Early vessel evolution and the diversification of wood function: Insights from Malagasy Canellales

Patrick J. Hudson, Jacqueline Razanatsoa, and Taylor S. Feild

2Department of Ecology and Evolutionary Biology, University of Tennessee, Knoxville, Tennessee 37919 USA; and 3Herbier du Parc Botanique et Zoologique de Tsimbazaza, Rue Fernand, Kasanga, Tsimbazaza, Antananarivo 101, Madagascar

Xylem vessels have long been proposed as a key innovation for the ecological diversification of angiosperms by providing a breakthrough in hydraulic efficiency to support high rates of photosynthesis and growth. However, recent studies demonstrated that angiosperm woods with structurally “primitive” vessels did not have greater whole stem hydraulic capacities as compared to vesselless angiosperms. As an alternative to the hydraulic superiority hypothesis, the heteroxylly hypothesis proposes that subtle hydraulic efficiencies of primitive vessels over tracheids enabled new directions of functional specialization in the wood. However, the functional properties of early heteroxyllyous wood remain unknown. We selected the two species of Canellales from Madagascar to test the heteroxylly hypothesis because Canellaceae (represented by Cinnamosma madagascariensis) produces wood with vessels of an ancestral form, while Winteraceae, the sister clade (represented by Takhtajania perrieri) is vesselless. We found that heteroxylly correlated with increased wood functional diversity related predominantly to biomechanical specialization. However, vessels were not associated with greater stem hydraulic efficiency or increased shoot hydraulic capacity. Our results support the heteroxylly hypothesis and highlight the importance integrating a broader ecological context to understand the evolution of vessels.

Key words: Canellales; heteroxylly; hydraulic conductivity; shade tolerance; xylem evolution; vessel development.

The early evolution and rise to ecological dominance by flowering plants (angiosperms) during the Mesozoic (130 to 65 million years ago) marked a profound change in the water- and carbon-use capacity of global vegetation (Bond, 1989; Becker, 2000; Feild and Arens, 2005, 2007; Boyce et al., 2009; Feild et al., 2009a). Specifically, the vast majority of ecologically important angiosperms today function at rates of leaf CO2 and transpiration that are considerably greater than all other known living and extinct land plant clades (Bond, 1989; Brodribb and Feild, 2000; Pammenter et al., 2004; Brodribb et al., 2007; Boyce et al., 2009). The high productivity and transpiration that angiosperm leaves can supply has (1) founded diverse biotas that directly or indirectly require angiosperm productivity, (2) may have entrained increased rates of forest turnover, and (3) irreversibly changed rates of global biogeochemical cycles through increased nitrogen use and weathering (Knoll and James, 1987; Volk, 1989; Grimaldi, 1999; Moreau et al., 2006; Crepet, 2008).

How and when angiosperms evolved their emblematic high rates of photosynthesis and growth remain enigmatic. However, the prerequisite that high CO2 uptake requires high transpiration means that understanding how the water conducting pipeline diversified during early angiosperm evolution represented a critical factor that enabled the evolution of angiosperm hyperproductivity (Sperry, 2003; Pammenter et al., 2004; Sperry et al., 2006; Brodribb et al., 2007; Feild et al., 2009a). In this vein, xylem vessels have been viewed as an important feature of the vascular network that helped spark the rapid ecological sweep of angiosperms across the planet during the Cretaceous (Bailey, 1944; Carquist, 1975; Doyle and Donoghue, 1986; Bond, 1989; Crane and Lidgard, 1989; Baas and Wheeler, 1996; Lupia et al., 1999; Sperry, 2003; Pammenter et al., 2004; Brodribb et al., 2005; Feild and Arens, 2005; McElwain et al., 2005). Vessels, by virtue of their larger conduit sizes, furnish a greater capacity to conduct water than tracheids—their evolutionary antecedents (Brodribb and Feild, 2000; Sperry, 2003; Sperry et al., 2006). In addition, increases in the efficiency of the xylem by vessels may enable the same hydraulic flow for a given amount of transpiring leaf area supported, but with less investment in wood (Sperry, 2003; Brodribb et al., 2005; Feild and Arens, 2007). Thus, vessels have been pointed to as innovations that allowed early angiosperms to take advantage of falling atmospheric CO2 during their Cretaceous radiation as compared to vesselless plants with more costly stems (McElwain et al., 2005). The hypothesis that angiosperms vessels resulted in increased flow capacity has in large part formed a conceptual cornerstone for an influential hypothesis that early angiosperms outcompeted most nonangiosperms during the Mesozoic (Doyle and Donoghue, 1986; Bond, 1989; Becker, 2000; Lupia et al., 1999; Pammenter et al., 2004; Feild and Arens, 2005; McElwain et al., 2005; Lusk et al., 2007).

Highly efficient vessels have been long viewed as appearing early during angiosperm evolution (Doyle and Donoghue, 1986; Bond, 1989; Pammenter et al., 2004; McElwain et al., 2005). Such vessels consisted of long tubes with thousands of short and
wide elements that were interconnected by simple perforation plates. However, extensive anatomical evidence indicates that such vessels were rare at the base of extant angiosperm phylogeny as well as during the Cretaceous (Frost, 1930; Bailey, 1944; Carlquist, 1975, 2004; Wheeler and Baas, 1991; Carlquist and Schneider, 2002). Instead, vessels of many basal lineages exhibited a range of small anatomical departures from vesselless angiosperm tracheids. Such vessels, referred to as “basal vessels” (Sperry et al., 2007), possessed incompletely lysed pit membranes, had little differentiation between lateral pitting and endwall pitting of vessel elements, and contained elongated scalariform perforation plates. Compared to the vessels of most derived angiosperm clades, basal vessels appear to be less streamlined for water flow (Bailey, 1944; Carlquist, 1975, 2004; Carlquist and Schneider, 2002). Supporting this hypothesis, measured hydraulic capacities of woods with basal vessels overlapped with those of vesselless angiosperm woods, and these capacities were much lower than woods of derived eudicots with vessels bearing simple perforation plates (Sperry et al., 2007). Hydraulic capacity in these comparisons was defined as hydraulic conductivity ($K_H$) divided by the cross-sectional area of xylem ($K_S = K_H / \text{sapwood area}$; Sperry et al., 2007).

If early vessels did not provide a leap in hydraulic efficiency over vesselless wood at the whole stem level, then what processes favored early vessel evolution in angiosperms? One untested hypothesis is that primitive angiosperm vessels permitted new developmental potential for xylem specialization (Feild and Arens, 2007; Sperry et al., 2007; Feild et al., 2009a). For example, on a per xylem conduit basis, hydraulic measurements indicated that basal vessels were on average more efficient than angiosperm tracheids (Hacke et al., 2007; Sperry et al., 2007). Therefore, fewer hydraulic cells appear to be necessary for a given $K_S$ in woods with basal vessels. Fewer cells dedicated to hydraulics in a given volume may permit the wood to function in other ways because $K_S$ would not be decreased by a loss of hydraulically dedicated wood volume. New wood functional properties promoted by vessels could result from the evolution of new cell types and/or altered proportions of cells dedicated to storage, radial transport, and mechanical functions relative to vesselless plants (Feild et al., 2009a). The manifestation of this effect, which vessels are a part of, is the broad phylogenetic trend of increasing heteroxylly with angiosperm diversification (Bailey, 1944; Carlquist, 1975, 2004; Wheeler and Baas, 1991; Carlquist and Schneider, 2002). However, the specific functional features in the wood that were involved during the early evolution of heteroxylly wood remain unknown.

The magnoliid lineage, Canellales, consisting of vessel-bearing Canellaceae (five to six genera, ~21 species) and its vesselless sister-group Winteraceae (four genera, ~65 species; Feild et al., 2000; Karol et al., 2000; Salazar and Nixon, 2008), the eastern tropical montane rainforests of Madagascar represent the only region where the distributions of Canellaceae (Cinnamomus) and Winteraceae (Takhtajania) come into close ecological contact (Schatz, 2000; Feild et al., 2002). The exceptional nature of the range overlap in Madagascar suggested that a comparative study of Canellales from the region might provide insights on the ecophysiological consequences of early vessel evolution. Such a suggestion was made because occurrence in the same climatic regime and biogeographic context may permit a clearer view of the possible ecophysiological advantages of early vessels (Feild et al., 2002). Although some systematic anatomy has been presented on Malagasy Canellales (Wilson, 1960; Carlquist, 2000), no comparative data exist on how the functional responsibilities of the xylem relate to tracheary element structure and ecological preference.

Our goal in the current study was to understand how early vessel evolution was integrated with overall wood function by comparing the growth habits, stem hydraulic and biomechanical structure–function relations, as well as shoot ecophysiological performances of two species of Canellales with and without vessels. We investigated three questions: (1) Do xylem vessels enable greater hydraulic efficiency relative to tracheids? (2) What are the functional consequences of an early experiment in heteroxylly vasculature evolution? (3) How are vesselless and vesselled woods related to leaf function? We discuss how our results bear on the early selective pressures for the tracheid–vessel element developmental transition in early angiosperms.

**Materials and Methods**

**Field sites and study species**—We studied two species: vessel-bearing Cinnamomus madagascariensis Dangay (Canellaceae) and vesselless Takhtajania perrieri (Capuron) Baranov & J.-P. Leroy (Winteraceae). These species were investigated at two relatively close (~40 km) sites in northeastern Madagascar and from one more distant site located in the central highlands of Madagascar (Table 1). Takhtajania perrieri is the only member of the genus and is endemic to Madagascar (Schatz, 2000). There are at least three species of Cinnamomus in Madagascar (Schatz, 2001). We focused on C. madagascariensis because the species occurs in wet, humid, evergreen, tropical forests along the eastern mountains of Madagascar, that are climatically most similar to the known populations of Takhtajania (Fig. 1) (Schatz, 2001). The other Cinnamomus species occur only in drier or warmer lowland, evergreen, tropical habitats (Schatz, 2001). However, some populations of C. madagascariensis occur in littoral tropical rainforests that are warmer and more evaporative than the sites we sampled (Table 1) (Bollen et al., 2004). We sampled two populations having climatic conditions that came closest to the climatic zone of Takhtajania (Table 1). Because of logistical constraints, we only conducted stem hydraulic measurements (see below) on the population of C. madagascariensis from Réserve Spécial Ambositantely (Table 1). The full complement of ecophysiological observations outlined below was made on C. madagascariensis from Parc National Marojejy and T. perrieri at Réserve Spécial Anjanaharibe-Sud (Table 1).

**Stem hydraulic conductivity**—We measured stem hydraulic conductivity ($K_H$; kg m$^{-2}$ s$^{-1}$ MPa$^{-1}$) using a portable flowmeter (Brodribb and Feild, 2000). $K_H$ was determined by measuring the pressure drop across a PEEK capillary tube (Upchurch Scientific, Oak Harbor, Washington, USA) of known conductance with the stem under a positive head pressure. We measured stems under 0.075 to 0.09 MPa using an air-filled captive air tank pressurized with a hand pump. We used an electronic manometer (± 0.001 MPa resolution; model 840081, SPER Scientific, Scottsdale, Arizona, USA) to measure the head pressure and pressure drop across the stem segment during flow. The flowmeter was filled with filtered (to 0.2 μm), degassed water from nearby streams.

Potassium and calcium ions can significantly influence stem $K_H$ (Zwieniecki et al., 2001). We were unable to control ionic concentrations for $K_H$ measurements. However, we found that greenhouse plants of Drimys winteri (Winteraceae) and Canella winterana (Canellaceae) both displayed approximately a...
Table 1. Details of field sites of the two species investigated. References for climatic and vegetation characteristics were: Ambobianyante (Pareliussen et al., 2006), Anjanaharibe-Sud (Goodman, 1998), and Marojejy (Goodman, 2000; Raxworthy et al., 2008).

<table>
<thead>
<tr>
<th>Locality information</th>
<th>Cinnamosma madagascariensis population 1</th>
<th>Cinnamosma madagascariensis population 2</th>
<th>Takhtajania perrieri</th>
</tr>
</thead>
<tbody>
<tr>
<td>Locality</td>
<td>Cinnamosma madagascariensis</td>
<td>Cinnamosma madagascariensis</td>
<td>Takhtajania perrieri</td>
</tr>
<tr>
<td>Site</td>
<td>Population 1</td>
<td>Population 2</td>
<td>Locality</td>
</tr>
<tr>
<td>Elevation (m a.s.l.)</td>
<td>PN Marojejy 1980 400-700</td>
<td>RS Ambobianyante 2008 1500</td>
<td>Anjanaharibe-Sud 2000</td>
</tr>
<tr>
<td>Climate</td>
<td>MAT (°C)</td>
<td>Rainfall (mm yr⁻¹)</td>
<td>Cloud input</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>2200</td>
<td>Low</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Moderate</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>High</td>
</tr>
<tr>
<td>Humidity</td>
<td>High</td>
<td>High</td>
<td>High</td>
</tr>
<tr>
<td>Vegetation characteristics</td>
<td>Tropical premontane forest</td>
<td>Tropical montane forest</td>
<td>Tropical montane forest</td>
</tr>
<tr>
<td>Canopy height (m)</td>
<td>21-25</td>
<td>15-20</td>
<td>10-15</td>
</tr>
</tbody>
</table>

Notes: MAT, mean annual temperature

25% increase in stem $K_H$ to perfusing solutions of KCl ranging from 15 to 100 mM as compared to distilled water (T. S. Feild, unpublished observations, 2001). These results suggest that our lack of control of ionic solution concentration would not have altered relative hydraulic differences between *C. madagascariensis* and Takhtajania.

We cut undamaged branches with fully expanded leaves from the subcanopy. We checked for native embolism by flushing several stem segments of each species with 0.2 MPA pressurized, degassed, and filtered water using a syringe. When plants were sampled on wet, cloudy mornings (0700-0830 hours; Feild, 2001). These results suggest that our lack of control of ionic solution concentrations would not have altered relative hydraulic differences between *C. madagascariensis* and Takhtajania.

From hydraulic flux data, we calculated sapwood-area-specific hydraulic conductivity ($K_s$; kg m⁻¹ s⁻¹ MPa⁻¹) as $K_s$ divided by the sapwood cross-sectional area with the pith area subtracted (Feild and Balun, 2008). Sapwood cross-sectional areas were determined on portions of measured stem tissue preserved in 50% ethanol using a Zeiss SterELO Discovery.V8 dissecting microscope (Carl Zeiss MicroImaging, Gottingen, Germany) and ImageJ software (National Institutes of Health, Bethesda, Maryland, USA). We calculated conduit-area-specific hydraulic conductivity ($K_v$; kg m⁻¹ s⁻¹ MPa⁻¹) as $K_v$ divided by the cross-sectional area of hydraulic lumens at a stem (Sperry et al., 2007). We calculated per conduit specific conductivity, defined as the average $K_v$ attributable to vessels and tracheids in *C. madagascariensis* as well as tracheids of Takhtajania. In these calculations, we assumed that fibers did not participate in hydraulic flow. Next, we divided $K_v$ by the number of conduits in a measured stem that were counted using criteria described below to specify cell types. We determined the leaf-area-specific hydraulic conductivity ($K_l$; kg m⁻¹ s⁻¹ MPa⁻¹) as $K_l$ divided by the surface area of leaves distal to the cut end of the segment. Using digital image analysis software on scanned images of leaves (ImageJ, NIH Image, Bethesda, MD, USA), we quantified leaf area. Shoot Huber value (HV) was calculated as sapwood area divided by the distal leaf area.

Xylem anatomy—We isolated tracheary elements using acetic acid, hydro- gen peroxide, and heat (Ruzin, 1999). Macerated wood was stained in 1% aqueous safranin, vortexed, and tracheary cells were pipetted onto slides. We imaged cells with an upright microscope (Axio Imager M1, Zeiss), and cells were analyzed for anatomical characteristics using ImageJ. The observations described below were made on three stems for each species.

For tracheary elements (including fibers of *C. madagascariensis*), we quantified mean length, lumen diameter, wall thickness, and the percentage of surface area devoted to pits (Hacke et al., 2007). Diameters and wall thicknesses of *T. perrieri* and *C. madagascariensis* conduits were derived from transverse sections from three stem segments of each species imaged from a microscope and 250 to 300 measurements of each variable per species (Hacke et al., 2007). Three cross sections of three stems for each species were imaged at 200x to estimate areas of cell types. Pit area was assessed on 50 cells for each class at 400x to 600x. To quantify tracheary element diameter, we measured each element’s lumen in a stem cross section was traced in ImageJ to determine the area of the conduit lumen. Then, the diameter of the circle with equal area was calculated to produce the lumen diameter for all cell types in both species. Tracheary element diameters were analyzed for frequency distribution in diameter classes of 5-μm increments for the relative contribution of each diameter class to the sum of all conduits raised to the fourth power of the radius. The sum was proportional to xylem hydraulic conductance, assuming that xylem conduits acted as ideal capillaries (i.e., Hagen–Poiseuille equation; Feild et al., 2002).

Mean hydraulic diameters of tracheary elements were calculated as described previously (Hacke et al., 2007). We distinguished vessel elements, fibers, and tracheids in *C. madagascariensis* on the basis of statistical differences in wall thicknesses that correlated with tracheary element pitting such that we could categorize the frequencies of cell types based on thickness measurements. Distinguishing tracheids from fibers is difficult because of blurry distinctions in pitting between the two cell types (Carlquist, 1975, 2004). However, we found that such a situation did not occur in *C. madagascariensis*. Tracheids had consistently different wall thicknesses and pitting characteristics from fibers (Table 2). Using these assessments, we were able to normalize the densities of cell types to a 1-mm² of sapwood for both species. With these results, we expressed $K_v$ in Cinnamosma on the basis of vessel- and tracheid-conduit areas. Finally, we quantified cross-sectional differences in ray tissue and xylem parenchyma between the two species on digital images (Pratt et al., 2007).

Wood density and stem biomechanics—Wood density ($\rho_{wood}$) was calculated as dry mass of stem divided by fresh volume (g cm⁻³) using volumetric displacement. We cut in half and removed the pith and bark of five 3-cm long and 1-cm thick pieces of wood. Next, a syringe needle was used to submerge each sample in a 60 mL beaker on a field portable balance (±0.001 g resolution, Denver Instruments, Denver, Colorado, USA), and the displacement of water measured. We dried the samples at 100°C in an oven overnight and measured them for dry mass. We accounted for the effect of temperature on the density of water by normalizing values to 20°C.

The elastic modulus of wood from each species was calculated using four points bending tests (Chapoton et al., 2006). The modulus of elasticity (E, GPa) was $E = \frac{Fa(3L^2 - 4a^2)(24y)}{I}$, where $F$ was the force (N) applied to each point, $L$ (m) was the distance between the two supports, $a$ (m) was the distance between the support and the point of force application, $I$ was the second moment of area of the stem section (m⁴), and $y$ (m) was the vertical deflection at the center of the segment. $I$ was calculated by approximating the stem cross section as an ellipse (Niklas, 1992). An appropriate force was used such that vertical deflection was small with respect to the length of the stem segment (less than 5%). We selected five straight stems for each species, cut the stems to 15 cm in length, and stripped them of bark before bending tests. Bark was removed to limit measured $E$ to a mechanical property of the wood. Stem diameters along the length were then measured with a micrometer to the nearest 0.1 mm. Pith was ignored in our calculations, as it should have only a minimal contribution to the mechanical properties of the stem (Niklas, 1992). To relate biomechanical measurements to measured stem hydraulic capacities, we conducted our bending tests on the most distal branches for both species.

Measurements of xylem energy content—Wood samples used for $\rho_{wood}$ were burned in an LECO AC-350 isoperibol calorimeter (LECO, St. Joseph,
Fig. 1. Study site locations and growth forms of the two species investigated. (A) Vesselless *Takhtajania perrieri* was sampled at Réserve Spécial Anjanaharibe-Sud Reserve (denoted as an open square, 14°44′S, 49°28′E, 1100 m a.s.l., 10 km from Andapa). Vessel-bearing *Cinnamosma madagascariensis* from two sites (denoted by open circles): Réserve Spécial Ambohitantely, 18°9′S 47°16′E, 1500 m a.s.l., Central Highlands and Parc National Marojejy, 14°26′S 49°45′E, 12 km from the village of Manatenina, 400–700 m a.s.l. The known distributions of *T. perrieri* (filled squares) and *C. madagascariensis* (filled circles) are provided (41 records; biodiversity occurrence data provided by Missouri Botanical Garden, accessed through GBIF [2009] Data Portal). (B) Growth habit of an adult *C. madagascariensis* tree from PN Marojejy. (C) Growth habit of an adult *T. perrieri* at RS Anjanaharibe-Sud. Scale bars: (A) 150 km, (B) 50 cm, (C) 30 cm.
Michigan, USA) to determine their heat of combustion. Samples were ground to a fine powder and then dried again at 75°C to a constant mass. Approximately 0.6 g of each sample was combusted in the bomb calorimeter in accordance with the ASTM D240-02(2007) standard test method for heat of combustion. Heat of combustion was expressed on a fresh volumetric basis, and dry mass basis represented a measure of wood construction cost. Five samples for energetic content from five different stems were measured for each species.

Leaf ecomorphic traits—Because of the remoteness of our field sites, we were unable to measure leaf gas exchange performances. Therefore, to infer relative differences in leaf gas-exchange capacity, we used a well-supported anatomical proxy of leaf water loss and CO₂ uptake (Parlange and Waggoner, 1970; Brodribb et al., 2007; Boyce et al., 2009; Kaiser, 2009). We inferred maximum leaf water vapor conductance (mmol H₂O m⁻² s⁻¹) from the structure of stomatal system and a previously published one-dimensional diffusion equation as

\[ g_{\text{max}} = \frac{1}{(d/ab) + 4(4b/ab)D} \]  

where \( a = \) guard cell pore length \( b = \) guard cell pore width \( d = \) guard cell pore depth \( D = \) diffusivity of water vapor in air \( N = \) guard cell density (Parlange and Waggoner, 1970; Brodribb and Hill, 1997). Measurements of \( d \) were made on cross sections of leaves fixed in formalin-acetic-alcohol (FAA; 1 : 0.5 : 5, in 3.5 parts DI water) and then embedded in a plastic resin (JB-4, Polycysnciences, Warrington, Pennsylvania, USA). The resin was polymerized according to the manufacturer’s instructions. We sectioned samples (7 µm thick) using a tungsten carbide blade on a rotary microtome (RM2245, Leica Microsystems, Wetzlar, Germany). Twenty guard cell pores from each species were imaged at 400× and measured with ImageJ. We determined \( a \) and \( N \) on safranin-stained, macerated cuticles that were isolated by using an accepted procedure (Feild et al., 2009b). Fifty guard cells were imaged at 400× and measured with Image J and, ten 4 mm × 4 mm sheets of cuticle were counted for \( N \) at 200×. The value \( b \) was taken as one-third of the guard cell pore length (Osborne et al., 2004). Geometric criteria for measuring guard cell pore length and depth followed Lawson and colleagues (1998).

The value \( g_{\text{max}} \) calculated from stomatal pore dimensions correlates well with measured rates of leaf water vapor loss (Brodribb and Hill, 1997; Kaiser, 2009). However, this equation does not take into account other resistors, such as intercellular conductance, the presence of internal cuticle, and ornamentations to the stomatal pore complex that pare back effective diffusional aperture (Brodribb and Hill, 1997; Kaiser, 2009). Because C. madagascariensis and T. perrieri did not possess stomatal plugs (Bongers, 1973; Feild et al., 2000), \( g_{\text{max}} \) based on stomatal pore dimensions is likely to be a relatively good approximation of leaf maximum gas-exchange capacity (Brodribb and Hill, 1997; Kaiser, 2009).

In addition, we made observations on the cross-sectional anatomy of leaves to test for differences in leaf structure related to the optical processing of photosynthetically active radiation, including mesophyll cell structure and leaf thickness (Smith et al., 1997; Smith and Hughes, 2009). Finally, leaf mass per area (LMA, g m⁻²) and leaf size (cm²) were determined for 20 leaves of each species. All leaves measured were undamaged and fully expanded. Leaves were scanned on a portable scanner, measured using ImageJ, and dry mass determined after drying for 2 d at 65°C.

Pressure-volume analysis of drying leaves—Pressure (\( \Psi_w \)) isopleths (bulk leaf water potential [\( \Psi_w \)] vs. relative water content [RWC]) of leaves were assessed by repeated measures (four to six observations per leaf) of leaf mass and \( \Psi \) using accepted procedures (Sack et al., 2003). We collected seven fully expanded and undamaged leaves for each species at maximum field hydration before sunrise (0600 to 0700 hours), and checked \( \Psi_w \) to verify that it was greater than −0.05 MPa before beginning a pressure–volume (PV) curve. From the initial point, leaves were desiccated, and alternately weighed and \( \Psi_w \) measured until the leaf was well below the turgor loss point. We weighed leaves on a field portable balance, and we determined \( \Psi_w \) and RWC at the turgor loss point using accepted curve-fitting procedures of PV data (Sack et al., 2003) in the program Sigma-Plot (Jandel Scientific, SPSS, Chicago, Illinois, USA).

Statistical analyses—All data were analyzed using a statistical analysis program (PASW Statistics 17, SPSS). Mann–Whitney \( U \) tests were applied to the ecophysiological and structural data to test for statistically significant differences between species.

RESULTS

Growth forms—Cinnamosma madagascariensis occurred as a treelet to small tree up to 8 m tall with a single stem bole up to 13 cm diameter at breast height (dbh). No evidence of basal sprouting was observed in either of the populations of C. madagascariensis that we studied. The canopies of C. madagascariensis trees were wide and deep with several interlacing, distichously branched distal branches (3 to 5 mm in diameter on most distal branches with little pith; Fig. 1B). Leaves were retained along much of the length of terminal shoots. Internodes of C. madagascariensis (1 to 2 cm long) were longer than T. perrieri. Plants occurred on flat areas of forested slopes in the understory to subcanopy and away from gaps.

In contrast, Takhtajania perrieri formed a shorter tree up to 5 m tall with stems up to 10 cm dbh. All adult plants we observed consisted of multiple trunks. Most plants sprouted from the base, with one to eight basal and pithy shoots. Plants occurred near trails, on cyclone-influenced ridge crest forests, and gap-phase vegetation. The canopies formed by T. perrieri were irregular due to leaning trunks, and the canopies were sparsely leaved with irregularly oriented candelabraform branches bearing leaves in tufted whorls at the tips (Fig. 1C). The terminal branches of Takhtajania, from 8 to 15 mm, were thicker than C. madagascariensis and consisted primarily of pith. Internodes of T. perrieri were short (0.4 to 0.9 mm long), and numerous leaf scars subtended each whorl.

Stem xylem anatomy and tracheary element structure—In transverse section, we found, as reported earlier, that the xylem of Takhtajania perrieri was homoxylous, with a dominance by tracheids of uniform lumen diameter and wall thickness (Fig. 2A, Table 2) (Carlquist, 2000). In contrast, transverse sections indicated that the wood of Cinnamosma madagascariensis was more heteroxylous (Fig. 2B) (Wilson, 1960). Compared to T. perrieri, axial cell types ranged widely in mean lumen diameter (from 8.9 µm in fibers to 36.7 µm in vessels) and mean wall thicknesses (1.5 µm in vessels to 4.3 µm in fibers; Table 2). In Takhtajania, we confirmed previous observations that tracheid endwalls had uniseriate and biseriate circular pits as well as occasionally dense scalariform pitting at the endwalls (Fig. 1C) (Carlquist, 2000). In C. madagascariensis, vessels were characterized by variably shaped, elongated, many-barred scalariform perforation plates, which accounted for on average 64.4% ± 13.8 SD of element length.

Tracheids of Takhtajania and Cinnamosma differed in structure (Table 2, Fig. 2C and D). Takhtajania’s tracheids were twice as long, 27% wider in lumen diameter, and had approximately 50% more surface area devoted to pits compared with C. madagascariensis tracheids (Table 2). Tracheid wall thicknesses of both species tracheids were similar (Table 2). Unlike Takhtajania tracheids, however, no C. madagascariensis tracheids possessed scalariform pitting. Compared to vessel elements and fibers, C. madagascariensis tracheids occupied an
Fig. 2. Comparative cross-sectional anatomy, tracheary element anatomy, and cell contents of *T. perrieri* and *C. madagascariensis*. (A) Transverse section of *T. perrieri* wood, showing tracheids of nearly uniform diameter. (B) Transverse section of *C. madagascariensis* wood illustrating the greater variation tracheary element diameters. (C) Endwalls of macerated *T. perrieri* tracheids. The two tracheids illustrate the extremes of endwall pitting anatomies that we observed. (D) Variations in endwall pitting of fibers, tracheids, and vessel elements in *C. madagascariensis*. (E, F) The percentages of the transverse surface area (SA) contributed by different cell types for (E) *T. perrieri* and (F) *C. madagascariensis*. We classified cell types in *C. madagascariensis* using wall thickness measurements as described in the Materials and Methods. Scale bars: (A, B) 100 μm, (C, D) 40 μm.
intermediate position in wall thickness as well as the area of an individual element devoted to pits (Table 2). The lateral pitting of these tracheids was most often uniseriate, with some biseriate pitting (Fig. 2D).

Tracheids composed 77% ± 6 SD of the transverse surface area of Takhtajania’s stem xylem, with 23% of the surface area occupied by rays (Fig. 2E). In contrast, we found that fibers dominated transverse xylem surface area (65% ± 5 SD) in C. madagascariensis, with rays (20% ± 6 s.d.), water-conducting cells (15% total; 13% ± 4 SD, vessels; 2% ± 1 SD, tracheids), and axial parenchyma (5% ± 3) at smaller fractions (Fig. 2F). Vascular parenchyma surrounded each vessel or vessel group (Fig. 2F).

**Stem hydraulics, biomechanics, and wood energy content**—Takhtajania possessed an approximately 65% greater average $K_e$ and HV than both populations of C. madagascariensis (Fig. 3A, B). However, we found that the average $K_e$ of C. madagascariensis from Marojejy was statistically indistinguishable from that of T. perrieri (Fig. 3C). Mean $K_s$, HV, and $K_l$ values from both C. madagascariensis did not differ significantly (Fig. 3). However, mean $K_l$ in branches from the Cinnamosma population from Ambohitandy was ~20% lower than T. perrieri (Fig. 3C). When normalized to conduit area, mean $K_C$ of the two populations of C. madagascariensis were both nearly three times greater than T. perrieri (Fig. 3D). Mean $K_C$ did not differ between the two populations of C. madagascariensis (Fig. 3). Vessels accounted for the majority (96%) of $K_C$ in both populations of C. madagascariensis (Fig. 3E). On a per capita basis, vessels possessed an order of magnitude greater conductivity than tracheids (Table 2).

For T. perrieri, the largest proportion of tracheids (mean = 39% ± 14 SD) fell in the 15–20 μm diameter class (Fig. 4A). By comparison, most of the vessels (30% ± 20 SD) in C. madagascariensis occurred in the 25–30 μm diameter class (Fig. 4B). When modeled as pipes, tracheids of the 15–20 μm size class was responsible for the largest proportion of hydraulic flow (34% ± 15 SD, Fig. 4C). The highest proportion of flow (33% ± 22 SD, Fig. 4D) occurred through vessels in the 30–35 μm diameter class. Vessels accounted for 96% ± 3 SD of idealized hydraulic flow, and tracheids were responsible for 4% ± 3 SD (Fig. 4D).

Both populations of C. madagascariensis possessed a 45% greater mean $\rho_{\text{wood}}$ as compared to T. perrieri (Fig. 5A). We measured a 35% greater $E$ in C. madagascariensis from Marojejy relative to T. perrieri (Fig. 5B). Consistent with this finding, we observed that branches that were bent to 40° relative to the stem axis snapped in T. perrieri, whereas twigs of C. madagascariensis did not snap with 90° bending.

The mean heat of combustion of C. madagascariensis dry wood on a mass basis was approximately 3% less than that of T. perrieri (Fig. 5C). However, when expressed on a volumetric basis, the amount of energy per volume of fresh wood in C. madagascariensis was 41% greater than T. perrieri wood (Fig. 5D).

**Leaf functional traits**—Both species had similar guard cell densities and guard cell pore depth, but the mean guard cell length of T. perrieri was larger (Table 3). Consequently, calculated $g_{\text{max}}$ based on guard cell dimensions and diffusional theory of C. madagascariensis was 39% lower than T. perrieri (Table 3). In cross section, we observed that C. madagascariensis developed a single layer of palisade parenchyma in the mesophyll, and some cells below the layer were columnar (Fig. 6A). A hypodermis was present above the palisade layer (Fig. 6A). In contrast, the leaf cross section of Takhtajania consisted only of spongy mesophyll tissue (Fig. 6B). Leaves of C. madagascariensis were 25% thinner than Takhtajania (Table 3). Leaves of C. madagascariensis were smaller in leaf mass per area as compared to Takhtajania (Table 3), but leaves of Takhtajania were larger than those of C. madagascariensis (Table 3). In addition, the leaves of C. madagascariensis were more resistant to desiccation in comparison to T. perrieri. Cinnamosma madagascariensis lost turgor pressure at $\Psi_w$ of ~2.1 MPa and RWC of 86.1% vs. $\Psi_w$ of ~1.2 MPa and 93.4% RWC for turgor loss in T. perrieri (Fig. 6C, D).

**DISCUSSION**

We found that vessels of an ancestral form, characterized by long and low-angle scalariform perforation plates with up to 120 pit bars (Frost, 1930; Bailey, 1944; Carlquist, 1975, 2004; Carlquist and Schneider, 2002), were associated with substantial divergence in wood function as compared to a relative without vessels. Vessels of C. madagascariensis provided approximately 3-fold greater $K_{II}$ normalized to the cross-sectional area of conduit lumens ($K_C$) in comparison to the vesselless
Although we were unable to determine the vessel length distribution in the field, we note that maximum vessel length in *C. madagascariensis* was ~20 cm by air injection. This observation suggests another significant increase in conduit efficiency of vessels relative to tracheids (Pittermann et al., 2006; Sperry et al., 2006, 2007; Hacke et al., 2007).

However, increased hydraulic efficiencies of basal vessels relative to tracheids occurred only at the conduit level because hydraulic efficiencies of whole stem (i.e., the sapwood level, $K_s$) of *C. madagascariensis* and *T. perrieri* were equivalent (Table 2, Fig. 3). The functional distinction between $K_C$ and $K_s$ wood of *T. perrieri*. Increased $K_C$ decreased by 75% the amount of wood cross-sectional area devoted to axial hydraulic flow as compared to *T. perrieri*. Although *C. madagascariensis* possessed tracheids (Wilson, 1960), we found that vessels were responsible for the vast majority of $K_C$. Consistent with greater conduit efficiency, the vessel elements of *C. madagascariensis* possessed several anatomical differences involved in greater flow efficiency in spite of a large decrease in the numerical density of hydraulic cells in the wood. These features included increased pit membrane area by perforation plates, ability to develop wider lumens, and thinner walls than *T. perrieri* tracheids. Although we were unable to determine the vessel length distribution in the field, we note that maximum vessel length in *C. madagascariensis* was ~20 cm by air injection. This observation suggests another significant increase in conduit efficiency of vessels relative to tracheids (Pittermann et al., 2006; Sperry et al., 2006, 2007; Hacke et al., 2007).

However, increased hydraulic efficiencies of basal vessels relative to tracheids occurred only at the conduit level because hydraulic efficiencies of whole stem (i.e., the sapwood level, $K_s$) of *C. madagascariensis* and *T. perrieri* were equivalent (Table 2, Fig. 3). The functional distinction between $K_C$ and $K_s$
Our results indicated that the majority of xylem cross-sectional area "freed up" by the basal vessels of *C. madagascariensis* was allocated to biomechanical function. This conclusion was supported by a dominance of sapwood cross-sectional area in *C. madagascariensis* by fibers having greater wall thicknesses as compared to vessels and tracheids (Table 2). The development of mechanically specialized fibers in *C. madagascariensis* most likely was responsible for the greater is important because differences in $K_C$ reflect overall wood allocation costs to supply transpiring leaves with water (Sperry, 2003; Baas et al., 2004; Sperry et al., 2006). In addition, vessels of *C. madagascariensis* were associated with lower capacity of the wood to supply transpiring leaves with water (lower $K_L$) in *C. madagascariensis* (Fig. 3). Instead of reducing overall shoot costs, we found that greater $K_C$ furnished by basal vessels increased the area available in the wood to function differently from vesselless Winteraceae wood.

**Functional divergence of Cinnamosma and Takhtajania wood**—Our results indicated that the majority of xylem cross-sectional area “freed up” by the basal vessels of *C. madagascariensis* was allocated to biomechanical function. This conclusion was supported by a dominance of sapwood cross-sectional area in *C. madagascariensis* by fibers having greater wall thicknesses as compared to vessels and tracheids (Table 2). The development of mechanically specialized fibers in *C. madagascariensis* most likely was responsible for the greater

---

**Fig. 4.** Comparisons of frequency distributions and hydraulic contributions of tracheary elements in *Cinnamosma madagascariensis* and *Takhtajania perrieri*. Distributions of conduit diameter frequencies for (A) *T. perrieri* tracheids and (B) tracheids (gray bars) and vessels (black bars) in *C. madagascariensis*. Distributions of conduit diameter frequencies and percentage contribution to calculated hydraulic flows for (C) *T. perrieri* tracheids and (D) *C. madagascariensis* tracheids (gray bars) and vessels (black bars). Sample sizes and hydraulic calculations using the Hagen–Poiseuille equation are provided in the Materials and Methods.
$p_{\text{wood}}$ and increased $E$ as compared to Takhtajania (Hacke et al., 2001; Jacobsen et al., 2005).

It is unknown if the $E$ and mechanically associated traits (e.g., wall thickness, pit area fraction) of fibers in *C. madagascariensis* represent properties extending outside the ranges capable by vesselless vasculatures in the Winteraceae. We note, however, that average $p_{\text{wood}}$ values of Canellaceae, including *C. madagascariensis*, *C. fragrans* (mean = 0.928 g cm$^{-3}$ ± 0.06 SD; $N$ = 10), and *Pleodendron costaricense*, were 9, 23, and 22% greater, respectively, than the reported maximum $p_{\text{wood}}$ found in 16 species of Winteraceae (Hacke et al., 2007; Moya-Roque et al., 2007; P. J. Hudson and T. S. Feild, personal observations, 2007, 2008). Because basal vessels furnished higher $K_C$, our results suggest that a greater $p_{\text{wood}}$ and $E$ can be achieved in *C. madagascariensis* for a given allocation of sapwood cross-sectional area in contrast to vesselless woods of Winteraceae (Hacke et al., 2001; Jacobsen et al., 2005; Preston et al., 2006; Swenson and Enquist, 2007; Chave et al., 2009). Mechanical traits also have consequences for the evolution of growth forms and regeneration ecologies.

---

**Table 3.** Comparative functional trait values (mean ± SD) of *Cinnamosma madagascariensis* and *Takhtajania perrieri* leaves. Asterisks denote significance of difference between species means: *$P<0.05$, **$P<0.01$, ***$P<0.001$ (Mann–Whitney $U$). NS denotes not significant at $\alpha=0.05$. Sample sizes for individual means are reported in the Materials and Methods.

<table>
<thead>
<tr>
<th>Leaf trait</th>
<th><em>C. madagascariensis</em></th>
<th><em>T. perrieri</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Stomatal density (mm$^{-2}$)</td>
<td>148.3 ± 7.4</td>
<td>147.8 ± 17.3 NS</td>
</tr>
<tr>
<td>Guard cell size (length x width, $\mu$m)</td>
<td>25.7 ± 2.4 × 21.2 ± 1.3</td>
<td>29.1 ± 2.2 × 22.5 ± 1.7***, **</td>
</tr>
<tr>
<td>Pore length ($\mu$m)</td>
<td>6.48 ± 0.85</td>
<td>8.32 ± 0.89***</td>
</tr>
<tr>
<td>Pore depth ($\mu$m)</td>
<td>10.16 ± 2.07</td>
<td>9.76 ± 0.92 NS</td>
</tr>
<tr>
<td>$\delta_{\text{max}}$ (mmol H$2O\cdot m^{-3} \cdot s^{-1}$)</td>
<td>125.49</td>
<td>203.23</td>
</tr>
<tr>
<td>Leaf size (cm$^2$)</td>
<td>32.2 ± 5.3</td>
<td>58.1 ± 18.3***</td>
</tr>
<tr>
<td>Leaf thickness ($\mu$m)</td>
<td>312.9 ± 7.7</td>
<td>404.5 ± 21.4***</td>
</tr>
<tr>
<td>LMA (g·m$^{-2}$)</td>
<td>100 ± 0.1</td>
<td>111 ± 0.1**</td>
</tr>
</tbody>
</table>
associated with a preference for densely shaded forest understory habitats. Greater mechanical strength can be advantageous in low light understories to develop thin diameter stems that support a broad leaf area to efficiently collect light (Sterck et al., 2001; Rowe and Speck, 2004; Osunkoya et al., 2007). Stems with greater $E$, for a given diameter can be more resilient to breakage, which can increase the probability that a costly investment in wood production is not lost by understory disturbance (Rowe and Speck, 2004; Jacobsen et al., 2005). Consistent with greater $\rho_{wood}$ and $E$, the growth form of C. madagascariensis appeared to be more demanding of mechanical support as compared to Takhtajania's (Fig. 1). Cinnamosma madagascariensis grew with a single and taller erect trunk that supported a broader and deeper canopy of thinner, densely leaved terminal branches (Fig. 1B). Also in line with an apparent understory preference, the values of $K_S$, $K_L$, and HV in both populations of C. madagascariensis nested in the ranges reported for shade-demanding woody plants from tropical forests (Brodribb and Feild, 2000; Feild and Holbrook, 2000; Santiago et al., 2004; Lusk et al., 2007; Campanello et al., 2008; Feild and Balun, 2008).

Surprisingly, we found that vesselless Takhtajania possessed a much greater $K_L$ than vessel-bearing C. madagascariensis.

---

Fig. 6. Comparative leaf anatomy and water relations in Cinnamosma madagascariensis and Takhtajania perrieri. In cross section, (A) C. madagascariensis leaves had a palisade layer as well as a hypodermis, whereas (B) T. perrieri leaves were composed of spongy mesophyll tissue. Scale bars: = 100 \( \mu \)m. (C) Moisture release and (D) pressure–volume curves demonstrate that leaves of C. madagascariensis (black circles) have a greater ability to maintain turgor under drought conditions than T. perrieri (white circles). Sample sizes are provided in the Materials and Methods.
(Table 1, Fig. 2). Consistent with greater $K_L$, calculated $g_{\text{max}}$ values were higher in T. perrieri, largely because guard cell pores were larger relative to C. madagascariensis (Table 3). Relative to other Winteraceae, the xylem hydraulics of T. perrieri were unusually high in capacity. For example, T. perrieri possessed 30% greater $K_S$ than the previously observed maximum in Winteraceae (Feild and Holbrook, 2000; Feild et al., 2002; Hacke et al., 2007). Supporting the hypothesis that hydraulic conductance and mechanics are closely traded off in hydraulic and mechanical responsibilities in the wood by in- turnute studies will be necessary to determine how other important components of heteroxylly evolution, such as parenchyma and rays, diversified and coevolved with hydraulics and biomechanics during early angiosperm evolution. The diversity of xylem vasculatures across extant basal angiosperms offers a diverse range of evolutionary “experiments” and considerable growth form variation to tease apart these interactions (Carlquist, 1975, 2004; Carlquist and Schneider, 2002; Feild and Arens, 2005, 2007; Feild et al., 2009a).

A limitation of our study is that recent phylogenetic studies make it clear that Takhtajania and C. madagascariensis have come to inhabit somewhat similar geographic and climatic envelopes through different biogeographic histories (Karol et al., 2000; Salazar and Nixon, 2008). While Takhtajania is placed phylogenetically as the sister to the remaining genera of Winteraceae, C. madagascariensis is well nested within Canellaceae, which results in considerable phylogenetic distance between the two taxa (Karol et al., 2000; Salazar and Nixon, 2008). Thus, a future broader comparative analysis of hydraulic and biomechanical coordinated evolution in relation to Canellales' habitats and growth forms will be needed to resolve how heteroxylly evolution relates to ecological diversification.

Although we did not measure resistance of the scalariform perforation plates, we posit that those of C. madagascariensis will be relatively high because of their long lengths with numerous pit bars (Sperry et al., 2007; Christman and Sperry, 2009). Because we found that vessels did not decrease $K_S$ relative to vesselless wood, our results support an emerging hypothesis that vessels only drove major cuts in the cost of $K_L$ through increased $K_L$ once elongate scalariform perforation plates became consolidated into a single aperture (Sperry et al., 2007; Christman and Sperry, 2009; Feild et al., 2009a). Therefore, understanding how early angiosperms acquired such high rates photosynthetic productivity will require a specific focus on perforation plate form and functional diversification, rather than vessels per se (Feild and Arens, 2007; Sperry et al., 2007; Feild et al., 2009a). Instead of devices immediately enabling access to high rates of water transport capacity and therefore photosynthesis, our results highlight the concept of vessels as traits that potentially enabled new properties of wood function.

**LITERATURE CITED**


