

Fossil evidence for Cretaceous escalation in angiosperm leaf vein evolution

Taylor S. Feild^{a,1}, Timothy J. Brodribb^b, Ari Iglesias^c, David S. Chatelet^d, Andres Baresch^e, Garland R. Upchurch, Jr.^f, Bernard Gomez^g, Barbara A. R. Mohr^h, Clement Coiffard^h, Jiri Kvacekⁱ, and Carlos Jaramillo^e

^aDepartment of Ecology and Evolutionary Biology, University of Tennessee, Knoxville, TN 37996; ^bDepartment of Plant Science, University of Tasmania, Hobart, 7001 Tasmania, Australia; ^cFacultad de Ciencias Naturales y Museo, Universidad Nacional de La Plata, 1900 La Plata, Argentina; ^dDepartment of Ecology and Evolutionary Biology, Brown University, Providence, RI 02912; ^eSmithsonian Tropical Research Institute, Balboa, Panama; ^fDepartment of Biology, Texas State University, San Marcos, TX 78666; ^gUniversité Lyon 1, Centre National de la Recherche Scientifique-Unité Mixte de Recherche 5125, Paléoenvironnements et Paléobiosphère, Villeurbanne, France; ^hCollections, Museum of Natural History, 10 115 Berlin, Germany; and ⁱNational Museum, Prague, Czech Republic

Edited by Peter Crane, Yale School of Forestry and Environmental Studies, New Haven, CT, and approved April 4, 2011 (received for review September 26, 2010)

The flowering plants that dominate modern vegetation possess leaf gas exchange potentials that far exceed those of all other living or extinct plants. The great divide in maximal ability to exchange CO₂ for water between leaves of nonangiosperms and angiosperms forms the mechanistic foundation for speculation about how angiosperms drove sweeping ecological and biogeochemical change during the Cretaceous. However, there is no empirical evidence that angiosperms evolved highly photosynthetically active leaves during the Cretaceous. Using vein density (D_v) measurements of fossil angiosperm leaves, we show that the leaf hydraulic capacities of angiosperms escalated several-fold during the Cretaceous. During the first 30 million years of angiosperm leaf evolution, angiosperm leaves exhibited uniformly low vein D_v that overlapped the D_v range of dominant Early Cretaceous ferns and gymnosperms. Fossil angiosperm vein densities reveal a subsequent biphasic increase in D_v . During the first mid-Cretaceous surge, angiosperm D_v first surpassed the upper bound of D_v limits for nonangiosperms. However, the upper limits of D_v typical of modern megathermal rainforest trees first appear during a second wave of increased D_v during the Cretaceous-Tertiary transition. Thus, our findings provide fossil evidence for the hypothesis that significant ecosystem change brought about by angiosperms lagged behind the Early Cretaceous taxonomic diversification of angiosperms.

angiosperm evolution | plant evolution | transpiration | tropical rainforest | venation

Photosynthesis and transpiration by leaves fundamentally influence the cycling of carbon and water in the terrestrial realm. Consequently, evolutionary changes in the rates at which leaves exchange water for carbon bear on the origin and maintenance of biodiversity by varying the size and resource stoichiometry of the primary productivity base. How leaves exchange gases also shape climate and atmospheric gas composition by changing the amounts of water vapor and carbon in the atmosphere (1–3). Recent evidence has suggested that the evolution of flowering plants involved a sharp rise in the capacity of leaves to transport water and extract CO₂ from the atmosphere (4, 5).

The evolution of unrivaled CO₂ uptake and transpirational output by angiosperm leaves form the mechanistic cornerstone for a multitude of hypotheses citing angiosperms as agents of expansive ecosystem change during the Cretaceous (6–8). These hypotheses include (i) intensified mineral weathering by angiosperms that decreased global atmospheric CO₂ concentration; (ii) heightened transpirational input to the atmosphere that increased regional rainfall and favored the spread and diversity of tropical rainforest vegetation; (iii) the nearly complete competitive exclusion by angiosperms of diverse gymnosperms and ferns from high-productivity sites worldwide; and (iv) the spread of novel fire regimes that entrained a positive feedback on angio-

sperm takeover (3, 4, 6, 9–15). In addition, increasing terrestrial productivity furnished by the rise of angiosperms with highly photosynthetically active leaves has been posited as a large-scale driver for the mid-Cretaceous turning point when terrestrial biodiversity eclipsed that of the marine realm (16). However, the actual timing for when angiosperms first evolved their high capacities for leaf photosynthetic gas exchange remains poorly resolved (4, 5, 17–19).

A fundamental shift in how angiosperm leaf venation functions has been proposed as a key mechanism in the evolution of high photosynthetic gas exchange potential (4, 5). Evidence for a shift in angiosperm venation structure was reconstructed from living phylogenetic diversity as a surge in the vein length density (D_v) of angiosperm leaves that rose above the densities found across all other vascular plants during the mid-Cretaceous (5). In contrast to nonangiosperms, leaves of modern ecologically dominant angiosperms routinely exhibit tremendous vein lengths, which are evenly reticulated through the lamina (8). Leaves of tropical pioneer angiosperms, which operate at the upper limits of C₃ gas exchange for woody vegetation, possess an average of 13 mm and a maximum of 20 mm of vein length within a single square millimeter of leaf area (4). By comparison, all known extant and extinct nonangiosperm leaves fall within the range of 0.1–6 mm of vein length per square millimeter (4). Increased vein branching furnishes greater photosynthetic capacity because adding more veins brings the xylem-based water supply closer to the sites of evaporation in the leaf. Hence, D_v defines the hydraulic supply limit of water vapor exchange that secondarily constrains maximum CO₂ assimilation by leaves (4, 5, 8, 20, 21).

Vein density evolution in the angiosperms has been argued to represent an innovation that transformed global terrestrial biogeochemistry and biodiversity (3–5, 15, 16). However, evidence for the timing of this Earth-changing shift in leaf function remains untested in the fossil record and is limited to phylogenetically based reconstruction of vein evolution from living angiosperms (5). Clarifying when densely veined angiosperm leaves became the dominant fixture of the vegetation is critical for disentangling how Mesozoic environmental change affected angiosperm evolution and how innovations in early angiosperm function restructured Cretaceous ecosystem function (6, 11–16). Fossil angiosperm leaves of Cretaceous age often faithfully

Author contributions: T.S.F. designed research; T.S.F., A.I., D.S.C., A.B., G.R.U., B.G., B.A.R.M., C.C., J.K., and C.J. performed research; T.S.F., T.J.B., and A.I. analyzed data; and T.S.F., T.J.B., A.I., G.R.U., B.G., B.A.R.M., C.C., J.K., and C.J. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

¹To whom correspondence should be addressed. E-mail: hedyosmum@gmail.com.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1014456108/-DCSupplemental.

preserve the fine details of leaf venation (17, 22). Thus, vein densities of fossil leaves may offer signatures for ancient maximal leaf hydraulic limits and their evolutionary changes during the early angiosperm radiation (4). Interestingly, there are reports that early angiosperms possessed low vein densities, but when high D_V leaves that dominate modern angiosperm forests first appear remains unknown (22). In this paper, we analyze fossil angiosperm leaves through the Cretaceous to determine the evolutionary timing of angiosperms' D_V innovation. We measured vein densities of 307 taxa (approximate morphotypes) of fossil early angiosperm leaves as well as diverse gymnosperms and ferns from the early phases of angiosperm diversification and ecological expansion (Early Cretaceous to earliest Tertiary) and plotted vein density patterns through this crucial period.

Results

D_V values of the oldest angiosperm fossil leaves sampled (Hauterivian to Early Albian, 136–112 Ma) averaged $3.31 \text{ mm mm}^{-2} \pm 0.93 \text{ SD}$ ($n = 29$). These angiosperm leaf fossils nested within the range of diverse co-occurring and coeval gymnosperms and ferns (Fig. 1). In contrast with nonangiosperm fossil vein densities, which remained uniform through the Cretaceous to Paleogene (mean = $2.43 \text{ mm mm}^{-2} \pm 1.19 \text{ SD}$; $n = 107$), angiosperm D_V values first increased above the nonangiosperm range by the

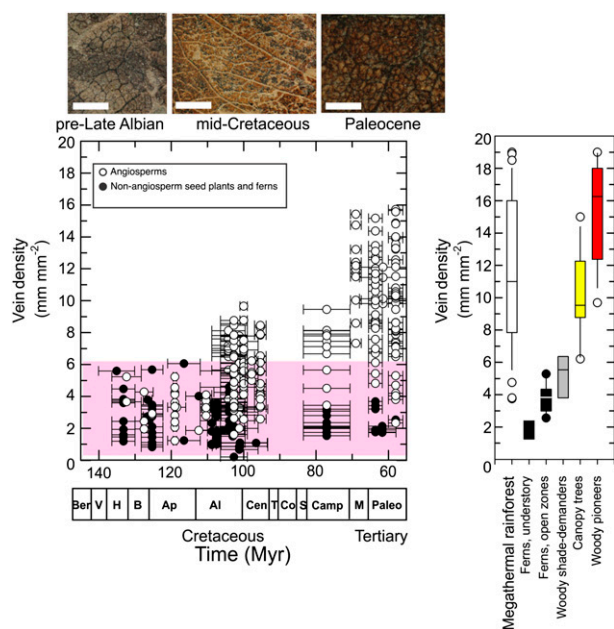


Fig. 1. Escalation in angiosperm D_V from Cretaceous to earliest Tertiary angiosperm fossil leaves (140–58 Ma). Angiosperm (○) and nonangiosperm (●) fossils represent mean D_V values per morphotype. Angiosperm D_V first increases above the nonangiosperm maximum at ~ 100 Ma (Late Albian). A second phase of angiosperm D_V increase occurred during the latest Cretaceous/earliest Tertiary (68–58 Ma). The fossil angiosperm D_V values during the second phase represent the earliest known densities comparable to the range of extant megathermal rainforest (Right box plot represents 25 species of woody plants across diverse regeneration guilds from Madang, Papua New Guinea). Representative fossil angiosperm leaves represent the pre-Late Albian (sparse D_V , *Ficophyllum crassinerve* = 3.5 mm mm^{-2}), mid-Cretaceous (moderately high D_V , *Sapindopsis magnolifolia* = 7.8 mm mm^{-2}), and Paleocene (dense D_V , morphotype CJ40 = 13.9 mm mm^{-2}) vein density shifts through time. Horizontal error bars around all data points represent the SD around the mean values for fossil ages. Geological ages: Ber, Berriasian; V, Valanginian; H, Hauterivian; B, Barremian; Ap, Aptian; Al, Albian; Cen, Cenomanian; T, Turonian; Co, Coniacian; S, Santonian; Camp, Campanian; M, Maastrichtian; Paleo, Paleocene. The shaded pink area denotes the range of D_V for all nonangiosperms, living and extinct (4). (Scale bar, 1 mm.)

Middle-Late Albian (106–100 Ma; Fig. 1). At 108–94 Ma (Late Albian-Cenomanian), mean angiosperm D_V ($5.55 \text{ mm mm}^{-2} \pm 1.79 \text{ SD}$, $n = 72$) became $\sim 2\times$ greater than that of nonangiosperms. Maximum D_V values for Late Albian-Early Cenomanian angiosperms increased to $\sim 40\%$ greater than the highest D_V values ($\sim 6 \text{ mm mm}^{-2}$) measured for nonangiosperms and were nearly $4\times$ greater than the nonangiosperm mean. The timing and magnitude for increased fossil angiosperm D_V during the mid-Cretaceous compared well to previous ancestral state reconstructions of D_V evolution across extant angiosperms (Fig. 2).

By the latest Cretaceous (Maastrichtian)-Paleocene, mean D_V values of angiosperms (mean = $9.76 \text{ mm mm}^{-2} \pm 3.0 \text{ SD}$; $n = 78$) increased 74% above the nonangiosperm sample through time, and the mean was 66% greater than the Hauterivian-Early Albian angiosperm mean. Several angiosperm D_V values from this timeframe became comparable to the vein densities found across extant canopy-dominants of the megathermal rainforest biome (Fig. 1).

Discussion

Fossil Evidence for a Cretaceous Surge in Angiosperm Vein Density.

Our measurements show fossil evidence that leaf hydraulic function changed dramatically during the Cretaceous radiation of angiosperms. We show that Early Cretaceous fossil angiosperm leaves shifted from possessing low vein densities to very high vein densities by the close of the Cretaceous. During the first 30 Myr of angiosperm leaf evolution seen in the fossil leaves from Brazil, China, and Europe, as well as North and South America across a large paleolatitudinal gradient (Dataset S1), angiosperm leaves exhibited uniformly low vein densities that fell in the range of dominant Early Cretaceous ferns and gymnosperms. These results support the conclusion that a widespread pattern of low leaf hydraulic potential existed for pre-Early Albian angiosperms (22).

By the latest Albian-Cenomanian (108–94 Ma), a rapid and sharp increase in angiosperm D_V became evident. Angiosperm D_V surpassed the upper bound of the physiological vein morphospace for living and fossil nonangiosperm vascular plant lineages during the mid-Cretaceous surge (4, 5). The highest fossil angiosperm D_V measured during the Late Albian approached 8 mm mm^{-2} and occurred in extinct platanoids (*Sapindopsis*) and Laurales. That these taxa defined the upper limits of mid-Cretaceous angiosperm vein densities supports earlier paleoecological evidence that platanoids and lauroids functioned with the most weedy, nonherbaceous ecologies at the time (17, 23). Interestingly, the timing and the amount of D_V increase found in mid-Cretaceous angiosperm fossils compared well to the pattern reconstructed from

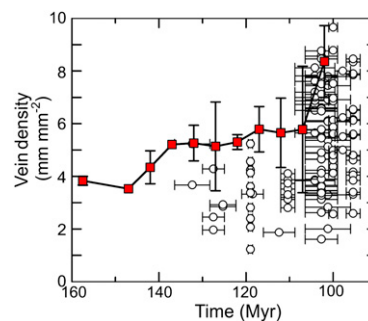


Fig. 2. The mid-Cretaceous increased angiosperm vein density based on fossil leaves (open circles) is comparable to the reconstructed vein density evolution based on ancestral states across extant angiosperm phylogeny (red squares) (5). Ancestral state values were averaged over 10-Myr slices with SD illustrated. Horizontal error bars around the fossil angiosperm data represent the SD around the mean values for fossil ages.

Conclusions

Our results indicate that a several-fold escalation in angiosperm leaf hydraulic capacity occurred during the Cretaceous. Increasing leaf hydraulic potential and linked high rates of transpiration by evolution of angiosperm vein densities exceeding those of nonangiosperms would have set in motion novel changes in the flows of carbon, water, and nutrients through the biosphere beginning in the mid-Cretaceous (3, 5, 15). However, the range of leaf hydraulic capacities representing the modern limits of woody angiosperm photosynthesis, which are those of capable of changing climates and foster today's enormous megathermal rainforest diversity and productivity, emerged long after angiosperm origin (3, 10, 13, 16, 19, 22, 23, 36, 37). Thus, our findings support previous interpretations of angiosperm paleoecology that despite tremendous phylogenetic diversification in the Early Cretaceous, transformative ecosystem change brought about by increased angiosperm productivity unfolded much later (24, 29).

Materials and Methods

D_v is the length of veins per unit leaf area (mm mm^{-2}). D_v was measured only on well-preserved compression fossils by tracing veins on fossil images

using ImageJ (<http://rsb.info.nih.gov/ij/>) on 5–12 mm^2 of leaf area (typically 2–4 areoles). We made three D_v measurements on each sampled fossil taxon or morphotype. We obtained images either by using a digital camera and macrolens or by digital measurements on previously figured fossils (Dataset S1). Fossils sampled included low, middle, and high paleolatitude belts from both hemispheres throughout much of the Cretaceous (Dataset S1). To examine how fossil angiosperms compare with megathermal lowland rainforest species, which operate at the modern limits of maximal leaf carbon/water exchange, we sampled 25 species across a range of regeneration guilds from a diverse lowland tropical rainforest in Madang, Papua New Guinea (mean annual temperature, 28 °C; annual rainfall, 4,500 mm with no significant dry season). We measured D_v of extant species on three samples per species using previously described techniques (4).

ACKNOWLEDGMENTS. We thank Lawong Balun for assistance during field work. Helpful comments were provided by Patrick Baker, Kevin Boyce, David Beerling, Claire Feild, Peter Wilf, and Hubert Feild. We thank Mauro Passalia, Griselda Puebla, Alejandra Gandolfo, and Elizabeth Hermsen for providing fossil leaf photography for D_v measurements. This work was supported by National Science Foundation Grant DEB-0919071, Consejo Nacional de Investigaciones Científicas y Técnicas (A.I.), and National Science Foundation Grant IOB-0714156 (to T.S.F.).

- Field CB, Behrenfeld MJ, Randerson JT, Falkowski P (1998) Primary production of the biosphere: Integrating terrestrial and oceanic components. *Science* 281:237–240.
- Kreft H, Jetz W (2007) Global patterns and determinants of vascular plant diversity. *Proc Natl Acad Sci USA* 104:5925–5930.
- Boyce CK, Lee JE (2010) An exceptional role for flowering plant physiology in the expansion of tropical rainforests and biodiversity. *Proc Biol Sci* 277:3437–3443.
- Boyce CK, Brodribb TJ, Feild TS, Zwieniecki MA (2009) Angiosperm leaf vein evolution was physiologically and environmentally transformative. *Proc Biol Sci* 276:1771–1776.
- Brodribb TJ, Feild TS (2010) Leaf hydraulic evolution led a surge in leaf photosynthetic capacity during early angiosperm diversification. *Ecol Lett* 13:175–183.
- Bond WJ (1989) The tortoise and the hare – ecology of angiosperm dominance and gymnosperm persistence. *Biol J Linn Soc Lond* 36:227–249.
- Körner C (1995) *Ecophysiology of Photosynthesis*, eds Schulze ED, Caldwell MM (Springer, Heidelberg), pp 463–490.
- Brodribb TJ, Feild TS, Jordan GJ (2007) Leaf maximum photosynthetic rate and venation are linked by hydraulics. *Plant Physiol* 144:1890–1898.
- Knoll AH (1986) *Community Ecology*, eds Diamond J, Case T (Harper and Row, New York), pp 126–144.
- Lidgard S, Crane PR (1988) Quantitative analyses of the early angiosperm radiation. *Nature* 331:344–346.
- Taylor LL, et al. (2009) Biological weathering and the long-term carbon cycle: Integrating mycorrhizal evolution and function into the current paradigm. *Geobiology* 7:171–191.
- Robinson JM (1994) Speculations on carbon dioxide starvation, Late Tertiary evolution of stomatal regulation and floristic modernization. *Plant Cell Environ* 17: 1–10.
- Lupia R, Lidgard S, Crane PR (1999) Comparing palynological abundance and diversity: Implications for biotic replacement during the Cretaceous angiosperm radiation. *Paleobiology* 25:305–340.
- McElwain JC, Willis KJ, Lupia R (2005) *A History of Atmospheric CO₂ and Its Effects on Plants, Animals, and Ecosystems*, eds Ehlering JR, Cerling TE, Dearing MD (Springer, Heidelberg), pp 133–165.
- Bond WJ, Scott AC (2010) Fire and the spread of flowering plants in the Cretaceous. *New Phytol* 188:1137–1150.
- Vermeij GJ, Grosberg RK (2010) The Great Divergence: When did diversity on land exceed that in the sea? *Integr Comp Biol* 50:1–8.
- Hickey LJ, Doyle JA (1977) Early Cretaceous fossil evidence for angiosperm evolution. *Bot Rev* 43:3–104.
- Feild TS, et al. (2004) Dark and disturbed: A new image of early angiosperm ecology. *Paleobiology* 30:82–107.
- Feild TS, Chatelet DS, Brodribb TJ (2009) Ancestral xerophobia: A hypothesis on the whole plant ecophysiology of early angiosperms. *Geobiology* 7:237–264.
- Sack L, Frole K (2006) Leaf structural diversity is related to hydraulic capacity in tropical rain forest trees. *Ecology* 87:483–491.
- McKown AD, Cochard H, Sack L (2010) Decoding leaf hydraulics with a spatially explicit model: Principles of venation architecture and implications for its evolution. *Am Nat* 175:447–460.
- Feild TS, et al. (2011) Fossil evidence for low gas exchange capacities for Early Cretaceous angiosperm leaves. *Paleobiology* 37:195–213.
- Upchurch GR, Wolfe JA (1993) Cretaceous vegetation of the Western Interior and adjacent regions of North America. *Geo Assoc Canada Spec Pub* 39:243–281.
- Wing SL, Boucher LD (1998) Ecological aspects of the Cretaceous flowering plant radiation. *Annu Rev Earth Planet Sci* 26:379–421.
- Wheeler EA, Baas P (1991) A survey of the fossil record for dicotyledonous wood and its significance for evolutionary and ecological wood anatomy. *IAWA J* 12:271–332.
- Philippe M, et al. (2008) Woody or not woody? Evidence for early angiosperm habit from the Early Cretaceous fossil wood record of Europe. *Palaeworld* 17:142–152.
- Oakley D, Falcon-Lang HJ (2009) Morphometric analysis of Cretaceous (Cenomanian) angiosperm woods from the Czech Republic. *Rev Palaeobot Palynol* 153:375–385.
- Wheeler EA, Lehman TM (2009) New Late Cretaceous and Paleocene dicot woods of Big Bend National Park, Texas and review of Cretaceous wood characteristics. *IAWA J* 30:293–318.
- Wing SL, Hickey LJ, Swisher CC (1993) Implications of an exceptional fossil flora for Late Cretaceous vegetation. *Nature* 363:342–344.
- Iglesias A, et al. (2007) A Paleocene lowland macroflora from Patagonia reveals significantly greater richness than North American analogs. *Geology* 35:947–950.
- Wing SL, et al. (2009) Late Paleocene fossils from the Cerrejón Formation, Colombia, are the earliest record of Neotropical rainforest. *Proc Natl Acad Sci USA* 106: 18627–18632.
- Gale J, Rachmilevitch S, Reuveni J, Volokita M (2001) The high oxygen atmosphere toward the end-Cretaceous; a possible contributing factor to the K/T boundary extinctions and to the emergence of C_4 species. *J Exp Bot* 52:801–809.
- Beerling DJ, Franks PJ (2010) Plant science: The hidden cost of transpiration. *Nature* 464:495–496.
- Barclay RS, McElwain JC, Sageman BB (2010) Carbon sequestration activated by a volcanic CO₂ pulse during Ocean Anoxic Event 2. *Nat Geosci* 3:205–208.
- Royer DL (2010) Fossil soils constrain ancient climate sensitivity. *Proc Natl Acad Sci USA* 107:517–518.
- Glasspool IJ, Scott AC (2010) Phanerozoic concentrations of atmospheric oxygen reconstructed from sedimentary charcoal. *Nat Geosci* 3:627–630.
- Smith SA, Beaulieu JM, Donoghue MJ (2010) An uncorrelated relaxed-clock analysis suggests an earlier origin for flowering plants. *Proc Natl Acad Sci USA* 107:5897–5902.