Ecology, 87(2), 2006, pp. 483–491

LEAF STRUCTURAL DIVERSITY IS RELATED TO HYDRAULIC CAPACITY IN TROPICAL RAIN FOREST TREES

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Abstract. The hydraulic resistance of the leaf (R_l) is a major bottleneck in the whole plant water transport pathway and may thus be linked with the enormous variation in leaf structure and function among tropical rain forest trees. A previous study found that R_l varied by an order of magnitude across 10 tree species of Panamanian tropical lowland rain forest. Here, correlations were tested between R_l and 24 traits relating to leaf venation and mesophyll structure, and to gross leaf form. Across species, R_l was related to both venation architecture and mesophyll structure. R_l was positively related to the theoretical axial resistivity of the midrib, determined from xylem conduit numbers and dimensions, and R_l was negatively related to venation density in nine of 10 species. R_l was also negatively related to both palisade mesophyll thickness and to the ratio of palisade to spongy mesophyll. By contrast, numerous leaf traits were independent of R_l, including area, shape, thickness, and density, demonstrating that leaves can be diverse in gross structure without intrinsic trade-offs in hydraulic capacity. Variation in both R_l-linked and R_l-independent traits related strongly to regeneration irradiance, indicating the potential importance of both types of traits in establishment ecology.

Key words: Barro Colorado Island Nature Monument (BCI); biological networks; high pressure flow meter (HPFM); leaf hydraulics; Panama; rain forest trees; shade tolerance; vasculature.

INTRODUCTION

Tree leaves vary enormously in structure and composition, contributing to functional differences that drive species coexistence (Abrams et al. 1994, Thomas and Bazzaz 1999). Diversity in leaf traits arises from phylogeny and adaptation, but it is constrained by trait correlations (Reich et al. 2003, Sack et al. 2003, Niinemets and Sack 2005). For example, leaf traits related to carbon economy are strongly coordinated within and across biomes; leaf mass per area (LMA) is positively related to leaf life span and negatively related to nitrogen and photosynthetic rate per leaf mass (e.g., Reich et al. 2003, Wright et al. 2004). Analogous linkages are increasingly reported among key traits and the leaf hydraulic resistance (R_l = the water potential driving force required for a given flow rate from petiole to evaporation sites; Sack and Tyree 2005)

R_l varies strongly across species, ranging by an order of magnitude across 10 coexisting tropical rain forest tree species on Barro Colorado Island, Panama (BCI; 9°9′ N, 79°51′ W; Leigh 1999, Sack et al. 2005). R_l constrains whole-plant hydraulic resistance (R_{pl}), which defines the capacity of a plant for water use. In a given microclimate and soil water supply, R_{pl} determines the leaf water potential at a given transpiration rate (Tyree and Zimmermann 2002). R_{pl} thus constrains how high stomatal conductance may be before desiccating the leaf, and correlates negatively with maximum stomatal conductance across species (e.g., Nardini and Salleo 2000). R_l is an important component, representing on average ~30% of R_{pl} in published data for a range of life forms (Sack et al. 2003). For given species during peak transpiration, the proportion can be much higher; R_l accounted for over 80% of R_{pl} in given species (Nardini and Salleo 2000, Brodribb et al. 2002). R_l is thus an important determinant of plant function; in tropical and temperate species, R_l correlated negatively with indices of stomatal pore area per leaf area, and with maximum rates of gas exchange (Aasamaa et al. 2001, Brodribb et al. 2005, Sack et al. 2005). In two studies, R_l also related to leaf form. For sets of temperate species, R_l correlated negatively with leaf thickness and lobiness, though it was independent of LMA (Sisó et al. 2001, Sack et al. 2003).

The diversity of leaf venation and mesophyll structure might also be associated with R_l. In recent studies, R_l was partitioned into hydraulic resistances of the xylem (R_x), including petiole and major and minor veins; and the pathways across the bundle sheath and across mesophyll cells to the airspaces (e.g., Cochard et al. 2004*b*, Gascó et al. 2004, Sack et al. 2004, 2005). A substantial proportion of R_l occurs within the leaf xylem; on average 64% for 10 tree species on BCI (Sack et al. 2005). This suggests that species variation in venation architecture would have consequences for R_l. Mesophyll structure may also be coordinated with venation and hydraulic properties. In several species sets

Manuscript received 5 May 2005; revised 1 August 2005; accepted 5 August 2005. Corresponding Editor: T. E. Dawson.

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vein density correlated positively with a thick palisade layer, and a high ratio of palisade to spongy mesophyll (Wylie 1946).

We focused on 10 diverse tree species on BCI, and tested for correlations of R_l with 24 traits relating to venation and mesophyll structure and gross leaf form. We hypothesized that R_x and R_l are dependent on xylem structure, and would be negatively related to numbers and dimensions of xylem conduits, and venation density. We also hypothesized that a palisade-rich mesophyll, associated with high area-based gas exchange rates (Vogelmann and Martin 1993, Smith et al. 1997, Kenzo et al. 2004), would be negatively related to R_l. Additionally, we tested for association of these traits with regeneration irradiance. Study species were chosen that either persist as juveniles for years in understory ($<$1–2% daylight; henceforth ‘‘shade-establishing’’) or establish typically in gaps or clearings (‘‘sun-establishing’’; Condit et al. 1996; R. Condit, *personal communication*; Fig. 1; Appendix A). Sun-establishing species have lower R_l than shade-establishing species (Sack et al. 2005) and thus may differ correspondingly in leaf structure.

MATERIALS AND METHODS

Species and sampling method

The 10 study species varied strongly in leaf size, shape, texture, and venation architecture (Fig. 1; see Plate 1). Leaves of the most recent mature cohort were sampled January–April 2003, from fully exposed branches $>$3 m high for three reproductively mature trees 5–10 m tall (see Sack et al. 2005).

Measurements of leaf hydraulic architecture

Leaf hydraulic resistance (R_l) was measured with the high pressure flow meter (Tyree et al. 1993, Sack et al. 2002; for full details, see Sack et al. 2005). Measured leaves were illuminated with $>$1200 μmol·m^{-2}·s^{-1} photosynthetically active radiation (LI-250 light meter; LI-COR Biosciences, Lincoln, Nebraska, USA), and maintained at approximately 25°C with a water bath. The components of R_l were determined by measuring resistance before and after applying vein cutting treatments, allowing measurement of the resistance of the leaf xylem (R_x) and also the resistance downstream of the minor veins, the extra-xylem resistance (R_{ox}; Gascó et al. 2004, Sack et al. 2004, 2005, Nardini et al. 2005*a*,*b*). R_x was partitioned into the resistances of petiole (R_p), major veins (R_{majv}), and minor veins (R_{minv}). Each of these hydraulic resistances was normalized by leaf area (units are MPa·mmol^{-1}·s·m^2).

Measurements of leaf structure and venation architecture

Gross leaf structure measurements were averaged for three to five leaves per tree. Measurements were made of area (LI-COR 3100C meter), volume (by immersion in a graduated cylinder), and dry mass, after drying at 70°C for $>$48 h. Lamina density was calculated as dry mass/volume. Scanned leaf images were analyzed (ImageJ; *available online*)[4] to determine shape indices: perimeter/area represents the relative amount of edge, and perimeter^2/area is a similar index independent of size (Sack et al. 2003).

We determined the vein order at which xylem vessels in the petiole ended. Air was pressurized to 100 kPa at the petiole, and veins were cut under water with a razor blade, beginning with minor veins and progressing back to the midrib. The vein orders at which air bubbled out had at least one vessel continuous from the petiole.

For determining venation traits, two leaves per tree were cleared (using the method of Berlyn and Miksche [1976]). Scanned images (Epson Perfection 3170; Epson, Long Beach, California, USA) were analyzed for the lengths of 1° and 2° veins (and 3° veins for *M. argentea*; ImageJ). The density of 3° veins (and 4° veins for *M. argentea*) was averaged for three approximately 2-cm^2 regions, centrally in the top, middle, and bottom thirds of the right side of the leaf. For *M. argentea*, measurements of 1° to 4° veins were made on uncleared leaves. The densities of higher order veins (including 2° and 3° veins for *C. longifolium*) were determined from cleared sections cut centrally on the right-hand side of each leaf; regions of approximately 2 mm^2 were imaged at 40× and analyzed using ImageJ.

Measurements of lamina and midrib cross-sectional anatomy

Anatomical measurements were made on cross-sections of midrib and adjacent lamina cut centrally on one leaf per tree (Leica CM 1850 cryostat; Leica, Bannockburn, Illinois, USA), imaged at 25–250×, and analyzed (ImageJ). Measurements were made of the thicknesses of the lamina, adaxial cuticle, epidermises, hypodermis, and palisade and spongy mesophyll. Minimum and maximum diameters were measured for each midrib xylem conduit and a theoretical midrib axial hydraulic resistivity (R^*_{mr}; units are MPa·mmol^{-1}·s·m) was calculated treating the conduits as ellipses, as

$$\left[\sum \frac{\pi a^3 b^3}{64\eta(a^2 + b^2)}\right]^{-1}$$

with a and b the long and short axes, and η the viscosity of water at 25°C (Lewis and Boose 1995, Cochard et al. 2004*b*), further normalizing by leaf area.

Statistics

Data for each trait were averaged for each tree (i.e., for overall $n = 3$ per species). Trait data were analyzed using a general linear model, with species nested within regeneration irradiance category (Minitab Release 14; Minitab, State College, Pennsylvania, USA). Bi-

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[4] ⟨http://rsb.info.nih.gov/ij/⟩

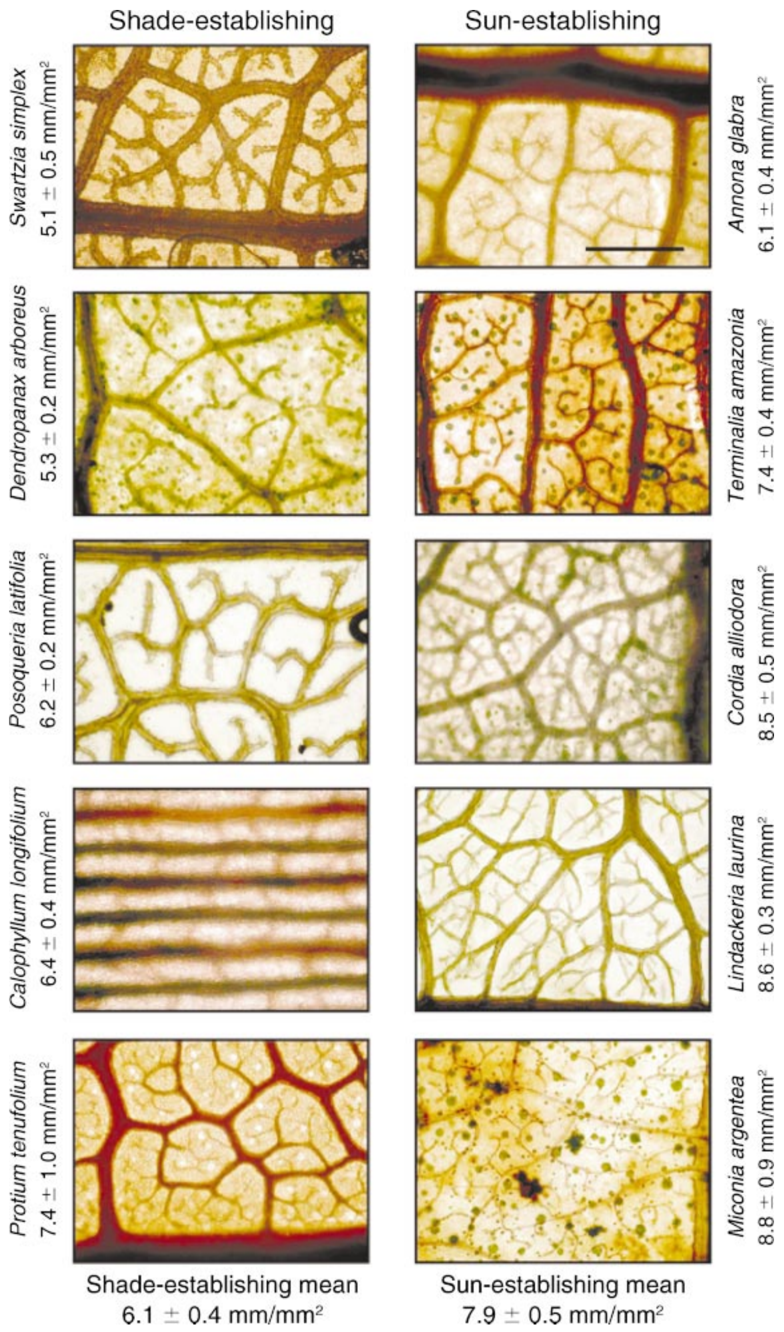

FIG. 1. High-order leaf venation architecture for tropical rain forest trees, ranked by total vein density (mean ± SE) for shade- and sun-establishing species. The largest vein shown is 2°, except for *D. arboreus*, *P. latifolia*, and *T. amazonia* (3°), and *M. argentea* (4°). The scale for all images is as shown for *A. glabra*, 0.5 mm.

variate relationships were assessed with parametric and Spearman rank correlation coefficients (r_p and r_S respectively), and lines fitted by model II regression (Sokal and Rohlf 1995, Sack et al. 2003). When relationships were apparently nonlinear, they were estimated as power laws by log transforming data for testing correlations or line fitting (Sokal and Rohlf 1995, Sack et al. 2003).

RESULTS

Variation in midrib xylem traits and relation to leaf hydraulic architecture

Species varied strongly in venation architecture (Tables A1 and A2, Figs. 1 and 2A–F). Species varied twofold in average midrib xylem conduit diameter, ninefold in the number of midrib conduits in cross sec-

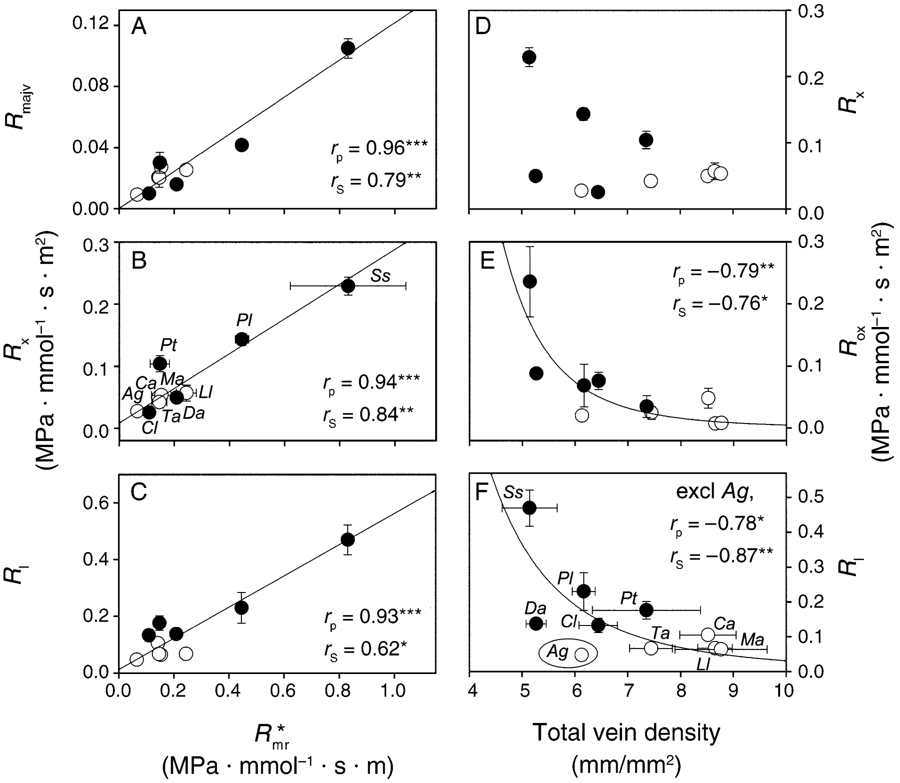

FIG. 2. The linkage of leaf hydraulic architecture with venation architecture for tropical rain forest tree species. Relationships of hydraulic resistances of (A) major veins, (B) leaf xylem, and (C) leaf with the midrib xylem axial resistivity, calculated from anatomical measurements, and relationships of hydraulic resistances of (D) leaf xylem, (E) tissue outside the xylem, and (F) leaf with total vein density. Open and solid circles indicate sun- and shade-establishing species respectively; species symbols are first letters of genus and species shown in Fig. 1. $R_l = 0.489\,R^*_{mr} + 0.0127$; $R_x = 0.248\,R^*_{mr} + 0.00842$; $R_{majv} = 0.108\,R^*_{mr} + 0.000269$. $R_{ox} = 1504$ vein density$^{-5.50}$; R_l (excluding outlier *A. glabra*) = 65.4 vein density$^{-3.21}$. Significance levels are: * $P \leq 0.05$; ** $P < 0.01$; *** $P < 0.001$.

tion, and 13-fold in R^*_{mr}. R^*_{mr} was significantly related across species to the mean conduit diameter ($r_S = -0.82$, $P = 0.004$; $r_p = -0.77$, $P = 0.009$), and not to number of conduits ($r_S = -0.22$, $P = 0.53$; $r_p = -0.27$, $P = 0.46$). Petiole xylem vessels ended in the midrib in *C. longifolium*, and in the 2° veins in other species, except in 3° veins in *C. alliodora* and *T. amazonia*.

Across species, R_l correlated with R^*_{mr}. This linkage arose from strong correlations between R^*_{mr}, R_{majv}, and R_x (Fig. 2A, B, and C). R^*_{mr} was not correlated with R_p ($r_S = 0.48$, $P = 0.16$; $r_p = 0.36$, $P = 0.31$), and not rank-correlated with R_{minv} ($r_S = 0.56$, $P = 0.12$; $r_p = 0.87$, $P = 0.003$) or R_{ox} ($r_S = 0.26$, $P = 0.47$; $r_p = 0.86$, $P = 0.001$).

Variation in vein arrangement and densities, and relation to leaf hydraulic architecture

Species were similar in several aspects of vein arrangement and diverse in others. All the species had pinnate 1° veins, and brochidodromous 2° veins, except *C. longifolium* and *M. argentea*, which respectively had parallel and basal acrodromous 2° veins (Leaf Architecture Working Group 1999). All species had three orders of major veins, and three to four orders of minor veins, except *C. longifolium*, which had only three orders of veins in total, with 2° veins most abundant and 3° minor veins (Fig. 1, Appendix A). Species varied substantially in 3° vein arrangement (Appendix A). Across species, excepting *C. longifolium*, the densities of 1°, 2°, and 3° veins, and of all major veins, each varied fourfold, and minor vein density and total vein density each varied twofold. The minor veins accounted for 86–97% of vein density (Tables A1 and A2; Fig. 1). The density of free vein endings varied fivefold, and correlated with minor vein density and total vein density (r_S = 0.82 and 0.73; r_p = 0.82 and 0.81 respectively; $P < 0.05$; Tables A1 and A2). In *C. longifolium*, free vein endings were absent.

Across species, R_l and its components were independent of major vein densities (r_S and r_p not significant at $P < 0.05$, including or excluding *C. longifolium*). The hydraulic resistances of xylem components (R^*_{mr}, R_p, R_{majv}, R_{minv}, and R_x) were also unrelated to the minor vein density, the density of free vein endings and total vein density ($r_p = -0.44$ to 0.29, $P = 0.24$ to 0.88; r_S

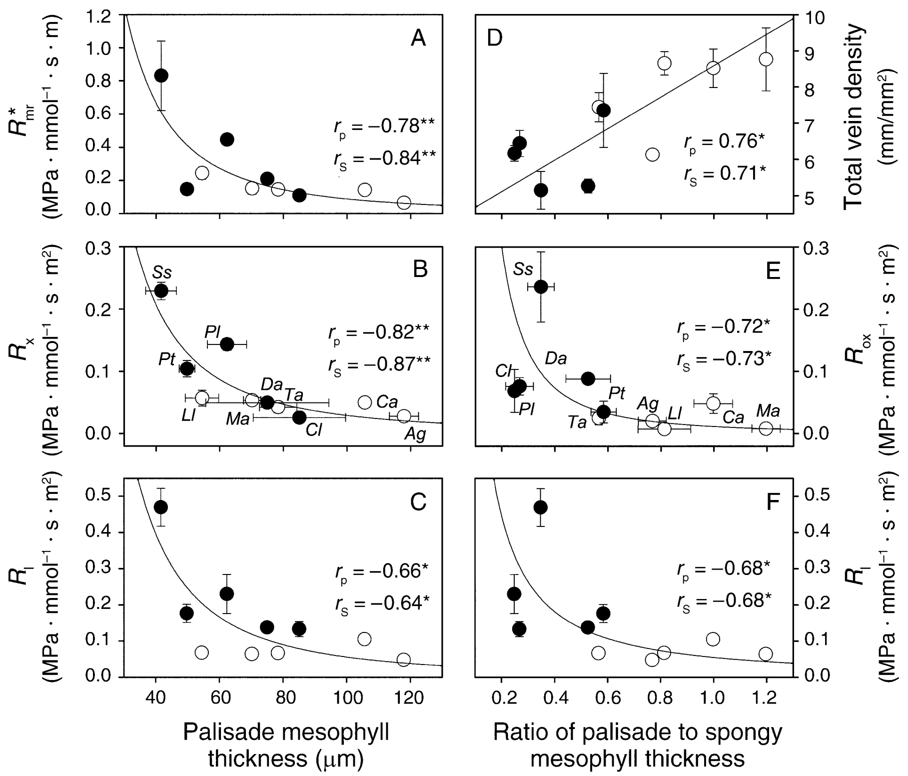

FIG. 3. The coordination of mesophyll tissue structure with venation architecture and hydraulic architecture. Relationships of palisade mesophyll thickness with (A) midrib xylem axial resistivity, (B) xylem hydraulic resistance, and (C) leaf hydraulic resistance, and of palisade to spongy mesophyll ratio with (D) total vein density, (E) hydraulic resistance of tissue outside the xylem, and (F) leaf hydraulic resistance. Symbols are as shown in Fig. 2. R^*_{mr} = 2608 palisade$^{-2.21}$; R_x = 564 palisade$^{-2.14}$; R_l = 1068 palisade$^{-2.14}$; Vein density = 4.35 palisade/spongy + 4.24; R_{ox} = 0.0114 palisade/spongy$^{-2.03}$; R_l = 0.0551 palisade/spongy$^{-1.30}$.

= −0.30 to 0.13; P = 0.43 to 0.98; Fig. 2D). In contrast, R_{ox} was negatively related to the densities of minor veins and free vein endings (excluding *C. longifolium*, in which 2° veins dominate; r_p = −0.73 to −0.78; r_S = −0.68 to −0.77, $P < 0.05$), and to total vein density across all species (Fig. 2E). The negative relationship between vein density and R_{ox} scaled up to a weak negative relationship between total vein density and R_l (r_p = −0.60, P = 0.07; r_S = −0.54, P = 0.14), significant for nine of 10 species excluding *A. glabra*, a sun-establishing species with distinctively low vein density (Fig. 2F).

Variation in mesophyll structure and relation to leaf venation and hydraulic architecture

Species varied strongly in the leaf tissue anatomy, ranging two- to 10-fold in thicknesses of adaxial cuticle, adaxial and abaxial epidermises, palisade and spongy mesophyll, whole mesophyll, and palisade to spongy mesophyll ratio (PSR; Appendix B, Appendix C). Across species, the thicknesses of abaxial epidermis, spongy mesophyll, and whole mesophyll correlated with that of the lamina (Appendix C). PSR was related to the thickness of spongy but not of palisade mesophyll (r_p = −0.82, P = 0.004; r_S = −0.78, P = 0.008, and r_p = 0.31, P = 0.38; r_S = 0.20, P = 0.58; respectively).

Mesophyll structure was coordinated with leaf hydraulic architecture and venation architecture (Fig. 3A to F). While R_l was uncorrelated across species with spongy mesophyll thickness (r_p = 0.30, P = 0.40; r_S = 0.21, P = 0.56), it correlated negatively with palisade thickness and with PSR (Fig. 3C and 3F). These correlations arose due to linkages of palisade mesophyll and PSR to respectively xylem and extra-xylem components. R^*_{mr}, R_{majv}, R_{minv}, and R_x were each negatively correlated with palisade thickness (r_S and r_p = −0.64 to −0.87; $P < 0.05$; Fig. 3A and B), but independent of spongy mesophyll thickness and PSR (r_S and r_p never both significant at $P < 0.05$). Conversely, R_{ox} was negatively related to PSR (Fig. 3E) but independent of palisade and spongy mesophyll thickness (respectively r_S = −0.14, P = 0.70; r_p = −0.21, P = 0.56; and r_S = 0.54, P = 0.11; r_p = 0.60, P = 0.06). Minor vein density, total vein density, and the density of free vein endings were positively to related to PSR (r_S and r_p = 0.64 to 0.78; P = 0.008 to 0.07; Fig. 3D) but independent of palisade (r_S and r_p = −0.20 to 0.17; P = 0.61 to 0.97), and generally negatively related to spongy mesophyll thickness (r_S and r_p = −0.60 to

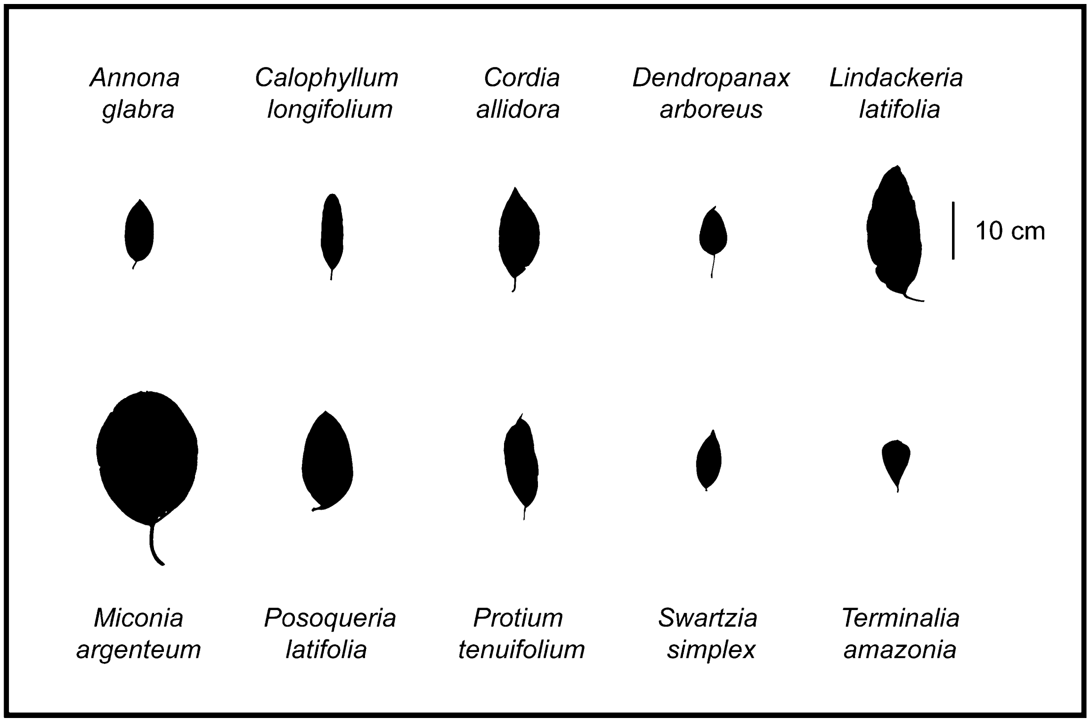

PLATE 1. Leaf silhouettes of the rain forest tree species sampled, diverse in size and shape, shown to scale. *P. tenuifolium* has compound leaves, and terminal leaflets were sampled.

-0.84; $P = 0.004$ to 0.07). R_l and its components were uncorrelated with the thicknesses of whole mesophyll, adaxial cuticle, and epidermises (r_S and r_p not both significant at $P < 0.05$).

The independence of aspects of gross leaf form from hydraulic architecture

Leaves varied strongly in gross morphology (Appendix B, Appendix D). Species' leaves ranged 12-fold in area, threefold in perimeter/area, 1.4-fold in perimeter^2/area, twofold in density, and threefold in thickness. These traits were uncorrelated with R_l and its components (r_S not significant at $P < 0.05$). The traits remained uncorrelated when also pooling data for sun and shade leaves of six species of temperate woody angiosperms (r_S not significant at $P < 0.05$; Appendix D).

Contrasts in leaf traits between sun and shade-establishing species

Sun- and shade-establishing tree species differed strongly in venation architecture, mesophyll structure, and leaf form (Tables A1 and A2; Figs. 1 and 2). Sun-establishing species had 74% more xylem conduits on average than shade-establishing species, 16% larger conduits, 57% lower R^*_{mr}, 34% higher minor vein density, 40% higher density of free vein endings (excluding *C. longifolium*), and 30% higher total vein density. Sun-establishing species had 75% larger leaves on average, with 36% thicker palisade, and 220% greater PSR, but only 74% as dense and 78% as thick overall, with 41%, 57%, and 77% the thickness of adaxial cuticle, spongy mesophyll, and whole mesophyll (Figs. 3, A1, and A2). Sun- and shade-establishing species were not statistically different in major vein density (excluding *C. longifolium* and *M. argentea*, which have very high and low densities of 2° veins respectively; Appendix A), or in perimeter/area, perimeter^2/area, or the thickness of epidermises (Figs. A1 and A2).

DISCUSSION

Coordination of leaf hydraulic resistance, venation architecture and mesophyll structure

The species' 10-fold variation in R_l was linked with strong variation in leaf venation and mesophyll structure. Leaf venation architecture was related to R_l in two independent ways. First, R^*_{mr}, determined by the number and size of midrib xylem conduits, was strongly correlated with R_l via relationships with R_{majv} and R_x. However, these correlations do not suggest that R^*_{mr} is a major component of R_l. The midrib would constitute only a minor proportion of R_{majv}, as water exits the large midrib xylem conduits into narrower conduits of higher resistance in the 2° and 3° veins, and crosses between conduits through highly resistant pits (Sperry et al. 2005); and R_{majv} itself represents only on average 25% of R_l for these species (Sack et al. 2005). Instead, the linkage is probably a case of scaling of resistances within the leaf, as shown previously in the linear scaling of R_{majv}, R_{minv}, R_{x}, R_{ox}, and R_{l} across the species set (Sack et al. 2005). Second, total vein density was negatively related to R_{ox}. R_{ox} represents the resistance to flow out of the xylem, across the bundle sheath, and through the mesophyll before exiting the leaf. This resistance would be lower when dense veins and vein endings confer a larger surface area for transfer out of the xylem. This relationship scaled up to a weak negative relationship between venation density and R_{l} (significant for nine of 10 species, excluding *A. glabra*). This trend is weakened due to species variation in the partitioning of R_{l} between xylem and extra-xylem components; despite the overall linear scaling described above the percentage of R_{l} in the xylem ranged across species from 26% to 89% (Sack et al. 2005). Notably, *major* vein density was independent of R_{l}. The arrangement and densities of major veins could reflect differences in mechanical support, as well as potential differences in tolerance of damage or drought, with greater redundancy allowing water transport around sites of damage or blockage (Roth-Nebelsick et al. 2001).

Mesophyll structure was also interlinked with R_{l} and its components. Palisade thickness was negatively correlated with R_{x}, while PSR was negatively correlated with R_{ox}; both were negatively correlated with low R_{l}. The positive correlation of venation density and PSR confirms the classical pattern (Wylie 1946). These relationships could arise, in part, due to functional linkage. For example, a higher PSR could contribute directly to a low R_{ox} if it is associated with shorter water flow paths through the mesophyll from veins to sites of evaporation (Sack and Tyree 2005). The most likely basis for the relationships is that hydraulic traits and mesophyll traits converge for effective whole-leaf design in given habitats. Species adapted to establishment in high irradiance would benefit from high vein density, conferring higher hydraulic capacity to supply these species higher maximum gas exchange rates, and also providing effective phloem translocation. These species would also benefit from the light harvesting capacity conferred by palisade-rich tissue (Smith et al. 1997).

The independence of hydraulic architecture from gross leaf morphology

As for temperate deciduous species previously studied (Sack et al. 2003), R_{l} was independent of leaf size, shape, and density for the ten tropical rain forest trees. R_{l} was also independent of thicknesses of the adaxial cuticle, epidermises, whole mesophyll, and whole lamina. Thus, leaves can be diverse in gross leaf form without intrinsically constraining R_{l}. We note that R_{l} can be related to leaf size and shape in specific contexts. For instance, for sun and shade leaves within tree canopies, R_{l} was correlated with leaf size (Sack et al. 2003); among temperate *Quercus* species, R_{l} correlated negatively with lobiness (Sisó et al. 2001). Those relationships could arise for leaves of a given species or genus in part from constrained xylem conduit numbers and dimensions, and vein densities; leaves that are smaller, or more lobed, will have all areas of mesophyll closer to major veins, supplied by shorter flow paths through minor veins and mesophyll (Zwieniecki et al. 2002). However, such relationships would not be expected given wide variation in vascular architecture due to phylogenetic and developmental diversity. Leaf size may be more generally associated with heat transfer properties, as well as impacts on shoot light capture (reviewed in Niinemets and Sack 2005).

Differences between sun- and shade-establishing species in leaf form and hydraulic architecture

Our findings indicate novel structural contrasts between shade- and sun-establishing tree species. Consistent with their 70% lower R_{l} (Sack et al. 2005), sun-establishing species had more numerous, larger xylem conduits in the midrib. Sun-establishing species also have, on average, substantially higher vein densities than shade-establishing tree species, analogous to the higher vein density for sun vs. shade leaves within a canopy (Wylie 1951, Roth-Nebelsick et al. 2001). Our findings also confirm previously shown differences among sun- and shade establishing species in tropical rain forests. The leaves of sun-establishing species had thicker palisade, and higher PSR, consistent with potential for faster gas exchange (Popma et al. 1992, Kenzo et al. 2004). By contrast, the leaves of shade-establishing species were denser and thicker, with thicker cuticle, spongy mesophyll, and total mesophyll, potentially contributing to mechanical protection and longer lifespan (Popma et al. 1992, Walters and Reich 1999). Across the study species, the divergence between sun- and shade-establishing species drove many of the major trait linkages; most relationships did not hold for either shade-establishing or sun-establishing species considered alone, but were strong across the whole set (e.g., Fig. 3A–F).

How might these differences impact on establishment ecology? Leaf traits for mature trees often correlate with seedling and sapling traits (Thomas and Bazzaz 1999, Cornelissen et al. 2003). The differences shown here could be even stronger for seedlings establishing in sun vs. shade, and have potential to be adaptive. The sun-establishing species' more numerous, larger, xylem conduits and higher vein densities confer a lower R_{l} that supplies a larger stomatal pore area, allowing high gas exchange rates and thus a higher relative growth rate for a given level of leaf area allocation (Kitajima 1994, Sack et al. 2005). The associated vascular construction costs would be repaid in fast-growing sun-establishing species, but not in shade-establishing species, which typically depend on long tissue life spans, low respiration rates and slow gas exchange and growth (Walters and Reich 1999).

Implication for leaf trait complexes

Whole-plant performance depends on the integrated function of complexes of leaf traits, such as the carbon economy traits associated with LMA (Ackerly 2004, Wright et al. 2004, Niinemets and Sack 2005). R_l is orthogonal to LMA, but interrelated with a suite of traits related to water flux and gas exchange per leaf area, including stomatal pore area, and, as shown by the relationships in this study, venation and mesophyll structure (Sack et al. 2003, Brodribb et al. 2005, Sack et al. 2005). Our study focused only on 10 species, but given their diverse phylogeny, morphology, and regeneration ecology, the relationships are likely to be robust. Future work is needed to confirm the relationships shown here for other species sets, and to allow detailed comparison across vegetation systems. We note that the leaves in this study were well hydrated and collected from trees growing in moist soil. During drought, R_l increases due to xylem cavitation and/or collapse (Bucci et al. 2003, Trifilo et al. 2003, Cochard et al. 2004*a*), and future studies will determine whether species diversity in leaf venation architecture and mesophyll structure has further consequences for comparative drought responses.

ACKNOWLEDGMENTS

Thanks to O. Acevedo, R. Barron, E. Manzane, D. Millan, and C. Nakahashi for assistance; to H. Cochard, R. Condit, M. Holbrook, E. Leigh, A. Nardini, M. Tyree, J. Wright, and G. Zotz for discussion; and the Smithsonian Tropical Research Institute for a Short Term Fellowship to L. Sack.

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APPENDIX A

A table reporting traits relating to leaf venation architecture for the 10 species, including mean ± SE for quantitative traits (*Ecological Archives* E087-026-A1).

APPENDIX B

A table showing the ANOVA results for leaf traits (*Ecological Archives* E087-026-A2).

APPENDIX C

A figure showing the relationships between the leaf lamina thickness and the thickness of component tissues for the 10 species (*Ecological Archives* E087-026-A3).

APPENDIX D

A figure showing the independence of leaf hydraulic resistance from gross leaf form (*Ecological Archives* E087-026-A4).