

HYDRAULIC ARCHITECTURE AND THE EVOLUTION OF SHOOT ALLOMETRY IN CONTRASTING CLIMATES¹

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We used pairs of congeneric shrub species from contrasting habitats to test for repeated evolutionary divergence in leaf–stem allometry and shoot hydraulic architecture in response to water availability. Allometric relationships and mean ratios between leaf size (individual and total area and mass per shoot) and stem cross-sectional area were compared between habitats using six species pairs representing three genera (*Arctostaphylos*, *Baccharis*, *Ceanothus*). We measured correlations among evolutionary changes in allometric, morphological, and physiological traits using phylogenetic independent contrasts. Allometric analysis revealed habitat differences: slopes were homogeneous among species (=1.46), but the more mesic-adapted species generally supported more leaf area at a common stem cross-sectional area. Reducing bivariate allometry to a ratio obscured this pattern because ratios varied with stem size, which was unrelated to habitat. Mean individual leaf size also was not correlated with either water availability or leaf–stem allometry. Stem hydraulic conductivity was generally lower in the xeric-adapted species of each pair, and its evolution mirrored changes in shoot allometry. This study provides evidence for repeated evolutionary divergence in shoot allometry and hydraulic architecture associated with water availability and demonstrates the importance of shoot allometry to water relations, independent of leaf size.

Key words: allometry; Huber value; hydraulic conductivity; phylogenetic independent contrasts; species pairs; specific leaf area (SLA).

Interspecific trait correlations are common in biology and arise from a combination of developmental mechanisms and fundamental adaptive relationships (Wagner and Altenberg, 1996; Brouat et al., 1998; West et al., 1999; but see Kozłowski and Weiner, 1997). One such relationship long observed in woody plants is that stem thickness is correlated with individual leaf size and with the total amount of leaf area or mass supported by that stem. These relationships are evident within communities (White, 1983), clades (Bond and Midgley, 1988; Ackerly and Donoghue, 1998), and species (Bond and Midgley, 1988; Gartner, 1991; Brouat and McKey, 2001).

Stem and leaf size correlations reflect the functional requirement for both biomechanical and hydraulic support of leaves (Shinozaki et al., 1964; Gartner, 1991; Niklas, 1994; Brouat and McKey, 2001). In dry hot climates, hydraulics should play a particularly strong role in the evolution of shoot morphology. High leaf temperature and low relative humidity increase vapor pressure deficits (VPD) and thus potential transpiration rates. This effect can be magnified in larger leaves, which have a thicker boundary layer and consequently greater leaf to air temperature differentials (Givnish, 1978; Chiariello, 1984). Moreover, low soil moisture increases the risk that the continuous hydraulic pathway from soil to leaf will break (i.e., cavitate), leading to hydraulic failure. Plants can control this risk by regulating water loss from their leaves relative to the capacity of the whole plant for water uptake and transport (Sper-

ry et al., 2002). Thus, in climates with limited soil moisture and high VPD, plant hydraulic function should best be maintained in shoots with reduced total leaf area relative to stem area.

Numerous studies support this prediction, showing that the amount of leaf area for a given conducting stem area declines with increasing evaporative demand or drought stress. This pattern has been observed in plastic responses to light (Maherali et al., 1997) and water availability (Li et al., 2000), in intraspecific differentiation along environmental gradients (Callaway et al., 1994; Villar-Salvador et al., 1997; Li et al., 2000), and in interspecific differences across habitats (conifers: Margolis et al., 1995; angiosperms: Vander Willigen et al., 2000; Cavender-Bares and Holbrook, 2001). Analyzing the leaf–stem size relationship as bivariate allometry or as mean leaf-to-stem area ratios (or, inversely, the Huber Value, e.g., Zimmermann, 1983) yields similar results.

Any functional analysis of leaf–stem size relationships must also consider that size only approximates the hydraulic capacity of the stem and the evaporative demands of the leaves. Anatomical and physiological traits mediate the intrinsic relationship between size and function, which is in turn sensitive to external environmental conditions, as discussed above. For example, the rate of water loss from leaves is modulated by stomatal conductance and various structural traits including stomate density and size (Reich, 1984; Abrams et al., 1994), leaf angle (Valladares and Pearcy, 1997), leaf shape (Givnish, 1978; Jones, 2001), and pubescence (Ehleringer and Mooney, 1978; Sandquist and Ehleringer, 1998). Similarly, the transport efficiency per unit cross-sectional area (specific conductivity) is generally reduced by xylem properties such as increased wall thickness relative to lumen diameter, which are associated with resistance to embolism under drought stress (Hacke and Sperry, 2001; Hacke et al., 2001). Thus, the interplay between shoot morphology and other hydraulic traits potentially has important consequences for water balance in the shoot and ultimately for the adaptive significance of shoot allometry.

In the present study, we examine variation in the size re-

¹ Manuscript received 7 January 2003; revision accepted 1 May 2003.

The authors thank R. Lanier Anderson, Nancy Anderson, Radika Bhaskar, Taryn Fransen, Angela Preston, Dylan Schwilk, and Susan Zeman West for help with data collection in the field; Carl Wishner and Earlyn Mosher (Soka University) for locating populations; E. Mosher, California State Parks, and the National Park Service for permission to collect material; R. Bhaskar, Jeanine Cavender-Bares, Jacques Dumais, and Dylan Schwilk for help with equipment in the lab; David Warton for providing the program for testing homogeneity of slopes; and R. L. Anderson, R. Bhaskar, D. Schwilk, and two anonymous reviewers for useful comments on the manuscript. This work was supported by a NSF Postdoctoral Research Fellowship to K. A. P. and NSF Grant 0078301 to D. D. A.

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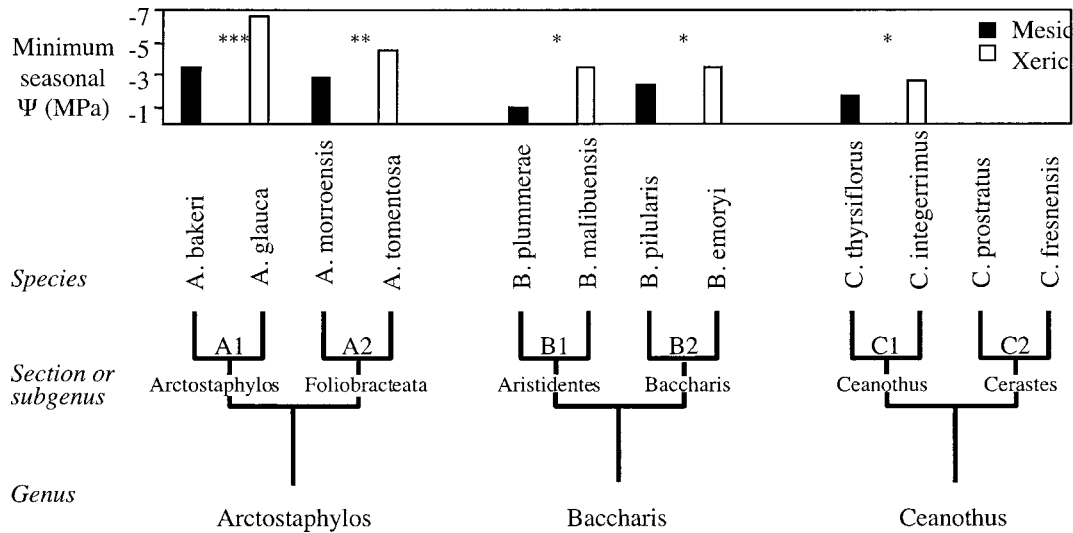


Fig. 1. Phylogenetic relationships among the species pairs measured in this study. Minimum seasonal water potential at midday is indicated by dark bars for the more mesic-adapted (left) and open bars for the more xeric-adapted species (right) in a pair. In each case, paired species differed significantly from one another in midday water potential (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

relationship between leaves and stems as a function of water availability using pairs of closely related species sampled from the phylogenetically diverse California flora. First, we asked whether there was evidence for repeated evolutionary shifts in allometry in response to water availability among the species examined. We predicted that the elevation (intercept) of the shoot–leaf area vs. stem–cross-sectional area relationship would be greater under more mesic than more xeric conditions, reflecting habitat differences in the total amount of leaf area that could be supported by a stem of a given size. The slope, or scaling relationship between these variables, was compared with the slopes predicted on purely hydraulic and purely static biomechanical grounds. Second, we tested the hypothesis that stem–leaf allometry has evolved independently of mean leaf size and not simply as a correlated response to selection for small leaves in dry habitats. Third, a series of physiological and anatomical traits related to water use were compared among species and between habitats. Individual structural traits were compared between habitats to test for divergence in response to water availability. Specific leaf area, stomatal density and size, and hydraulic capacity of stems were all predicted to be greater with higher water availability, whereas higher wood density and greater leaf-specific hydraulic supply were expected under more xeric conditions. Pairwise correlations were also calculated for a larger set of physiological and structural traits related to hydraulic function to test for correlated evolution among these traits and with allometric relationships.

MATERIALS AND METHODS

Species pairs—The three genera chosen for this study, *Arctostaphylos*, *Baccharis*, and *Ceanothus*, are geographically widespread and native to California. We examined 12 taxa representing nine phylogenetic independent contrasts: in each genus, two pairs of closely related species (four species total) were chosen from environments of contrasting water availability (Fig. 1). A species pair was drawn from each of the two monophyletic subgenera of *Ceanothus* (Jeong et al., 1997; Hardig et al., 2000), two morphologically distinct taxonomic sections of *Baccharis* (as defined by Nesom, 1990), and two sections of *Arctostaphylos*. In the genus *Arctostaphylos*, the taxonomy is

not well reflected in the current phylogenetic hypothesis (Markos et al., 1998). Although the two pairs of *Arctostaphylos* species do provide environmental contrasts, their use as evolutionary contrasts should be considered provisional. Because each species pair represents an independent evolutionary divergence and an independent radiation into contrasting habitats, each provides an independent test of whether differences in leaf size and in stem–leaf allometry have resulted from evolution in contrasting environments.

Habitat contrasts were based on estimates of the regional climate within a species range, the typical microclimate occupied by the species, and measures of plant water status. Climate means and extremes for specific collection sites were calculated using data available from the Numerical Terradynamic Simulation Group at the University of Montana, School of Forestry (www.daymet.org; Thornton et al., 1997), which included daily values for 1980 through 1997 at a spatial resolution of 1 km². Climate data averaged over each species range were taken from the analysis by Knight and Ackerly (2002). During the study, microsite water availability was estimated for ten of the species by measuring predawn leaf water potential at the end of the growing season when water was least available (for locations and climate data, see Supplementary Data accompanying the online version of this article). Predawn leaf water potential reflects both the abiotic environment (soil water levels and soil properties) and an individual plant's growth and allocation within that environment (e.g., rooting depth). Water potential at midday was used to estimate the minimum seasonal water potential experienced by plants in the field, which has been shown to be correlated with drought tolerance (resistance to drought-induced embolism; Pockman and Sperry, 2000; Ackerly, 2004). For all species pairs, the majority of climate and water status variables supported the habitat contrast.

Allometry—We measured the relationship between leaf and stem size using bivariate allometry rather than a simple ratio between the traits, such as the Huber value (sapwood cross-sectional area per total distal leaf area; Zimmermann, 1983). Allometric analysis preserves crucial information about size dependence of the leaf-to-stem size ratio and avoids some statistical problems associated with ratios (Jasienski and Bazzaz, 1999), and so it is usually more informative than ratios (see discussions in Müller et al., 2000; Preston and Ackerly, 2003).

First-year shoots were collected for allometric analysis from mature plants in natural populations throughout California (see Supplementary Data). Several species are endemics with extremely small ranges (*A. bakeri* subsp. *bakeri*, *A. morroensis*, *B. malibuensis*) and are represented in the data by a single population. Shoots of more widespread species were collected from more than

one location. Eight to 33 individuals per species were sampled in early summer (May, early June) and at the end of the growing season before the onset of winter rains (late September–mid-October) in both 1999 and 2000. Wherever possible, a species was sampled at all four collecting periods. Single first-year shoots were collected from each individual, wrapped in wet paper towels, and transported in sealed bags in a cooler back to the laboratory.

Stem cross-sectional area was measured at two points on each shoot: the internode below the most basal leaf on the stem (“basal area”) and the internode below the most recently matured leaf (“tip area”). At each point, diameter was measured in two directions and the area of the ellipse was calculated. Leaves were counted and total fresh mass and area were measured for all leaves on a shoot (using an LI-3100 leaf area meter, LI-COR, Lincoln, Nebraska, USA). We avoided collecting shoots with well-developed axillary branches, but when present, leaves from nascent axillary shoots were weighed and measured separately from main axis leaves. These were not used to calculate mean individual leaf area or mass, but they were included in the total leaf area or mass on a shoot. Dry mass of leaves was used to calculate specific leaf area (SLA, in square centimeters per gram). Xylem cross-sectional area was measured for a subset of shoots (4–7 per species) by examining hand sections using a light microscope with an ocular scale.

A load parameter (L_p) was also calculated for each shoot, taking into account stem and leaf biomass and stem length. Following Niklas (1990) and Farnsworth and Van Gardingen (1995), L_p was defined as $L_p = W l^2 A^{-2} E^{-1}$, where W is the total mass of the shoot, l is the shoot length, and A is the stem cross-sectional area. The elastic modulus, E , was excluded from the calculations because we assumed it to be constant within species and therefore not to affect estimates of the slope of L_p on stem cross-sectional area.

Field measurements—At the end of the growing season in 2000, gas exchange was measured in the field both midmorning and midday using a LI-6400 Portable Photosynthesis System fitted with an arabidopsis chamber (LI-COR). Instantaneous water use efficiency (WUE) was calculated from these measurements as the ratio of area-based photosynthetic rate to transpiration. Leaf water potential was measured predawn and midday using a pressure chamber (Soil Moisture, Santa Barbara, California, USA). Stomatal impressions made with clear fingernail polish were taken from the leaves used for midday gas exchange measurements. First-year shoots from five individuals per species were collected under water and stored with their cut ends in water in sealed bags in a cooler until they could be used to measure stem hydraulic conductivity. Due to sampling restrictions, some physiological measurements were not made on *A. glauca*, *C. prostratus*, and *C. fresnensis*.

Hydraulic conductivity—The hydraulic conductivity of the stems (K ; in gram meters per second per kilopascal) was measured directly as the flow rate through a stem segment under known pressure (following Gartner, 1991). Segments 6 cm long were cut under water from the current year’s growth of shoots collected in the field. Segments were secured in a manifold of tubing, and filtered degassed water was forced through each individual segment and into a graduated cylinder on a balance (Acculab LA110). Flow rate was monitored with a computer connected to the balance, and readings were taken once they had stabilized, typically after 1–3 min. The small diameters (<0.3 cm) of the first-year stems imposed high resistance to flow, and a pressure of 0.3 MPa was required to bring readings into the optimal range for our system. As a consequence, any natively embolized vessels were flushed, and our measurements reflect maximum conductivity. Leaf-specific conductivity (K_l ; in grams per second per meter per kilopascal) was determined by dividing hydraulic conductivity by the total area of leaves distal to the measured segment. The cross-sectional areas of the stem and the sapwood at the distal end of the stem segment were divided by hydraulic conductivity to yield stem- and xylem-specific conductivity (K_s and K_x ; in grams per second per meter per kilopascal).

Leaf area-specific whole-plant conductance (G_l) (Meinzer et al., 1995), was estimated from the field physiological measurements as transpiration (E ; in millimoles per square meter per second) per water potential differential ($\Psi_{\text{predawn}} - \Psi_{\text{midday}}$, in megapascals), assuming steady-state conditions. This measure of leaf-specific conductance is not directly comparable to the leaf-

specific hydraulic conductivity of stem segments because G_l incorporates the entire path of water movement, whereas the conductivity of stem segments (K) does not include resistance from other parts of the soil/root/leaf pathway. In addition, native embolisms will reduce the value of G_l whereas embolized vessels were flushed during the measurement of K . Nevertheless, to the degree that both measures reflect xylem characteristics, G_l and K_l should be correlated across species.

Anatomical measurements—Stomate density (number of stomata per square centimeter) of both abaxial and adaxial leaf surfaces was estimated from impressions taken in the field, based on 1–3 samples per leaf surface (microscope field = 0.0113 cm² viewed at 125 \times). The total (two-sided) density of stomata is the sum of the adaxial and abaxial densities. Aperture length was measured to the nearest micrometer for three stomata per leaf at 500 \times power using a calibrated ocular scale.

Wood density was used as a proxy for xylem anatomy, because wood will be densest in species with small vessel diameters and/or thick vessel walls. These traits enhance mechanical strength (Wagner et al., 1998) and are associated with reduced xylem vulnerability to cavitation under water stress (Davis et al., 1999; Hacke et al., 2001). Bark and pith were removed from short lengths of dried stem in their first year of growth. The remaining tissue (primarily xylem) was weighed and its volume measured by displacement of 70% isopropyl alcohol in a closed-tipped 2-mL pipette.

Statistical analysis—Allometric relationships between stem size (tip area and basal area) and each of the leaf size variables (individual and total area and mass) and the load parameter were derived from reduced major axis (= standard major axis) Model II regression of log-transformed variables. Model II is less biased than Model I regression in its estimate of the functional relationship between two variables that are both subject to error (Sokal and Rohlf, 1995). The linear form of the standard allometric equation, $\log(Y) = \log(a) + \beta \times \log(X)$, provides a slope and an intercept that can be treated statistically. Allometric equations and the confidence intervals around their slopes and intercepts were calculated using SAS version 8.2 (SAS Institute, Cary, North Carolina, USA) and equations from Sokal and Rohlf (1995). Hydraulic models predict a slope of 1 (isometry) for the relationship between total leaf area on a shoot and sapwood cross-sectional area, assuming that leaf and stem conductivity are invariant with size (Zimmermann, 1983; Niklas, 1994; Farnsworth and Van Gardingen, 1995; Brouat et al., 1998). Bio-mechanical principles predict a slope of 2 for the L_p vs. stem cross-sectional area relationship (Niklas, 1990; Farnsworth and Van Gardingen, 1995).

Homogeneity of allometric slopes was tested following the methods of Warton and Weber (2002). Where slopes are homogeneous it is possible to characterize allometry as an elevation or lateral shift, i.e., the predicted value of a leaf-size variable at a common stem size. Comparing predicted values at the grand mean stem cross-sectional area, rather than at the y intercept, minimizes their standard errors, which increase with distance from their species means (Sokal and Rohlf, 1995). For each species, predicted values of total leaf area and total leaf mass per shoot were calculated at the grand mean stem size of all species. These values, the “adjusted leaf area” and “adjusted leaf mass” are measures of the amount of leaf area or mass supported at a common stem cross-sectional area. Species-adjusted leaf area and mass were compared across habitats using Wilcoxon signed ranks tests (the univariate procedure in SAS). Confidence intervals were used to test for significant differences between paired species (Sokal and Rohlf, 1995). Analysis of variance was used to test whether phylogenetic relatedness explained species variation in allometry. The main effects in this analysis were genus and species pair nested within genus. Because of their prominence in the literature, mean ratios of total leaf area to stem area (inverse of the Huber value) were also calculated for each species for comparison with adjusted leaf area.

Other anatomical and morphological traits that may influence water use were compared between habitats and among species pairs using a general linear model (the GLM procedure in SAS). Where significant interactions were found between habitat and species pair, the effect of habitat was tested for each trait pair separately, and a sequential Bonferroni correction was applied to the significance values (Rice, 1989). Species means of leaf size var-

TABLE 1. Traits used in the pairwise correlations. A subset of these traits was also compared between habitats and among species pairs.

Trait	Units
Stem cross-sectional area	
Base	mm ²
Tip	mm ²
Leaf size	
Mean leaf area	cm ²
Total shoot leaf area	cm ²
Mean leaf mass	g
Total shoot leaf mass	g
Adjusted leaf area	cm ²
Number of leaves	no.
Specific leaf area (SLA)	cm ² /g
Water potential (Ψ)	
Predawn	MPa
Midday	MPa
Soil-leaf gradient (Ψ _{predawn} - Ψ _{midday})	MPa
Wood density	
	g/cm ³
Hydraulic conductivity	
Leaf-specific (K _l)	g · s ⁻¹ · m ⁻¹ · kPa ⁻¹
Stem-specific (K _s)	g · s ⁻¹ · m ⁻¹ · kPa ⁻¹
Leaf-specific whole-plant (G _l)	mol · m ⁻² · s ⁻¹ · MPa ⁻¹
Photosynthesis (A)	
Transpiration (E)	μmol · m ⁻² · s ⁻¹
Water use efficiency (WUE, A/E)	μmol/mol
Stomatal aperture length	μm
Stomatal density	no./cm ²

ables were also compared between habitats using a Wilcoxon signed ranks test. Although this test is much less powerful than GLM, it permitted comparisons with the analyses of adjusted leaf area and mass.

Pairwise correlations among species means and independent contrasts were calculated for 21 traits (Table 1). Phylogenetic independent contrasts were calculated for all traits as described by Garland et al. (1992) using the program CACTUS (Schwilk, 2000; Schwilk and Ackerly, 2001). Contrasts were calculated between species in a pair and between pairs within a genus, but not between genera, due to the relatively large evolutionary distance between them. Contrast calculations were based on equal branch lengths, which is a robust approximation when lengths are unknown (Ackerly, 2000). For each correlation, phylogenies were pruned to include only species with data for both traits. This procedure limited the number of contrasts available for generating correlations with some traits, and to accommodate the resulting low power, alpha levels were adjusted to 0.1 for these cases. All leaf and stem size variables and both leaf- and stem-specific hydraulic conductivities were log-transformed.

RESULTS

Allometry—Allometric slopes for each of the four leaf size variables regressed against stem size were homogeneous among species (Table 2). Individual leaf area and mass varied isometrically with stem cross-sectional area, having a common slope of 1.12 and 1.00, respectively. Total shoot leaf area and mass, however, increased disproportionately with stem cross-sectional area, with common slopes near 1.5. For the regression of total shoot leaf area on stem cross-sectional area, slopes of five species differed from one (*P* < 0.05); for total leaf mass, seven species differed from one. Allometric slopes for the load parameter vs. stem cross-sectional area were also homogeneous among species, with a common slope of 2.65, greater than the predicted slope of 2. Within species, the re-

TABLE 2. Slopes and associated 95% confidence intervals, R², and significance of the Model II regression of leaf size variables and load parameter on stem cross-sectional area for 12 species representing three genera (*Arctostaphylos*, *Baccharis*, and *Ceanothus*). Adjusted leaf area and mass with 95% confidence intervals are also shown. Common slopes for each regression are reported along with the probability that the slopes are homogeneous among species. * *P* < 0.05, ** *P* < 0.01, *** *P* < 0.005.

Species	N	log(Total shoot leaf area) vs. log(Stem cross-sectional area)			log(Total shoot leaf mass) vs. log(Stem cross-sectional area)			log(Load parameter) vs. log(Stem cross-sectional area)						
		Slope	95% CI	R ²	Adjusted leaf area	Adjusted leaf mass	Slope	95% CI	R ²					
<i>A. bakeri</i>	16	1.601	0.47	0.724***	1.411	0.10	1.905	0.45	0.815***	0.120	0.10	3.698	1.83	0.202*
<i>A. glauca</i>	11	1.483	0.75	0.505**	1.366	0.21	1.793	0.89	0.524**	-0.118	0.25	3.162	2.18	0.070
<i>A. morroensis</i>	21	1.470	0.42	0.634***	1.242	0.08	1.506	0.38	0.714***	-0.243	0.08	2.250	0.85	0.341***
<i>A. tomentosa</i>	13	1.470	0.65	0.511***	1.366	0.15	1.572	0.67	0.557***	-0.137	0.15	2.797	1.67	0.114
<i>B. malibuensis</i>	20	1.349	0.25	0.854***	1.015	0.06	1.422	0.22	0.901***	-0.438	0.05	2.331	0.75	0.588***
<i>B. plummerae</i>	30	1.346	0.37	0.468***	1.235	0.10	1.525	0.33	0.669***	-0.405	0.09	2.419	0.80	0.235***
<i>B. emoryi</i>	8	1.326	0.96	0.384	1.015	0.32	1.424	0.92	0.515*	-0.349	0.31	2.708	2.67	-0.140
<i>B. pitularis</i>	33	1.494	0.28	0.723***	1.206	0.10	1.497	0.28	0.730***	-0.324	0.10	1.964	0.70	0.036
<i>C. integriramus</i>	29	1.246	0.45	0.135*	1.206	0.10	1.276	0.41	0.330***	-0.425	0.09	3.716	1.47	-0.036
<i>C. thysiflorus</i>	22	1.750	0.67	0.298**	1.497	0.20	1.870	0.67	0.378***	-0.180	0.20	2.311	1.00	0.088
<i>C. fresnensis</i>	11	1.534	0.82	0.417*	0.501	0.15	1.497	0.75	0.492*	-0.893	0.14	3.670	2.20	0.300*
<i>C. prostratus</i>	8	1.665	0.67	0.812***	1.161	0.23	1.672	0.56	0.870***	-0.232	0.19	3.323	2.64	0.263
Common slope		1.461					1.535					2.650		
Probability of homogeneity		0.983					0.772					0.502		

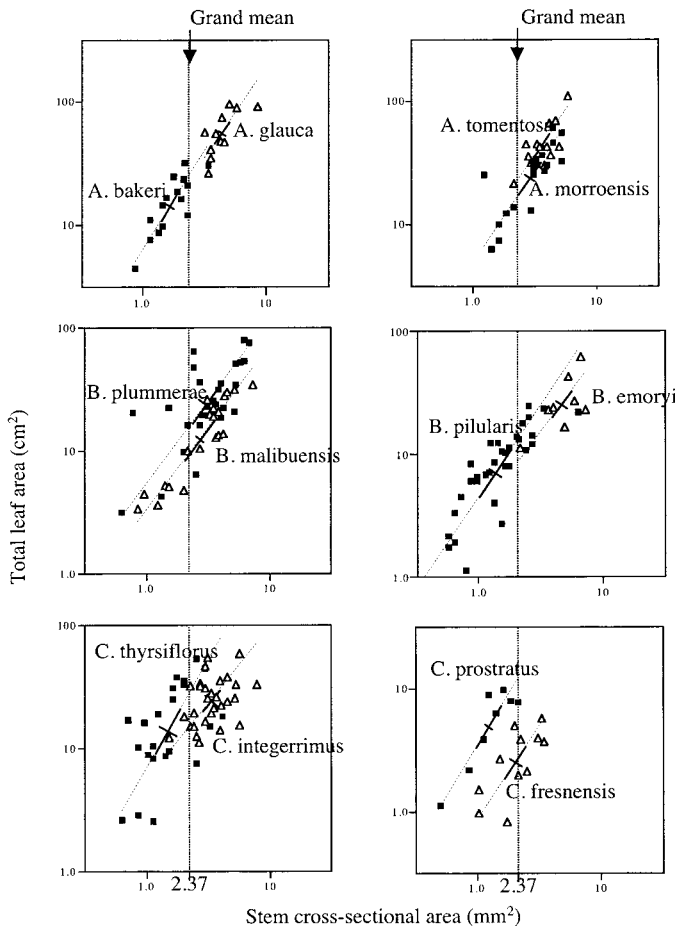


Fig. 2. Intraspecific allometric relationships for each of the 12 species examined. Mesic species are shown as black squares and xeric species as open triangles. Dashed lines represent Model 2 regressions, and solid lines show 95% confidence intervals for major and minor axes. The vertical hairline marks the grand mean stem size where adjusted leaf area was estimated. All y-axes span two units on a log scale.

relationship between L_p and stem size was always weaker than the corresponding relationship between leaf area and stem size (Table 2).

Adjusted leaf area and adjusted leaf mass, which were derived from allometric elevations, both varied 10-fold among species (Table 2 and Fig. 2). Overall, adjusted leaf area was significantly lower in the xeric-adapted species than in their mesic-adapted counterparts (five of six pairs; one-tailed Wilcoxon signed-ranks test, $P = 0.0469$). In pairwise comparisons, three of the six species pairs differed significantly in adjusted leaf area (one-tailed tests, $P < 0.05$; Fig. 3, left). Adjusted leaf mass also tended to be lower in xeric-adapted species, but the overall trend was less pronounced (four of six pairs, one-tailed Wilcoxon signed-rank test, $P = 0.0781$). The stronger effect of habitat on adjusted area than on adjusted mass was mediated by differences in specific leaf area (SLA, in square centimeter per gram). In four species pairs, SLA was significantly higher in the species with the greater adjusted leaf area (Table 3), which reduced the difference in adjusted leaf mass. The opposite pattern held for one species pair (*A. bakeri* vs. *A. glauca*), in which the higher SLA was combined with the lower adjusted leaf area in the xeric-adapted species, which magnified the habitat effect on adjusted leaf mass.

Mean leaf-area-to-stem-area ratio (the inverse of the Huber value) was highly correlated with adjusted leaf area across species (Pearson's $R = 0.835$). Unlike the adjusted leaf areas, however, leaf-area-to-stem-area ratios showed no association with habitat (Fig. 3, center). In three of the species pairs, the leaf-to-stem ratio varied as predicted and was higher in the mesic-adapted species; however the other three species pairs either varied in the opposite direction (*A. morroensis* vs. *A. tomentosus*; *A. bakeri* vs. *A. glauca*) or were virtually identical in leaf-to-stem ratio (*Baccharis pilularis* vs. *B. emoryi*). The contrast in leaf-to-stem ratio differed most dramatically from the allometric contrast in those species pairs that also differed in overall shoot size. When the species with the lower adjusted leaf area also had smaller stems and less shoot leaf area, then the two measurements did not differ qualitatively (*B. plummerae*–*B. malibuensis*; both *Ceanothus* pairs); however, when the opposite was true, the habitat contrast was eliminated (*B. pilularis*–*B. emoryi*) or reversed (*A. morroensis*–*A. tomentosus*). Leaf-to-stem ratios had large confidence intervals, and none of the observed pairwise differences was statistically significant.

There was no significant effect of genus or species pair within a genus on either adjusted leaf area or adjusted leaf mass, indicating that closely related species were not more likely than distant relatives to have similar allometric relationships. However, four of the five species with the greatest adjusted leaf area belong to the genus *Arctostaphylos*.

Habitat variation in morphological and anatomical traits—In contrast to the allometric differences, there was no consistent relationship between water availability and the absolute values of any of the leaf size variables (Wilcoxon signed ranks test, $P > 0.469$; Fig. 3, right). In four of the six species pairs, the greater individual leaf size (both area and mass) was found in the species from the more xeric habitat, a result that deviates from the typical pattern of reduced leaf size in dry habitats (Poole and Miller, 1981; Fonseca et al., 2000).

In general, the main effect of habitat on several other stem and leaf traits was weak or inconsistent across species pairs (Table 3), but several traits differed significantly among species pairs. As predicted, specific leaf area (SLA, in square centimeters per gram) was greater in mesic than in xeric habitats for four of the six species pairs; but the pattern was reversed in both *Arctostaphylos* pairs, generating a significant interaction between habitat and species pair. Stomatal aperture length differed among species pairs but was unrelated to water availability. The effect of habitat on stomatal density was not consistent. Of the two significant pairwise comparisons, the mesic-adapted species had the higher density in one of the pairs (*C. thyrsoflorus* > *C. integerrimus*), and the opposite pattern held for the other (*B. emoryi* > *B. pilularis*). Wood density tended to be higher where water availability was low, but this trend was not quite significant. In pairwise comparisons, the xeric-adapted species *B. malibuensis* was found to have denser wood than its mesic counterpart *B. plummerae* subsp. *plummerae*. Reduced conductivity resulting from denser wood could be offset by an increase in the proportion of conducting tissue in the stem cross-sectional area; however, the effect of habitat on proportion of xylem was inconsistent. In half of the pairs, percentage xylem was greater in species from xeric habitats, but this difference was significant for two of them (*B. emoryi* > *B. pilularis*, *C. fresnensis* > *C. prostratus*), resulting in a significant overall habitat difference. If the species mean

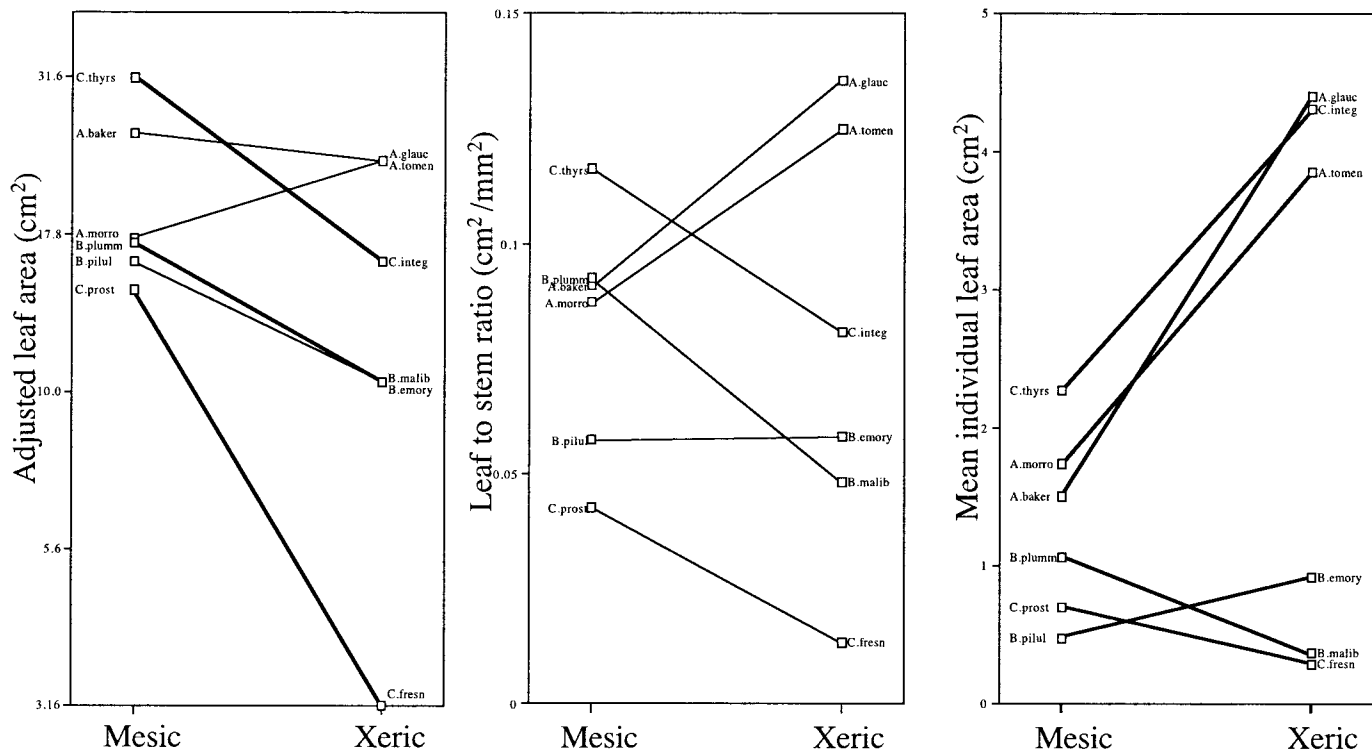


Fig. 3. Habitat contrasts for adjusted leaf area (left), leaf-to-stem area ratio (center), and mean individual leaf area (right). Lines connect paired species. Thick lines connect species that differ significantly ($P < 0.05$).

values for percentage xylem are used to convert stem-leaf allometry to sapwood-leaf allometry, the overall effect is to exaggerate the observed differences between habitats.

In four of five measured species pairs, stem-specific hydraulic conductivity (K_s) was lower in the more xeric-adapted species of the pair (Fig. 4). In three of five pairs, leaf area-specific hydraulic conductivity (K_l) was greater in the xeric than in the mesic-adapted species. Neither of these trends was significant, however. In pairwise comparisons, leaf- and stem-specific conductivities differed significantly in one pair, and the difference was in the direction opposite to our predictions: the mesic species, *A. morroensis*, showed lower stem-specific and higher leaf-specific conductivities than the xeric-adapted species, *A. tomentosa* (contrast A2 in Fig. 4).

TABLE 3. Significance values from analyses of variance testing the effects of habitat and taxonomic group (species pair) on a series of structural traits. M, mesic; X, xeric.

Structural trait	Habitat	Species pair	Habitat × species pair
Specific leaf area	0.0060 M>X	0.0001	0.0001
Stomatal aperture	0.5201	0.0001	0.5021
Stomatal density	0.0024 M>X	0.0001	0.0001
Wood density	0.0648	0.1824	0.0483
Sapwood/stem cross-sectional area	0.0021 X>M	0.0732	0.0001
Hydraulic conductivity			
Stem-specific	0.4446	0.0267	0.0179
Xylem-specific	0.9025	0.7059	0.3550
Leaf-specific	0.2247	0.0001	0.0131
G_t	0.8231	0.0001	0.9154

Correlations among traits—Significant correlations among independent contrasts were found for 20 traits (Fig. 5; for all pairwise coefficients see table available as Supplementary Data accompanying the online version of this article). Because this data set is phylogenetically clustered, it is particularly important to examine trait correlations using independent contrasts, which provide estimates of correlated evolutionary change. The six contrasts between paired species involve planned habitat contrasts, whereas the three contrasts between congeneric pairs do not. Except where stated, correlations re-

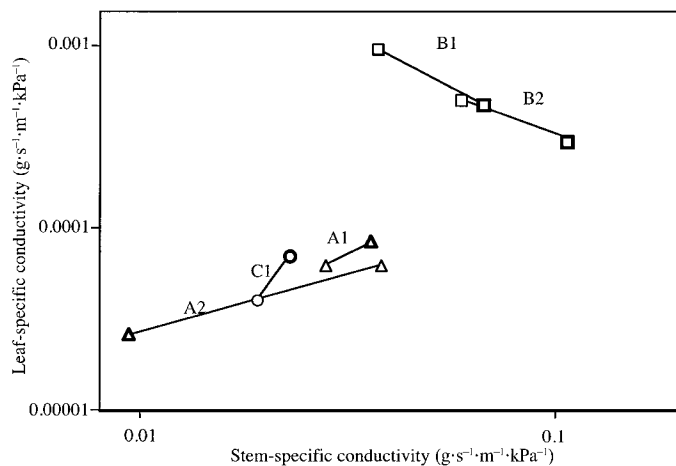


Fig. 4. Species mean leaf-specific conductivity vs. stem-specific conductivity. Species pairs are labeled as in Fig. 1. Symbols indicate genera: *Arc-tostaphylos*, Δ ; *Baccharis*, \square ; *Ceanothus*, \circ . Bold symbols indicate the mesic-adapted species of each pair.

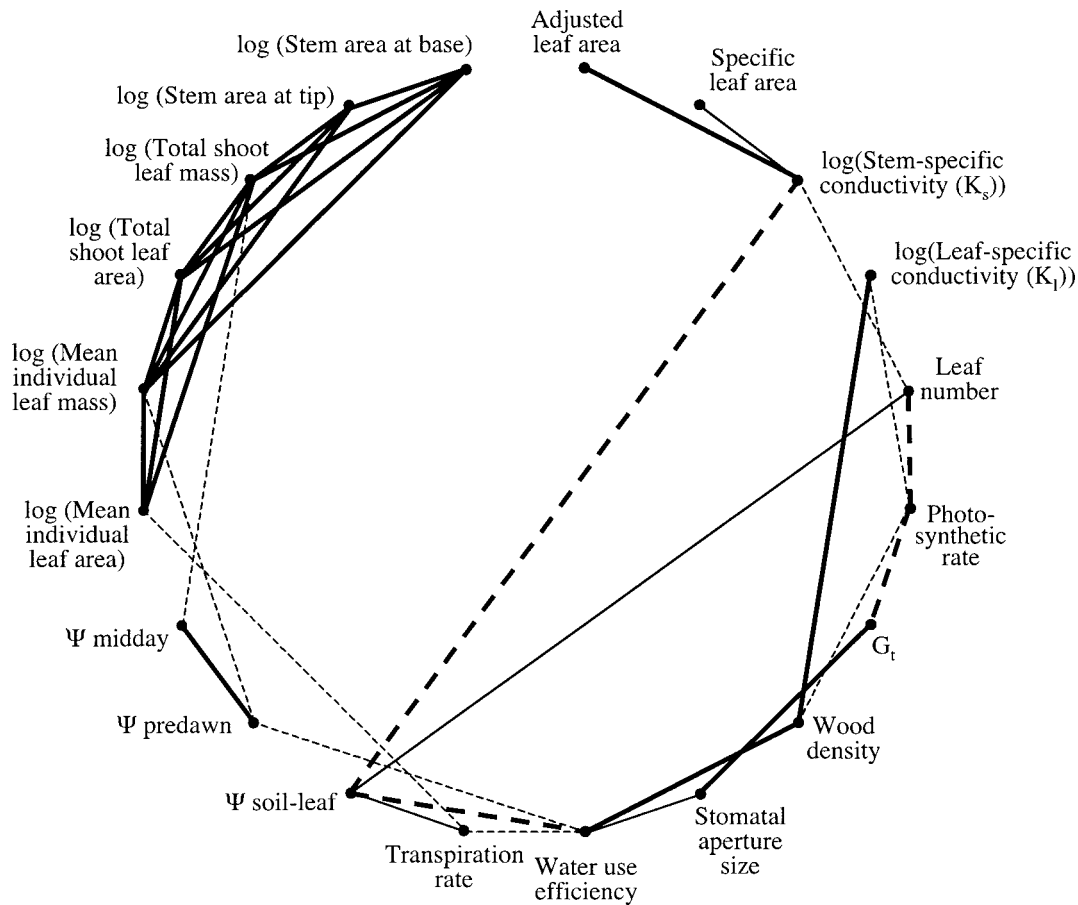


Fig. 5. Pairwise contrast correlations for 20 structural and physiological traits. Solid lines indicate positive correlations and dashed lines indicate negative correlations between traits. Thick lines, $P < 0.05$; thin lines, $P < 0.1$.

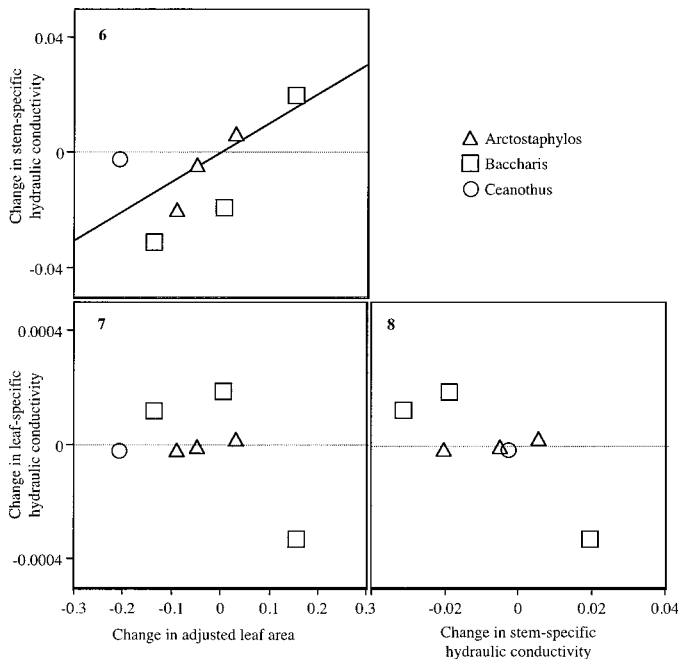
ported below are evolutionary correlations based on independent contrasts; across-species correlations are provided online as Supplementary Data.

Strong correlations were found among a suite of positively coevolving stem and leaf size traits, including stem cross-sectional area at the base and the tip of the shoot, individual leaf area and mass, and total leaf area and mass on a shoot. All but two of the 15 pairwise correlations among these traits were significant (Pearson's $R \geq 0.668$, $P < 0.05$). Adjusted leaf area, however, was notably absent from this suite of correlated traits. Thus our data suggest that the absolute values of total shoot leaf area and stem cross-sectional area have been tightly coupled evolutionarily, but the allometric relationship between them (characterized as adjusted leaf area) appears to have evolved independently of both traits.

A weaker set of correlations formed a loose-knit suite of physiological and structural traits related to hydraulic architecture (Fig. 5, right side). Increased stem hydraulic capacity (K_s) was associated with greater adjusted leaf area ($R = 0.772$, $df = 6$, $P = 0.025$), but not with increased potential rate of hydraulic supply per unit leaf area (K_l , leaf-specific conductivity) (Figs. 6 and 8). Consequently, leaf-specific conductivity either declined (as in *Baccharis*) or remained virtually unchanged with adjusted leaf area (Fig. 7). Both measures of area-based leaf water supply, leaf-specific conductivity (K_l) and leaf-specific whole-plant conductance (G_t), were negatively correlated with photosynthetic rate ($R = -0.758$ and

-0.903 , respectively). K_l and G_t measure slightly different properties of the hydraulic pathway, and they were not correlated with one another in the contrast data ($R = 0.657$, $P = 0.228$). G_t was also correlated with larger stomatal apertures ($R = 0.986$). Despite its purported effect on hydraulic efficiency, increased wood density was not associated with decreased stem-specific conductivity ($R = -0.128$), however it was correlated with increased leaf-specific conductivity (K_l , $R = 0.657$). Changes in wood density and photosynthetic rate were negatively correlated; but increased wood density was also associated with greater water use efficiency. Stomatal density showed no significant relationships with any other trait, but stomatal aperture length was correlated with leaf-specific whole-plant conductance (G_t) and water use efficiency. Specific leaf area, which typically increases with water and nutrient availability, showed no pattern of correlated evolution with any other traits except stem-specific conductivity ($R = 0.632$, $P = 0.093$).

The strength and sign of pairwise correlations were generally similar between the interspecific and the independent contrast data, and the correlation coefficients for trait pairs were themselves correlated (Pearson's $R = 0.707$). One important difference between them was that adjusted leaf area showed a much stronger association with the suite of leaf size traits in the interspecific than in the contrast analysis. This result reflects the fact that the three species pairs with the largest leaves



Figs. 6–8. Correlations between contrasts in conductivity and adjusted leaf area. **6.** Evolutionary change in stem-specific hydraulic conductivity vs. the change in adjusted leaf area. **7.** Evolutionary change in leaf-specific hydraulic conductivity vs. the change in adjusted leaf area. **8.** Evolutionary changes in leaf- vs. stem-specific hydraulic conductivity. Symbols show contrasts within genera: *Arctostaphylos*, Δ ; *Baccharis*, \square ; *Ceanothus*, \circ .

also had the highest adjusted leaf areas and that contrasts between pairs broke this correlation (Fig. 3).

Another important difference was that the evolutionary independence of leaf- and stem-specific hydraulic conductivity seen in the contrast data was not apparent when species values were considered. Instead, there was a significant positive relationship between them ($R = 0.673$, $P = 0.033$), indicating that species varied in their overall rate of water transport. Leaf-specific conductivity was negatively correlated with average individual leaf area and mass. The interspecific and contrast results also differed with regard to the relationship between hydraulics and allometry. Across species, adjusted leaf area was not significantly correlated with K_s but covaried negatively with K_1 ($R = -0.746$, $P = 0.013$).

DISCUSSION

Our results show that a taxonomically diverse set of native California shrub species has undergone repeated independent evolutionary divergence in shoot allometry associated with habitat water availability. Among these 12 species, evolutionary changes in the relationship between leaf area and stem cross-sectional area have been largely independent of changes in either individual leaf size per se or the allocation of leaf biomass to photosynthetic and evaporative area (SLA). Rather, divergence in stem-leaf allometry was shown to be most closely associated with the evolution of stem hydraulic capacity.

Evolution of allometry—The results of our study are consistent with the overall trend that emerges from the literature showing an association between habitat and the relationship between stem size and total leaf area. Moreover, by using a phylogenetic comparative approach, we were able to find ev-

idence that stem-leaf allometry has evolved repeatedly and independently in the direction predicted on functional grounds. In this data set, a pattern would not have emerged from a cross-species comparison of adjusted leaf areas in mesic vs. xeric habitats. Habitat was defined relative to each pair, and there was overlap between the habitat groups in both climate variables and measured water potentials (Fig. 1). More importantly, adjusted leaf area varied widely among species within a habitat group and was related to overall shoot size. Thus the pattern emerged only when phylogenetically paired species were contrasted (Ackerly, 1999).

In five of six species pairs, the more xeric-adapted species supported less total leaf area for the same stem cross-sectional area than its mesic-adapted counterpart. The one exception to this pattern was the contrast between *Arctostaphylos tomentosa* and *A. morroensis*. In light of this result, it is interesting that the classification of these two species as mesic- or xeric-adapted was the least straightforward of the pairs. Although *A. tomentosa* had much more negative predawn water potentials than *A. morroensis*, both the mean and the maximum vapor pressure deficit estimated for the collection sites was higher for *A. morroensis* (mean 1396.3, maximum 3631.8; vs. mean 1185.2, maximum 3353.2).

Quantifying stem-leaf relationships—The two most common methods for quantifying stem-leaf relationships yielded different results in our study. In contrast to the allometric results discussed above, the leaf-to-stem area ratio (inverse Huber value) was not related to habitat. Moreover, the ratios between untransformed values increased with shoot size, as revealed by the allometric slopes of the log-transformed variables, which were greater than one for each of the species. The mean ratio for a species was therefore sensitive to the actual shoot size distribution of the species and also more sensitive than allometry to sampling distribution. The size dependence of the ratio probably also contributed to its variance, and the 95% confidence intervals were much greater relative to the species means for leaf-to-stem ratios than for the adjusted leaf areas (data not shown). This discrepancy between results derived from ratios and results based on allometric analysis demonstrates the importance of retaining as much information as practical about the size distributions of variables, both individually and relative to one another.

Another important aspect of the leaf-stem relationship is its slope, which describes the way two variables scale relative to one another (Niklas, 1994). The 12 species measured in this study were remarkably uniform in the way total shoot leaf area scaled with stem cross-sectional area; intraspecific slopes of the log-linear relationships were all approximately 1.5. By comparison, most other studies report shallower intraspecific slopes of around one (e.g., Whitehead et al., 1984; Bond and Midgley, 1988; Callaway et al., 1994; Maherali et al., 1997; Brouat and McKey, 2001), which is the relationship predicted by hydraulic models that assume that area-based hydraulic capacity and demand are size-independent (Shinozaki et al., 1964; Zimmermann, 1983; Niklas, 1994; Farnsworth and VanGardingen, 1995; Brouat et al., 1998).

The departure of our observed slopes from the predicted value has several potential explanations. First, the observed allometry could more strongly reflect biomechanical than hydraulic demands; however, this explanation is undermined by the extremely weak relationships between stem size and estimated biomechanical load (L_p) found for all of the species.

Second, Model I regression (used in most studies) typically estimates shallower slopes than Model II (used here), but the models converge as the underlying relationship gets stronger (R^2 approaches 1). Given that the leaf–stem size relationship is generally very strong, the bias introduced by Model I regression in other studies is likely to be small, and in any case it cannot explain the departure of Model II slopes from isometry. Finally, our measure of stem cross-sectional area included bark and pith, rather than just sapwood area, which could violate the hydraulic model's assumption of size-independent hydraulic properties. The relationship between leaf area and sapwood area in subsamples of stems from each species was not consistently more shallow than the leaf–stem or sapwood–stem relationships. There was also no evidence that the proportion of the stem occupied by sapwood increased as a function of stem cross-sectional area in these first-year shoots. Even when sapwood area does not vary with stem size, it is still possible for stem-specific hydraulic conductivity to increase via changes in xylem structure (Maherali et al., 1997; K. A. Preston, unpublished data). Addressing this possibility would require hydraulic data from many more stems over a greater range of sizes than were measured here.

Allometry and the evolution of individual leaf size—Although there is ample evidence that individual leaf size declines as soil water becomes more limited (e.g., Poole and Miller, 1981; Cunningham et al., 1999; Fonseca et al., 2000), previous studies have not asked whether observed differences in total leaf area relative to stem size are also associated with differences in mean individual leaf size. Such a result would raise the possibility that selection was acting primarily on leaf size with indirect effects on shoot allometry. In the species examined here, evolutionary changes in total leaf area on a shoot were very strongly correlated with changes in mean leaf area and virtually independent of changes in leaf number. As a result, changes in absolute total leaf area occurred primarily through changes in individual leaf size. A recent study found a similar pattern among a large set of woody species (Westoby and Wright, 2003). Nevertheless, when shoot leaf area was considered relative to stem cross-sectional area (i.e., as adjusted leaf area), it did not show a strong pattern of correlated evolution with mean individual leaf area, and the association between adjusted leaf area and total shoot leaf area was even weaker. These results demonstrate that the evolution of shoot allometry in these species has not been a simple consequence of changes in mean leaf size. Rather, it appears that the allometric relationship has itself been a target of selection.

Another mechanism by which shifts in leaf area relative to stem size could occur is through changes in the leaf area to mass ratio (SLA), which generally declines with reduced soil moisture (Cunningham et al., 1999; Fonseca et al., 2000). For example, if proportional allocation to leaf and stem biomass within a shoot were developmentally constrained, then an increase in SLA could increase total leaf area within these constraints. If so, paired species should differ in adjusted leaf area but not in adjusted leaf mass, and evolutionary increases in adjusted leaf area should covary tightly with increases in SLA. In our study, SLA differed between habitats in four of six species pairs, but SLA and adjusted leaf area showed no pattern of correlated evolutionary change.

Habitat and pairwise trait correlations—One objective of this study was to relate evolutionary changes in allometry with

variation in several structural and physiological traits hypothesized to influence shoot water relations. We approached this problem in two ways. Individual structural traits were compared between habitats to test for repeated divergence in response to water availability mirroring the observed allometric shifts. Pairwise correlations were also calculated for a larger set of physiological and structural traits, including adjusted leaf area, that potentially interact in their effects on hydraulic function. These correlations were calculated for species means as a measure of overall association and for phylogenetic independent contrasts to test for patterns of correlated evolutionary change.

In general, structural traits showed weak or inconsistent relationships with habitat. Stronger relationships were found with taxonomic pair, and the effect of habitat often differed among the pairs. When traits did show a trend with habitat, it was usually in the expected direction. Specific leaf area was generally higher in mesic than xeric habitats, whereas wood density and the proportion of the stem in sapwood tended to be higher in xeric than mesic habitats.

In the contrast analysis, the only significant pairwise trait relationship with adjusted leaf area was a positive correlation with stem-specific hydraulic conductivity. Many other studies have shown that variation in the stem–leaf relationship is similarly associated with variation in hydraulic properties (Whitehead et al., 1984; Margolis et al., 1995; Maherali et al., 1997; Villar-Salvador et al., 1997; Tausend et al., 2000; Vander Willigen et al., 2000; Cavender-Bares and Holbrook, 2001). To our knowledge, however, ours is the first study using phylogenetically structured sampling to demonstrate correlated evolution of stem–leaf allometry and stem hydraulic properties across a diverse set of angiosperms.

Correlated evolution of stem hydraulic capacity with the amount of leaf area supported will necessarily have an effect on leaf-specific potential hydraulic supply (K_l). In most species pairs examined, changes in stem hydraulics and shoot morphology compensated one another, and as a result, leaf-specific conductivity (K_l) showed essentially no change with habitat divergence (Fig. 8). In both *Baccharis* species pairs (and the contrast between them) evolutionary reductions in stem-specific conductivity have been relatively greater than the reductions in leaf area for a given stem size. Consequently, leaf-specific conductivity appears to have increased in the two *Baccharis* species adapted to dry habitats (Fig. 4, and note negative correlation for *Baccharis* in Fig. 8). It is not clear why the *Baccharis* species alone should show this pattern, as they do not experience particularly large differences in VPD compared to other species pairs in the study. Other studies that have linked hydraulic parameters to habitat-based differences in allometry or leaf-stem ratios have also found either increases in potential hydraulic supply to leaves (Maherali et al., 1997; Tausend et al., 2000) or no difference, due to compensatory changes in stem hydraulic capacity when moisture is limited or evaporative demand is high (Whitehead et al., 1984; Margolis et al., 1995; Villar-Salvador et al., 1997; Vander Willigen et al., 2000; Cavender-Bares and Holbrook, 2001).

Allometry and phenotypic integration—One of the main results presented here also bears on recent work concerning phenotypic integration. In most studies, traits are considered phenotypically integrated if they covary, and evolutionary changes in integration appear as changes in the presence (and sometimes strength or sign) of trait correlations (Callahan and

Waller, 2000; Murren et al., 2002). A number of studies have demonstrated decoupling of the evolution of trait integration from the evolution of individual trait means (e.g., Murren et al., 2002). Here we also report evolutionary independence of trait relationships (adjusted leaf area) from trait means (total leaf area and stem cross-sectional area). In our data, however, the functionally relevant difference among species involved the form of this relationship, not its strength. Specifically, the elevation (adjusted leaf area) varied between habitats, which had important consequences for shoot water relations and was associated with changes in stem hydraulic properties. We argue, therefore, that the functional and evolutionary significance of phenotypic integration derives from both the strength and the form (i.e., slope, elevation) of trait relationships. Allometric analysis promises to be a powerful method for investigating such patterns of multivariate evolution and phenotypic integration, especially within an ecological and phylogenetic framework (e.g., Preston and Ackerly, 2003).

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