{leaf} and A_{max} values for leaves developed by plants grown under high irradiance were higher than those for plants under low irradiance (ANOVA, $P < 0.01$; Fig. 2; Tables 3, S2). By contrast, no differences were found in g_s measured under saturating irradiance for plants grown in the two irradiance treatments. Notably, one of the six species showed a contrary response: *C. leptostegia* showed a 2.5-fold higher K_{leaf} in plants grown in low vs high light. For the species that showed a strong significant positive plasticity of K_{leaf} with irradiance, because g_s remained stable, K_{leaf} : g_s was greater for plants grown at higher irradiance (Fig 2, inset). Similarly, species with greater K_{leaf} RDPI values also exhibited greater plasticity in K_{leaf} : g_s and K_{leaf} : A_{max} ratios ($r^2 = 0.96$ and 0.99, respectively, $P < 0.01$).

Species' values for hydraulic plasticity were strongly correlated with A_{max}. Across species, the RDPI of K_{leaf} from low to high growth irradiance was strongly positively related to A_{max} ($r^2 = 0.85$, $P = 0.009$; Fig. 3).
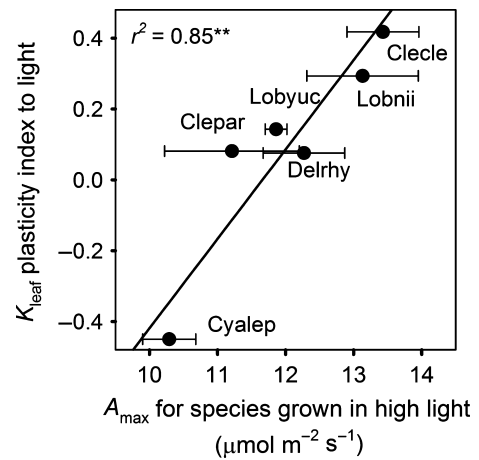

Fig. 3 Strong correlation of the leaf hydraulic response to growth irradiance (relative distance plasticity index; see the Materials and Methods section) and light-saturated photosynthetic rate per leaf area (A_{max}) for plants under high growth irradiance, a proxy for native light habitat (Givnish *et al.*, 2004) across six ecologically diverse species of Hawaiian lobeliads. Fitted standard major axis: K_{leaf} light response $= 0.25 \times A_{area} - 2.94$. Clecle, *Clermontia clermontioides*; Clepar, *Clermontia parviflora*; Cyalep, *Cyanea leptostegia*; Delrhy, *Delissea rhytidosperma*; Lobnii, *Lobelia niihauensis*; and Lobyuc, *L. yuccoides*. Data are means ± SE; **, $P < 0.01$.

Plasticity across growth irradiances in leaf structure, venation, and composition

Numerous plastic differences in responses to light were found for measured leaf physiological, structure, venation, anatomical, and compositional traits, that is, for 22 of the 42 measured traits (52%) ($P < 0.05$ in ANOVAs after correction for multiple tests; Tables 3–5). These plastic shifts occurred in the expected directions, based on previous studies of other species, for 15 of these 22 traits (68%). Of the four potential anatomical drivers of K_{leaf}, major and minor VLA and A_{mes}/A showed the expected sun/shade plasticity ($P < 0.05$; Figs 1, 4; Tables 5, S2). For plants grown under high irradiance, leaf area was on average 1.7-fold smaller, leaves tended to be 1.2-fold thicker and 1.6-fold more dense, yielding 2.2-fold higher LMA values. No significant plastic differences were found in species' FEVs across growth irradiances ($P > 0.05$; Table 5), a trait that was correlated with K_{leaf} in some species sets (Sack & Scoffoni, 2013). Across species, there was a significant increase of the upper and lower cuticle thicknesses, the palisade thickness, and the number of palisade and spongy cell layers from low to high irradiance, and of total mesophyll, spongy, palisade, and bundle sheath surface areas per leaf area (Fig. 4; Tables 5, S2). However, no differences between light treatments were found in xylem anatomical traits, except that, contrary to expectation, the mean midrib xylem conduit diameter was smaller in species grown under high irradiance (Tables 5, S2). Finally, as expected, plants grown under high irradiance had less negative values for $\delta^{13}C$ and higher A_{area} : N_{area} (Fig. 4; Tables 4, S2). We found no significant differences in N_{area} and N_{mass} across growth irradiances and, contrary to expectation, lower N : C under high than under low irradiance (Tables 4, S2).
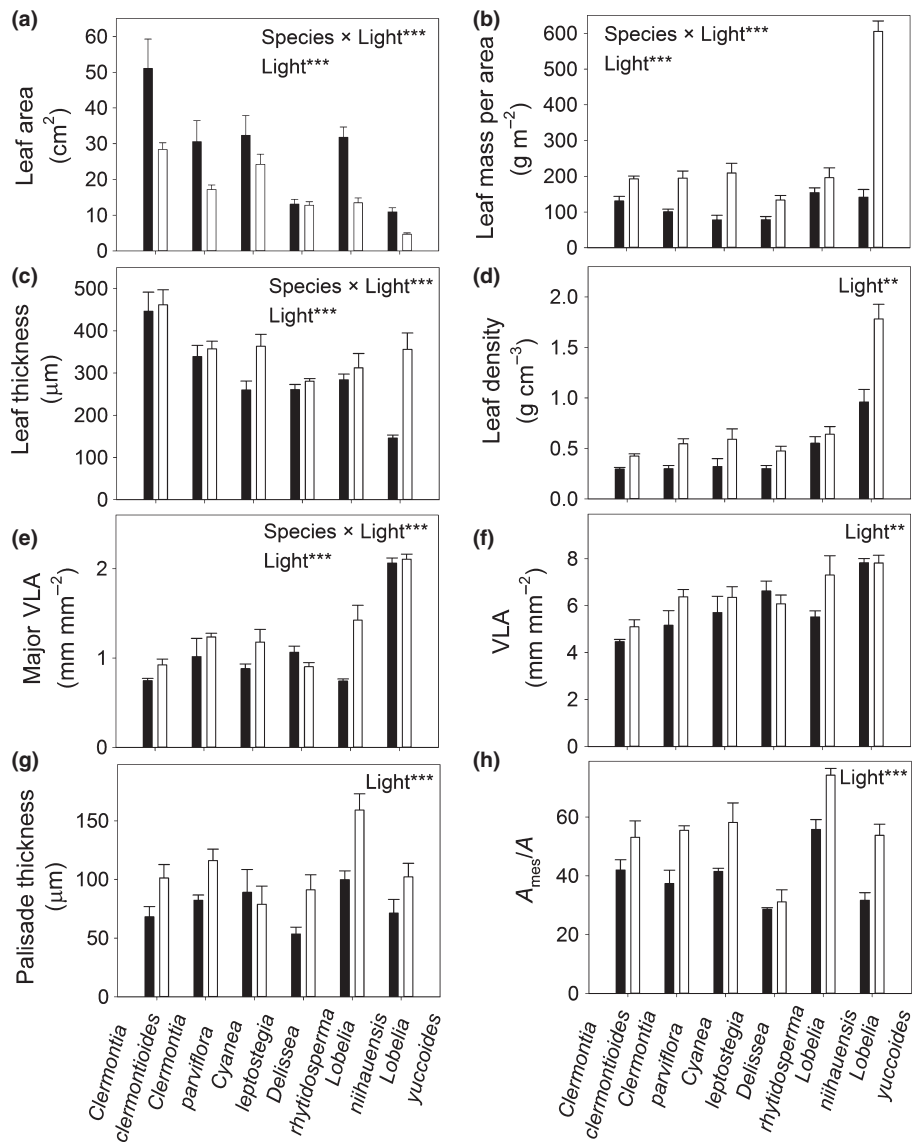
Fig. 4 Plastic response of anatomical and structural traits to growth for six ecologically diverse species of Hawaiian lobeliads. Mean ± SE values for leaf area (a), leaf mass per area (LMA) (b), leaf thickness (c), leaf density (d), major vein length per area (major VLA) (e), total vein length per area (VLA) (f), palisade tissue thickness (g), and mesophyll surface area per leaf area (A_{mes}/A) (h) for individuals grown under low irradiance (closed bars) and high irradiance (open bars). All these traits varied significantly across species ($P < 0.01$; ANOVA, Table 2: **, $P < 0.01$; ***, $P < 0.001$).

Averaging the absolute RDPI values across all the 22 traits showing plastic responses indicated the overall differences across species in-shade trait plasticity. *L. yuccoides* showed the highest plasticity (RDPI = 0.23), followed by *L. niihauensis* (RDPI = 0.18), *C. clermontioides* and *C. parviflora* (RDPI = 0.15), *C. leptostegia* (RDPI = 0.14), and *D. rhytidosperma* (RDPI = 0.13). Species varied in the degree of their plastic responses of structural and anatomical traits. Thus, species differed in the plasticity of leaf size and thickness, LMA, major VLA, midrib diameter, percentage air space in the spongy mesophyll, xylem conduit diameter, theoretical midrib conductivity and conduit sizes ($P < 0.05$ for species × growth irradiance interaction; Tables 4, 5). Additionally, species differed in the plasticity of upper cuticle and epidermal thickness and the percentage air space in the spongy mesophyll.

Species native to higher irradiance had greater plasticity in traits in addition to K_{leaf}. Thus, species with higher A_{max} in high light also had a greater plastic response of the $K_{leaf} : A_{max}$ ratio ($r_p = 0.90$, $r_s = 0.83$, $P < 0.05$), N : C ratio ($r_p = 0.85$, $r_s = 0.83$, $P < 0.05$), percentage air space in the palisade ($r_p = 0.91$, $r_s = 0.94$, $P < 0.05$), palisade cell size ($r_p = 0.92$, $r_s = 0.89$, $P < 0.05$), and bundle sheath surface area per leaf area ($r_p = 0.93$, $r_s = 1.0$, $P < 0.01$).

Structural and anatomical drivers for variation in hydraulic capacity among species and irradiance treatments

Across species, whether considering the high or low irradiance treatments individually, or both treatments together, K_{leaf} was not significantly correlated with individual anatomical drivers:

major and minor VLA, the theoretical hydraulic conductivity through the midrib (K_t), or the mesophyll surface area per leaf area (A_{mes}/A) ($P > 0.05$; Table S2). However, K_{leaf} was significantly correlated with multiple factors; combining the effect of each of these anatomical traits on K_{leaf} using Eqn 3 (obtained from multiple regressions; see the Materials and Methods section) explained 40% of the variation observed in K_{leaf} ($P < 0.01$; Fig. 5):

$$K_{leaf,pred.} = -0.41 + 0.19 \times majorVLA + 0.09 A_{mes}/A \qquad \text{Eqn 3}$$

Discussion

Our results provide a demonstration of sun/shade plasticity in K_{leaf}, the first for multiple species (tripling the data available for K_{leaf} plasticity; Table 1). Equally importantly, this study clarifies the variation in hydraulic plasticity across species of an adaptive radiation, including rare and endangered species. Our findings clearly demonstrate that the K_{leaf} responses to growth irradiance can vary strongly even among closely related species, explaining the discrepancy between the two previous studies of single species, in which one showed an increase in K_{leaf} with growth irradiance, while the other did not. Moreover, this study provides a new level of detail for sun/shade leaf plasticity in general, by providing a comprehensive view of plastic shifts in hydraulics, gas exchange, leaf mesophyll and xylem anatomy, venation architecture and leaf composition in relation to conditioning light regime
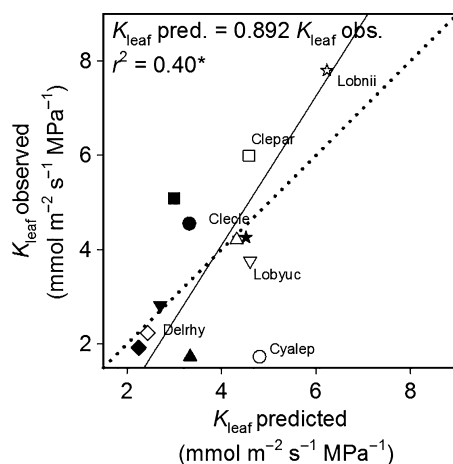

Fig. 5 Relating leaf hydraulic conductance (K_{leaf}) to leaf anatomy for six ecologically diverse species of Hawaiian lobeliads grown at two different irradiances. On the y-axis, K_{leaf} is predicted from major vein length per area (major VLA) and mesophyll surface area per leaf area (A_{mes}/A) for mean values of species grown under low and high irradiance, using Eqn 3. The plotted dashed line was forced through the origin to allow comparison with the 1 : 1 line. The presented r^2-value is for the line fitted through the points (solid line). *, $P = 0.027$. *Clermontia clermontioides* (Clecle; triangles); *Clermontia parviflora* (Clepar, squares); *Cyanea leptostegia* (Cyalep, circles); *Delissea rhytidosperma* (Delrhy, diamonds); *Lobelia niihauensis*; (Lobnii, stars); *Lobelia yuccoides* (Lobyuc, reverse triangles). Open and closed symbols are for plants grown under high and low irradiance, respectively.

and to the native light regime of individual species. Additionally, our results provide further evidence of the magnitude of physiological diversification that has evolved rapidly during species diversification in the Hawaiian lobeliads. Our results have implications for the hydraulic control of gas exchange during plasticity and adaptation to contrasting light regimes.

Plastic response of the K_{leaf} to growth irradiance and anatomical drivers

K_{leaf} showed a strong and variable plastic increase with light supply across six species and with A_{max}, a proxy for the light supply in a species' native habitat. Indeed, the A_{max} is an index of adaptation to high irradiance, shown in a previous study to be positively correlated with the average photon flux density of individual species in their native habitat across 11 lobeliad species, including four from the present study (Givnish *et al.*, 2004; Montgomery & Givnish, 2008). This substantially extends and resolves the contrasting findings of two recent studies of single species (Raimondo *et al.*, 2009; Murphy *et al.*, 2012) by examining several species and by relating the strength of the plastic response to those species' natural distribution along a light gradient and likely exposure to increasing VPD and heat loads (Givnish *et al.*, 2004; Givnish & Montgomery, 2014).

What causes K_{leaf} to differ with growth irradiance? A species can increase its K_{leaf} by modifying its xylem anatomy, its mesophyll anatomy, and/or its biochemistry. Across species, anatomical changes statistically explained 40% of observed variation in K_{leaf}. Plants of a given species grown under higher irradiance tended to develop smaller and thicker leaves with higher major VLA and mesophyll surface area per leaf area (A_{mes}/A), which are traits that would increase both vein xylem conductivities (McKown *et al.*, 2010) and outside-xylem conductivity (an increase in A_{mes}/A could increase the evaporative surface inside the leaf; Sack & Scoffoni, 2013). Future studies are required to focus on sun/shade plasticity in leaf biochemistry, such as changes in the amount of aquaporin expression and/or distribution throughout the outside-xylem pathways (Kim & Steudle, 2007; Shatil-Cohen *et al.*, 2011), which our results suggest play an additional important role in the plasticity of K_{leaf}. There is an increasing need for genetic mapping to allow for molecular approaches in physiological studies, especially to enable high-resolution understanding of evolution within adaptive radiations such as the Hawaiian lobeliads. Such future work will clarify the mechanisms of physiological evolution, and could also contribute detailed information regarding physiologically important genetic variation within species, of great potential use for potential conservation of rare and endangered species.

Physiological benefits of the plastic response of the K_{leaf}

In the Hawaiian lobeliads, while K_{leaf} tended to increase in higher irradiance, g_s did not shift upward correspondingly, consistent with the previous finding of little differences in g_s across species measured in the field for 11 Hawaiian lobeliad species, including four species of this study (Givnish *et al.*, 2004). This finding

contrasts with two previous studies of intracanopy plasticity for two species, in which g_s increased proportionately to higher K_{leaf} for sun-exposed vs shaded leaves within canopies (Sellin & Kupper, 2007; Brodribb & Jordan, 2011). The greater plasticity of K_{leaf} than g_s in our study would provide excess hydraulic supply, which would buffer the greater evaporative load induced by higher VPD under higher irradiance and could provide additional tolerance of environmental stresses such as heat load and drought (Brodribb & Jordan, 2008; Scoffoni et al., 2011). The lobeliad species in this study occur in moist montane forests where heat load may not be a critical issue. They do, however, differ in potential exposure to drought, with L. niihauensis occurring in drier sites than congener L. yuccoides, and C. clermontioides occurring in drier sites than congener C. parviflora (Wagner et al., 1999b). Notably, the differences we found for these species are consistent with their distribution, with the species from drier sites having greater hydraulic plasticity.

Adaptive significance of species differences in their sun/shade plasticity in leaf hydraulics

The six Hawaiian lobeliad species differed in their sun/shade plasticity of K_{leaf}. The K_{leaf} increased from low to high irradiance by 16% in D. rhytidosperma to 144% in C. clermontioides, but decreased by 164% from low to high irradiance in C. leptostegia. As expected from previous studies, A_{max} increased for plants grown under higher photon flux densities, and this was coupled in five of six species with an increase in K_{leaf} and with K_{leaf}/A_{max}. The later patterns were expected because, not only does stronger illumination drive more evaporation, but it is also often associated with higher VPD and thus higher hydraulic demand even at a given g_s (Young & Smith, 1979; Smith, 1981; Ellsworth & Reich, 1992; Maherali et al., 1997). The exception to the rule, C. leptostegia, showed a plastic drop in hydraulic conductance with light availability. We suggest that this decrease in hydraulic conductance with exposure to brighter light is a mechanism for the unique decline in A_{max} with light availability in C. leptostegia among the lobeliads studied (Givnish & Montgomery, 2014), which presumably contributes to its exclusion from bright environments. These results point to a potential significance of sun/shade K_{leaf} plasticity in determining differences among species in the ability to establish under contrasting light habitats.

Integrated plasticity in response to sun vs shade of leaf physiological and anatomical traits

In addition to the sun/shade plasticity of K_{leaf} and assimilation rates, our results point to the integrated plasticity of a wide complex of leaf anatomical and compositional traits. Across the six measured Hawaiian lobeliad species, traits benefiting differential performance in sun vs shade shifted together in a coordinated way. Such coordinated shifts are expected from optimality theory, because an improvement in one component only would cause increasing performance limitation by others, whereas improvement in multiple components can lead to nonlinear

disproportionate increases in performance (McKown et al., 2010; Sack & Scoffoni, 2013; Sack et al., 2013b). Consistent with the many studies looking at sun vs shade leaves, or sun vs shade establishing species, leaves adapted or acclimated to high irradiance tended to be smaller, thicker and denser, yielding higher LMA, and thus allowing leaves, when grown under high irradiance, to capture direct light more efficiently and to have thinner boundary layers, reducing the heat load (e.g. Givnish, 1988; Popma et al., 1992; Bragg & Westoby, 2002; Sack & Frole, 2006). Consistent with developmental constraints, smaller sun leaves had higher major VLA and smaller midrib diameter, which improve dehydration tolerance and biomechanical support relative to investment cost (Sack et al., 2012). The shifts in leaf thickness corresponded to increases in the thickness of the palisade tissue and the numbers of palisade and spongy cell layers, providing more photosynthetic tissue and cell/intercellular air space contact for CO_2 and water exchange, consistent with the higher A_{mes}/A values (Kenzo et al., 2004). Thicker leaves have less evaporative surface, but also involve more internal competition and redundant structure for light and CO_2 absorption and so should be favored only in sunnier or effectively drier environments (Givnish, 1978). Our study points to a wider suite of leaf physiological and anatomical traits that have undergone coordinated shifts during plasticity and adaptation to ecologically diverse light regimes and that would act to optimize performance from low to high irradiance. This integrated suite of traits includes hydraulic, stomatal, photosynthetic, and structural and anatomical features. Coordinated shifts in all these traits would improve carbon gain under high irradiance, reduce construction and maintenance costs under low irradiance, and potentially improve tolerance of additional stresses experienced in given light regimes, such as high VPD and water stress under high irradiance.

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Supporting Information

Additional supporting information may be found in the online version of this article.

Table S1 Results for sequential Bonferroni analyses, testing the tablewide significance of physiological, structural and anatomical trait variance

Table S2 Mean and standard errors for the 42 traits of this study

Methods S1 Plant germination and growth.

Methods S2 Measuring leaf hydraulic conductance using the evaporative flux method with a flow meter.

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