

LETTER

Leaf hydraulic evolution led a surge in leaf photosynthetic capacity during early angiosperm diversification

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Abstract

Angiosperm evolution transformed global ecology, and much of this impact derives from the unrivalled vegetative productivity of dominant angiosperm clades. However, the origins of high photosynthetic capacity in angiosperms remain unknown. In this study, we describe the steep trajectory of leaf vein density (D_v) evolution in angiosperms, and predict that this leaf plumbing innovation enabled a major shift in the capacity of leaves to assimilate CO₂. Reconstructing leaf vein evolution from an examination of 504 angiosperm species we found a rapid three- to fourfold increase in D_v occurred during the early evolution of angiosperms. We demonstrate how this major shift in leaf vein architecture potentially allowed the maximum photosynthetic capacity in angiosperms to rise above competing groups 140–100 Ma. Our data suggest that early terrestrial angiosperms produced leaves with low photosynthetic rates, but that subsequent angiosperm success is linked to a surge in photosynthetic capacity during their early diversification.

Keywords

Angiosperms, atmospheric CO₂, dominance, evolution, gas exchange, gymnosperms, hydraulics, leaf, photosynthesis, veins.

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INTRODUCTION

The maximum limits of photosynthesis and growth in angiosperms are unrivalled among land plants. High rates of photosynthesis in the leaves of angiosperm crops and forest trees form the productivity base for most terrestrial ecosystems as well as for human civilization (Field *et al.* 1998). Despite the significance of high angiosperm productivity to biosphere function, there is little information about when and how the capacity for very high rates of photosynthetic CO₂ assimilation evolved within the angiosperm clade. The classical view of early angiosperms as opportunistic weedy plants with high rates of growth (Stebbins 1974; Hickey & Doyle 1977; Wing & Boucher 1998) assumes high productivity as the ancestral state for angiosperms. However, this view has been challenged recently by evidence suggesting that angiosperms originated as forest understorey species (Feild *et al.* 2004) with inferred low photosynthetic capacity (Feild *et al.* 2009). These contrasting hypotheses have very different implications for understanding the timing and mechanisms behind terrestrial productivity evolution on

Earth. Such a knowledge gap remains a major obstacle for understanding how angiosperm radiation in the Cretaceous period transformed the ecological and biogeochemical function of the biosphere (Taylor *et al.* 2009).

High productivity in angiosperms is often cited as a major contributor to their success over other plant groups (Bond 1989; Berendse & Scheffer 2009). In turn, several innovations in the angiosperm vegetative system have been hypothesized as adaptive triggers for photosynthetic evolution, including xylem vessels (Carlquist 1975), phloem specialization (Esau *et al.* 1953; Turgeon *et al.* 2001; Van Bel 2003) and expanded root/mycorrhizal complexity (Brundrett 2002). The assumption underlying all of these hypotheses is that prior to angiosperm evolution, plant productivity was relatively low, and that by virtue of an evolutionary leap in vegetative function, angiosperms became prevalent by achieving rates of productivity that were higher than other competing land plants. This assumption remains untested and there has been no attempt to understand the early course of photosynthetic evolution in angiosperms with C3 photosynthesis.

Current models of photosynthetic rate through geological time are parameterized using palaeo-temperature, CO₂ and O₂ reconstructions that range so widely that these uncertain inputs ultimately drive the output of the model (Beerling & Woodward 1997). Consequently, this modelling approach has a very limited ability to view the impact of adaptive changes in leaf function that potentially change the competitiveness of one plant group over another. In this study, we take a new approach to reconstructing photosynthetic rate evolution in angiosperm leaves by observing long-term changes in leaf vein density as a robust proxy for evolutionary changes in leaf gas exchange capacity. Our photosynthetic metric describes the maximum capacity for photosynthesis (on an area basis) under static atmospheric conditions (photosynthetic capacity, P_c), rather than making absolute predictions of photosynthetic rate from reconstructed atmospheric conditions. Normalizing photosynthetic capacity in this way allowed us to differentiate the effects of functional evolution from the poorly understood course of changing atmospheric CO₂ over the Phanerozoic epoch (Kuypers *et al.* 1999; Berner & Kothavala 2001; Fletcher *et al.* 2008).

The hydraulic approach employed in this study is based on recent studies demonstrating a strong functional linkage between the hydraulic and photosynthetic performance of leaves (Brodribb *et al.* 2007; Boyce *et al.* 2009). Data from a large range of living plant species has verified that the maximum photosynthetic rate of leaves can be reliably predicted from the density of veins in the leaf lamina (D_v) (Sack & Frole 2006; Brodribb *et al.* 2007; Noblin *et al.* 2008; Boyce *et al.* 2009). Water transport and photosynthetic systems are coupled because vascular plants exchange water and CO₂ through a common pathway; the stomatal pores in the leaf (Fig. 1) (Wong *et al.* 1979). Stomatal dimensions themselves could be used to estimate the diffusion limitation to photosynthesis, but leaf epidermal features such as stomatal encryption and waxy deposits often disrupt the relationship between pore dimensions and gas exchange capacity (Roth-Nebelsick 2007). Alternatively, a hydraulic approach examines the supply of water as a sensitive proxy for the maximum limit for photosynthetic gas exchange. The hydraulic capacity of leaves is largely determined by vein architecture because increased vein branching brings specialized water transport xylem tissue closer to the sites of evaporation in the leaf (Sack & Holbrook 2006; Brodribb *et al.* 2007; Noblin *et al.* 2008). Maximum hydraulic conductance would be achieved if veins branched such that they contacted all living cells in the leaf, but no plant makes such an enormously expensive and complex architectural investment. Instead, leaves trade-off between the structural investment in minor veins and photosynthetic enhancement achieved by increased D_v . Of course other traits besides veins – such as leaf tissue optics, stomatal morphology and

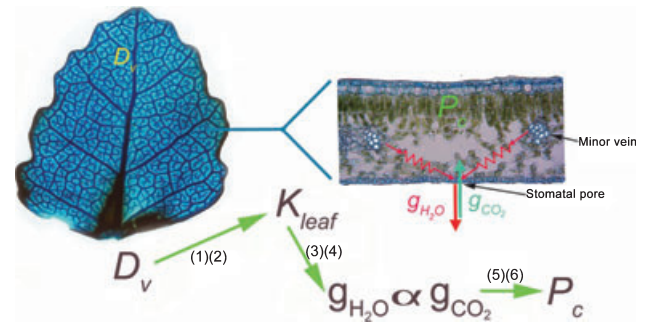


Figure 1 A diagrammatic explanation of how vein density in the leaf lamina (D_v) limits the photosynthetic capacity of the leaf (P_c). Green arrows indicate how anatomical, hydraulic, diffusive and biochemical processes interact to link D_v and P_c , and numbers indicate the physical relationships between processes (see Supporting information for equations). In passing from the ends of the veins to the stomata, the transpiration stream (red arrows) encounters a large resistance to flow as it moves out of specialized transport tissue (xylem) and through the living mesophyll cells. The size of this resistance depends on the hydraulic distance from the vein endings to the stomata, and hydraulic efficiency is thus determined by D_v . In leaves with high D_v the leaf hydraulic conductivity is large enabling the leaf stomata to exchange large quantities of water vapour and CO₂. Conversely, low D_v cannot support high rates of leaf gas exchange.

photosynthetic biochemistry – can also influence P_c (Smith *et al.* 1997). However, vein function sets an upper limit of P_c for any terrestrial leaf because D_v sets a biophysical hydraulic supply constraint that cannot be exceeded.

In this study, we integrate current knowledge of angiosperm phylogeny with hydraulically based reconstructions of photosynthesis in 759 species of living and fossil plants to determine the trajectory of photosynthetic evolution during early angiosperm diversification. Our analysis suggests that the leaves of early angiosperms possessed low photosynthetic rates, but that a rapid period of leaf vein evolution in the Late Cretaceous enabled angiosperms to occupy a new level of photosynthetic performance unattainable in other competing land plants.

MATERIALS AND METHODS

Dataset and leaf D_v determination

A large data set of 759 vascular plant species was compiled to investigate the evolutionary diversification of leaf D_v , defined as the length of veins (mm) divided by the surface area of lamina (mm²). Our dataset included extant angiosperms (504 species) as well as both living (89 species) and extinct (166 species) non-angiosperms. Data from 255 non-angiosperm taxa and 186 core eudicot taxa were taken from Boyce *et al.* (2009) and D_v values, sample sources and

ecologies of the new data for 318 additional species reported here can be found in the Table S1. To determine the early evolutionary diversification of D_v in angiosperms, we focused our sampling on taxa belonging to major phylogenetic divergences resolved at the base of extant angiosperm phylogeny by the most recent molecular studies (Table S2). These major lineages included: basal lineages (*Amborella*, Nymphaeales, Austrobaileyales), Chloranthales, Magnoliidae (Canellales, Laurales, Magnoliales, Piperales), basal eudicots (Buxales, Gunnerales, Ranunculales, Proteales, Sabiales, Trochodendrales) and basal monocots (Acorales, Arales; classification as in Table S1) as well as a diversity of core eudicot and monocot lineages. We excluded basal angiosperm aquatics (such as *Ceratophyllum*) with totally submerged leaves from our analyses because aquatic and aerial environments are not comparable with respect to hydraulic transport (Feild *et al.* 2009).

For leaves collected from the field, we selected fully expanded, undamaged leaves from the most sun-exposed environment available. Fieldwork sampling was conducted from 2001 to 2009 in Australia, China, Costa Rica, Dominican Republic, Fiji, French Polynesia, Jamaica, Madagascar, New Caledonia, New Zealand, Papua New Guinea, Peru, Thailand, USA and Vietnam. Finally, we also sampled leaves of several difficult to collect taxa from the National Cleared Leaf Collection housed at the Smithsonian Institution, Washington, DC, USA.

To determine D_v , we cleared sections of leaf tissue ($\approx 3 \text{ cm}^2$) using standard procedures that use sodium hydroxide and sodium hypochlorite for tissue bleaching followed by safranin or toluidine blue for vein staining and mounting in xylene-Permount for microscopic observations (Boyce *et al.* 2009). We imaged cleared leaves with a digital camera (AxioCAM; Carl Zeiss, Germany) at 20–40 \times using an upright microscope (AxioImager M1; Carl-Zeiss, Germany). Vein lengths on digital images were determined using IMAGEJ (NIH Image, Bethesda, MD, USA). Five measurements of D_v were made from a minimum of three leaves from three individuals of each species.

Reconstruction of angiosperm D_v through time

We assembled 36 molecular phylogenies of angiosperms into a composite phylogenetic tree by grafting them onto two well supported ‘backbone’ topologies (Jansen *et al.* 2007; Moore *et al.* 2007; Endress & Doyle 2009). The two selected backbone topologies used in our analyses have been the focus of extensive comparative research and represented the current accepted topologies in the assessment of phylogenetic patterns of trait evolution at the base of extant angiosperm phylogeny. The two backbone topologies differed in the placement of Chloranthales and monocots, and we included such phylogenetic uncertainty in our

analyses. Then, we pruned the species level phylogenies grafted onto both the backbone topologies to leave only the species for which we had D_v values. The composite phylogenetic tree was constructed using MESQUITE version 2.6 (Maddison & Maddison 2008). Ancestral state values of D_v were reconstructed using weighted squared-change parsimony (SqP) over species terminals of the two supertrees using MESQUITE. SqP minimizes the sum of squared change along all branches of the tree to reconstruct the values of internal nodes based on the trait values of the species terminals (Maddison & Maddison 2008). We used SqP to calculate ancestral states because most of the species that we sampled did not have specific molecular sequence data. In addition, we used SqP because the method enabled us to include out-group fossil taxa to infer ancestral states for the angiosperm stem-lineage including Bennettiales, Caytoniales, glossopterids and peltasperms (Table S3). In cases where the phylogenetic relations of clades were uncertain, we used MESQUITE to randomly resolve phylogenetic relations within clades. Our procedure involved randomizing polytomies 100 times in MESQUITE and the D_v values for the ancestral state for a clade averaged and a standard deviation calculated. We also included our results based on the non-randomized consensus tree in our average value of D_v for all phylogenetic node estimates. In all cases, we found differences between these estimates of D_v ancestral state were very small ($< 10\%$).

To infer the evolution of angiosperm D_v through time, we integrated the current published data on dating the phylogenetic divergences within angiosperms using fossil-calibrated molecular clock approaches to infer the timings for the ancestral state values of D_v through time (Table S3). As an alternative to molecular divergence-based age estimates, we compiled age data for phylogenetically assessed fossils that have been placed on stem or crown lineages of interest. We then applied these fossils as a minimum age estimates (Table S3). The minimum age approach represents a more conservative approach over using molecular divergence times because it makes fewer assumptions that are often difficult to evaluate (Crepet 2008).

Modelling photosynthetic capacity in living and fossil leaves

Photosynthetic capacity was reconstructed in the 759 species of living and fossil leaves using a model that combined hydraulic, diffusive and biochemical limitations to photosynthesis (see Appendix 1). The basis of the model is that under non-limiting conditions of soil water availability, leaf hydraulic conductance (K_{leaf} : $\text{mmol m}^{-2} \text{ s}^{-1}$) can be accurately calculated from the distance water must flow from the vein terminals to the sites of evaporation

(Brodribb *et al.* 2007). Knowledge of K_{leaf} enables maximum stomatal conductance to water to be calculated which in turn provides a basis for the calculation of P_c .

To focus our reconstructed P_c on productive canopy dominant and open-habitat clades, we sub-sampled reconstructed D_v from nodes in the top 10% of the D_v range at any 10 Myr time slice. This was performed to eliminate clades that have radiated into low productivity habitats such as oligotrophic soil, shade, cold or desert environments. Using the upper 10% of the data (shown in Fig. 2), we found that a sigmoid function best summarized the trajectory of reconstructed angiosperm D_v against time. The equation for this sigmoidal function [$D_v = 3.3 + 5.30/(1 + e^{-(time-130)/-8.7})$; $r^2 = 0.93$] was then substituted into our photosynthetic model, allowing the trajectory of P_c to be reconstructed over time for a range of leaf morphologies (see Supporting information Appendix 1).

Although P_c was calculated at current atmospheric concentrations of CO_2 and O_2 , we also investigated the possible impact of different CO_2 concentrations equivalent to that suggested to occur from the Late Cretaceous to present (Fletcher *et al.* 2008). Maximum reconstructed P_c from early angiosperms and non-angiosperms were compared with those of derived angiosperm clades under 200, 400, 600, 800 and 1000 $\mu mol mol^{-1}$ [CO_2].

Modelled optimum vein density

Changes in paleo- CO_2 have been suggested as a trigger for angiosperm evolution. Hence we were interested to model the possible influence of $[CO_2]_{atm}$ on vein density evolution in angiosperms. Our analysis was based on the assumption that that leaf veins are costly to produce and hence the evolutionary pressure to increase D_v will only be strong while the photosynthetic benefits are significant. Because the relationship between D_v and photosynthesis is saturating at high vein densities (Fig. S4), it is possible to define an 'optimal' investment in leaf venation as the value of D_v that achieved 95% saturation of P . Different values of $[CO_2]_{atm}$ yielded different relationships between D_v and P (Fig. S4), thereby allowing optimal D_v to be predicted at a range of $[CO_2]_{atm}$. For $[CO_2]_{atm}$ we used the same range as above.

Leaf gas exchange

Gas exchange rates (CO_2 and water vapour) were measured on a sample of 35 species including eudicots, magnoliids, basal angiosperms and non-angiosperms to test the predictions of our hydraulic model with empirical measures. Leaves were measured under saturating light (cuvette light intensity set to match full sun conditions at the level of the sample leaf) using an open gas exchange analyser (Li6400; Licor Biosciences, Lincoln, NE, USA) in plants growing in natural

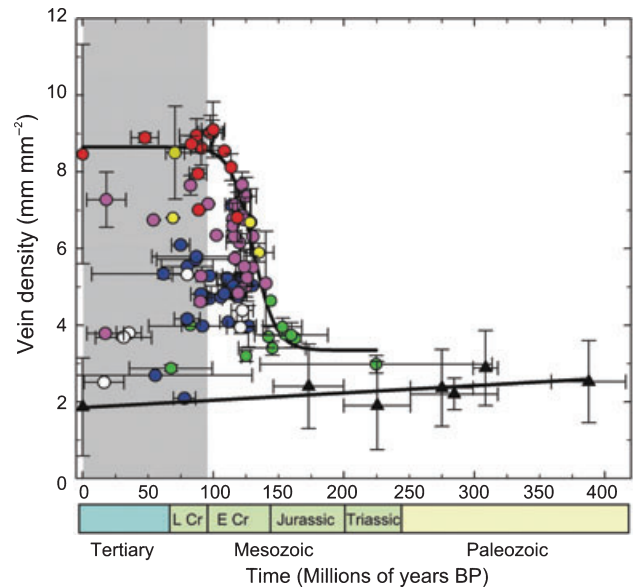


Figure 2 A Cretaceous vein density surge in angiosperms. Leaf vein density (D_v) reconstructed for key nodes of extant angiosperm phylogeny (circles) provides evidence for a dramatic increase during the Mid to Late Cretaceous from low values of 3 to 9 $mm mm^{-2}$. No such pattern was observed for a sample of non-angiosperm fossil and living plants (triangles) which showed no significant trend in D_v over a period of 400 Ma to the present. The reconstructed peak in angiosperm D_v occurred *c.* 5 Ma before the massive rise in angiosperm diversification, and greatly increased angiosperm ecological dominance (noted by the shaded box) documented in global fossil inventories (Teslenko 1967). A timeline of angiosperm D_v evolution is denoted by a solid line regression through the upper ten percent of D_v values at any 5 Ma time slice. The D_v values for major deep-level nodes of extant angiosperms reconstructed include basal eudicots (blue circles), basal-most lineages (green circles), Chloranthales (white circles), core eudicots [including the mean and SD ($n = 193$) of extant species; red circles], magnoliids (pink circles) and monocots (yellow circles). Angiosperm reconstructions here are based on data from extant species, but the pattern for fossil species is very similar (Fig. S1). Horizontal error bars around the angiosperm points represent standard deviation around the mean values of the molecular divergence time estimates and phylogenetic reconstructions of D_v . Non-angiosperm D_v values through time represent time-averages (error bars \pm SD, black triangles) for a dataset of fossils and extant plants ($y = 1.85 + 1.90x$; $r^2 = 0.43$).

forest under optimal conditions of water availability. Leaf temperatures were in the range 24–28 °C, CO_2 and O_2 were maintained at ambient levels (380 $\mu mol mol^{-1}$ and 21%) and atmospheric vapour pressure deficit (VPD) was maintained between 1.5 and 2.5 kPa. Mean instantaneous rates of photosynthesis and water loss were measured on 10 leaves from 3 to 5 individual plants between 0900 and 1100 h.

RESULTS

Reconstructed vein density evolution

From an examination of leaf veins in 504 angiosperm species, we found evidence of a major transformation in leaf vein architecture during the early evolution of angiosperms. The reconstructed D_v for basal angiosperm clades indicated low values not dissimilar to non-angiosperm clades (Fig. 2). However, 40–60 Ma after origin of the angiosperm crown group, maximum D_v in angiosperm leaves increased dramatically to high values far above D_v maxima in the non-angiosperm flora (Fig. 2). By the Late Cretaceous (90–100 Ma) reconstructed D_v for angiosperm leaves appears to have risen from $\approx 3.3 \text{ mm mm}^{-2}$ to a peak of 8.6 mm mm^{-2} (Fig. 2). This maximum D_v in angiosperm leaves represents a threefold increase above the static 380 Ma average for non-angiosperm vascular plants. Our reconstructed pattern of D_v corresponded well with observed shifts in venation complexity observed previously (Hickey & Doyle 1977) in early angiosperm fossil leaves from the Early Cretaceous (Figs S1 and S2).

Modelled evolution of photosynthetic capacity

The observed transformation in vein architecture of leaves was used to reconstruct the functional maximum rates of water loss and CO_2 uptake in angiosperm leaves using a linked hydraulic-photosynthetic model. The threefold rise in maximum D_v that occurred during the early evolution of angiosperms is predicted to have increased the hydraulic efficiency of leaves more than twofold, thereby allowing leaves to transpire more water through the stomata (Fig. S3). Leaf permeability to water vapour and CO_2 are directly related and hence the maximum photosynthetic uptake of CO_2 is reconstructed to rise in parallel with stomatal conductance, increasing by 174% during the diversification of angiosperms. At its peak, P_c in angiosperms would have risen to more than double the reconstructed photosynthetic maximum in non-angiosperms (Fig. 3). Expressed in terms of modern day atmospheric CO_2 and O_2 concentrations, this represents an important increase in maximum photosynthetic rates from $12.1 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ in non-angiosperms before the Cretaceous, to $26.3 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ in Late Cretaceous angiosperms. In contrast to angiosperms, the reconstructed P_c for ferns and gymnosperms remains static over the 400 Ma period examined. Maximum P_c measured in extant ferns and gymnosperms with laminate leaves remains in the predicted range, only *Pinus* leaves have managed to exceed this range by producing needle leaves (Brodrribb & Feild 2008) which partially overcome vein density limitations (Fig. 3).

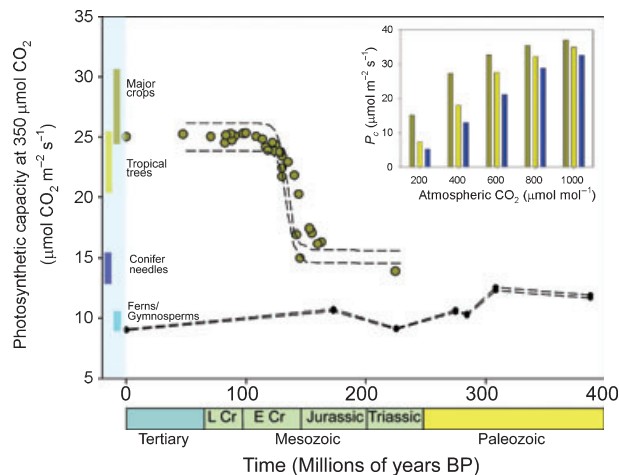


Figure 3 A Cretaceous surge in angiosperm photosynthetic capacity. Trajectories of reconstructed leaf photosynthetic capacity (P_c) in C3 angiosperms (khaki circles, dashed curve) and non-angiosperm vascular plants (black dots, broken line) are shown. An abrupt surge in maximum angiosperm photosynthesis is evident in the Late Cretaceous, rising from levels close to the non-angiosperm maximum, to levels far beyond other clades. Relatively high P_c in extant angiosperms (green bars show P_c maxima for rice, wheat and soybean as well as tropical trees) are thought to contribute significantly to their success over competing clades, and data here trace this advantage back to the Late Cretaceous. Photosynthetic capacities are reconstructed values from D_v (leaf vein density; Fig. 2) and a coupled hydraulic-photosynthetic model (see Supporting information Appendix 1). A constant value for atmospheric $[\text{CO}_2]$ of $350 \mu\text{mol mol}^{-1}$ was used when modelling P_c , to emphasize the comparative advantage of angiosperms over non-angiosperm groups. Modelled rates of photosynthesis under different levels atmospheric CO_2 (khaki, derived angiosperms; yellow, basal angiosperms; blue, non-angiosperms) demonstrate an increase in the relative photosynthetic advantage of high D_v when atmospheric $[\text{CO}_2]$ is $< 1000 \mu\text{mol mol}^{-1}$.

Impacts of varying atmospheric $[\text{CO}_2]$

Enhanced D_v in angiosperms is predicted to have a major impact on maximum photosynthetic rate when modelled at an ambient $[\text{CO}_2]$ of $350 \mu\text{mol mol}^{-1}$. However, our hydraulic model predicts that the advantage derived from vein elaboration is sensitive to ambient $[\text{CO}_2]$. Under low atmospheric CO_2 concentrations typical of Cenozoic glacial periods [$200 \mu\text{mol mol}^{-1} \text{CO}_2$ (Nefel *et al.* 1982)], angiosperm leaves with high D_v are predicted to have a photosynthetic maximum 270% higher than non-angiosperms (Fig. 3 insert), however this advantage was found to diminish to 13% at an atmospheric $[\text{CO}_2]$ of $1000 \mu\text{mol mol}^{-1}$.

As a result of a saturating relationship between D_v and photosynthetic rate in leaves (Fig. S4), plants experience

rapidly diminishing photosynthetic returns from vein investments that yield > 95% of the maximum possible photosynthetic rate. We defined optimum investment in vein elaboration as the D_v at 95% of maximum photosynthesis. Optimum D_v was found to increase greatly with decreasing $[\text{CO}_2]_{\text{atm}}$. A 10-fold range of optimum D_v was predicted to occur, from 1.7 mm mm^{-2} at a $[\text{CO}_2]_{\text{atm}}$ of 1500 $\mu\text{mol mol}^{-1}$, to 13.1 mm mm^{-2} at a $[\text{CO}_2]_{\text{atm}}$ of 200 $\mu\text{mol mol}^{-1}$ (Fig. 4).

DISCUSSION

The proficiency with which leaves fix CO_2 is a central axis of plant selection (Stebbins 1974; Sperry 2003) and our data demonstrate how a profound change in the structure of angiosperm leaves is likely to have expanded the maximum photosynthetic capacity of land plants. Along with the evolution of stomata, vascular tissue and C4 photosynthesis (Sage 2004), the dense elaboration of leaf veins appears to

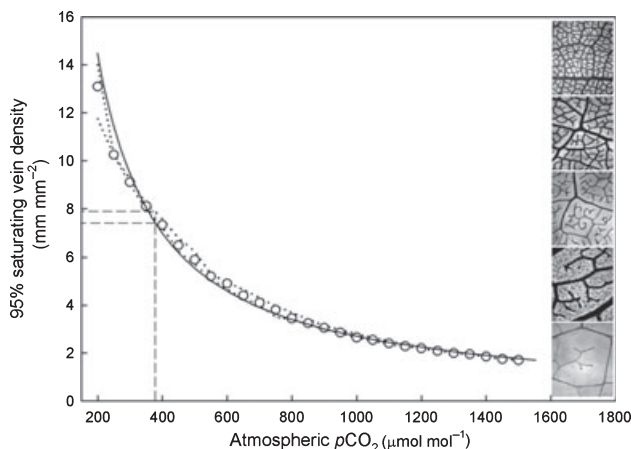


Figure 4 Optimal vein density under different atmospheric CO_2 regimes. Optimal leaf vein density (D_v) for sun leaves, defined as D_v required to 95% saturate photosynthetic capacity, is shown to be highly sensitive to the atmospheric CO_2 concentration $[\text{CO}_2]_{\text{atm}}$. Open circles show modelled optimal D_v at 50 $\mu\text{mol mol}^{-1}$ steps in $[\text{CO}_2]_{\text{atm}}$ over the median range of $[\text{CO}_2]_{\text{atm}}$ estimated since the evolution of angiosperms. Evolutionary pressure to increase D_v is predicted to become increasingly strong as $[\text{CO}_2]_{\text{atm}}$ falls below 1000 $\mu\text{mol mol}^{-1}$. Dashed lines show that the optimal D_v predicted for present day $[\text{CO}_2]_{\text{atm}}$ is only slightly below the mean maximum D_v observed in our angiosperm sample (Fig. 2). Our analysis suggests that declining $[\text{CO}_2]_{\text{atm}}$ during early angiosperm evolution may have catalysed the rise of angiosperms at the expense of other vascular plants. A series of micrographs demonstrate the range of D_v occurring in our sample of extant angiosperms. Taken at the same magnification (4 \times) D_v increases linearly from the most basal living angiosperm (*Amborella trichopoda*; $D_v = 2.3 \text{ mm mm}^{-2}$) to a derived Fabaceae (*Bauhinia binata*; $D_v = 19.1 \text{ mm mm}^{-2}$).

have been a landmark event in the history of land plant functional evolution.

Angiosperm evolution

Increased photosynthetic rates provide plants with more carbon for growth, reproductive output, carbon storage and synthesis of secondary metabolites. It is in these measures of plant function that modern angiosperms seem to outperform their competitors (Stebbins 1974; Regal 1977; Gottsburger 1988; Bond 1989; Verdu 2002; Williams 2008). A widely cited hypothesis for how angiosperms were able to displace their predecessors focuses on the superior growth characteristics of angiosperms compared with other terrestrial plant groups (Bond 1989). According to this hypothesis, angiosperms find success in environments wherever abiotic stresses such as cold and low nutrients do not limit potential productivity. Our reconstructed trajectories of P_c through time strongly support the hypothesis that photosynthetic rate evolution in angiosperms was a major factor influencing changes in the relative dominance of angiosperms over other groups. However, we found that high photosynthetic capacity is not the ancestral condition in terrestrial angiosperms. Instead, our analysis suggests P_c evolution occurred synchronously with angiosperm radiation.

Our results suggest that evolutionary innovation in angiosperm leaves opened up a range of P_c that has been inaccessible to other clades of plants. Reconstructions of leaf D_v evolution here provide evidence that by the Cenomanian [*c.* 40 Ma after the earliest evidence of angiosperms (Lupia *et al.* 1999)] the increased capacity for water transport and photosynthesis would have given angiosperms a substantial competitive advantage in productive environments (Figs 3 and S1). However, we also show that high P_c , which today is emblematic of angiosperm canopy trees, arose after a prolonged period of diversification at low P_c in terrestrial habitats (Fig. 3). Consistent with the hypothesis that high P_c evolved after a considerable temporal lag following an early radiation, we found evidence supporting a new ecophysiological explanation for the major evolutionary trend of increasing leaf rank in fossil leaves (Figs S1 and S2). Leaf rank describes the maximum vein order at which branching angles and areole size are uniform, and many workers have noted that leaf rank increases in fossil angiosperm leaves during the Early Cretaceous (Barremian to Cenomanian) (Hickey & Doyle 1977; Wing & Boucher 1998; Cuneo & Gandolfo 2005). We observed that D_v is positively correlated with leaf rank in extant angiosperms (Fig. S2). Thus, our reconstructed trajectory of D_v evolution is strongly convergent with the timings for the first appearance of highly organized venation patterns in Cretaceous fossil angiosperm leaves (i.e. at leaf rank 4 and corresponding to a D_v from 10 to 14 mm mm^{-2} in

extant plants). Agreement between these two measures of vein architecture strongly supports our conclusion that the Cenomanian represented the key geological interval for the first appearance of hyper-productive angiosperm leaves.

Photosynthetic evolution

Framed in the context of human agricultural crops or plants that dominate highly productive lowland tropical rainforests, the photosynthetic transformation reconstructed here is crucial. We predict that during early angiosperm diversification P_c increased from values below those of any important tropical rainforest tree or crop plant, to a peak equivalent to lowland tropical rainforest dominants such as dipterocarp trees, and staple C3 crop plants such as wheat, soybeans and rice, which range from 24 to 32 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ (Fig. 3). It is significant to note that without the evolution in D_v that occurred in angiosperms, land plants would not have the physical (hydraulic) capability to drive the high productivity that underpins modern terrestrial biology and human civilization. The reason for this is that under relatively low atmospheric $[\text{CO}_2]_{\text{atm}}$ like those existing at present, substrate (CO_2) limitation of photosynthesis is large, with the result that hydraulic efficiency exerts substantial leverage over the rate of photosynthesis (see below). Hence, without the vein density surge and linked rise in leaf hydraulic efficiency that occurred during angiosperm evolution, we predict that leaf photosynthesis would be almost twofold lower than present (Fig. 3).

Uncertainty remains as to why it took *c.* 250 Ma for plants to undergo the radical increase in D_v that would greatly expand leaf performance. It has long been conjectured that declining $[\text{CO}_2]_{\text{atm}}$ during the Cretaceous was a major stimulus for the radiation of angiosperms (Teslenko 1967; Robinson 1994; McElwain *et al.* 2005) but the mechanisms explaining early angiosperm diversification in the face of falling $[\text{CO}_2]_{\text{atm}}$ remain enigmatic. The large increase in D_v reconstructed here during the mid-Cretaceous finally provides the mechanistic link between a postulated decline in $[\text{CO}_2]_{\text{atm}}$ and angiosperm evolution. Falling $[\text{CO}_2]_{\text{atm}}$ inevitably increases the ratio of water transpired to carbon assimilation (Polley *et al.* 1993; Sperry 2003). Thus, under conditions of declining $[\text{CO}_2]_{\text{atm}}$ the only means for C3 plants to attenuate declining photosynthetic rate is to increase the conductance of the epidermis to CO_2 (increasing the density and aperture of stomata) thereby incurring greater transpirational losses. The resultant increase in hydraulic demand at the leaf means that D_v must be elaborated to ensure water transport efficiency matches increased transpirational demand (Sperry 2000). As such it would be expected that evolutionary pressure to increase D_v under these circumstances would be significant (Figs 4 and S4).

Angiosperms would have been ideally suited to respond to atmospheric conditions that increased the hydraulic cost of photosynthesis, either from falling $[\text{CO}_2]_{\text{atm}}$ or water vapour concentration, or a combination of both. The ability in angiosperms to develop xylem vessels in leaf veins (Bierhorst & Zamora 1965) creates an economical means of expanding the hierarchical organization (Coomes *et al.* 2008) and density of leaf veins. We suggest that leaf hydraulic innovations such as vessels and high D_v were selected in angiosperms because of their influence over P_c . Other changes in leaf physiology such as increased leaf N and stomatal density (Franks & Beerling 2009) may have also occurred in parallel with D_v to sustain rising P_c during angiosperm evolution. However, the evidence here points to major structural innovation in the hydraulics of angiosperm leaves as playing a central role in P_c evolution by ameliorating a fundamental biophysical (water transport) constraint on P_c .

Given that the relative photosynthetic benefit of increasing D_v increases greatly as $[\text{CO}_2]_{\text{atm}}$ falls below 1000 $\mu\text{mol mol}^{-1}$ (Fig. 3 insert) our data suggest that declining $[\text{CO}_2]_{\text{atm}}$ (below mid Cretaceous highs of $> 1000 \mu\text{mol mol}^{-1}$) would be required for the observed pattern of angiosperm leaf evolution to emerge. Global biogeochemical carbon models and several palaeo-proxies of $[\text{CO}_2]_{\text{atm}}$ also indicate substantial drops in $[\text{CO}_2]_{\text{atm}}$ over the Cretaceous timeframe but the data are far from concordant on trends in $[\text{CO}_2]_{\text{atm}}$ during this time (Kuypers *et al.* 1999; Berner & Kothavala 2001; Royer *et al.* 2001; Fletcher *et al.* 2008). In particular, rapid perturbation events that are invisible in geological models may be significantly involved. For example, the global ocean-anoxic event II at the Cenomanian–Turonian boundary, which may have resulted in $[\text{CO}_2]_{\text{atm}}$ as low as 500 $\mu\text{mol mol}^{-1}$ for 1 Ma, may have provided an environmental opportunity to catapult the dominance of high D_v leaves over the globe (Kuypers *et al.* 1999). Interactions between $[\text{CO}_2]_{\text{atm}}$ and other atmospheric gases such as water vapour and O_2 are also likely to be significant in their influence on D_v evolution. A scenario where both atmospheric humidity and $[\text{CO}_2]$ were declining (Boer & Yu 2003) would have the effect of compounding the hydraulic costs of assimilation. These conditions may explain the evolution of leaves with D_v that exceed our model predictions (D_v exceeds 20 mm mm^{-2} in some tropical canopy species).

CONCLUSIONS

The fundamental role played by angiosperms in the biological and atmospheric function of earth provides a major impetus for research into the adaptive processes responsible for early angiosperm diversification. Until recently most inquiry has focused on reproductive functions

and plant–animal interactions as the engines of today’s biodiversity (Crepet 2008; Williams 2008). To the extent that high leaf P_c is linked to canopy productivity (Bonan 2008), our results suggest that the Early to mid-Cretaceous transition in leaf architecture was an event of profound significance in the functional evolution and modernization of terrestrial vegetation. We posit that angiosperm leaves, by virtue of their veins, provided a Cretaceous productivity stimulus package that reverberated throughout the biosphere. Elevated P_c and the linked high rates of transpiration are likely to have enhanced the flows of energy, water and nutrients through the biosphere, which cascaded into new opportunities for diverse organisms in geologically young, angiosperm-dominated ecosystems (Berendse & Scheffer 2009; Boyce *et al.* 2009).

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Figure S1 D_v trends in fossil leaves.

Figure S2 A relationship between vein density and leaf rank for 27 species of angiosperms.

Figure S3 A relationship between vein density (D_v) and the largest distance between minor veins and the stomata (d_x).

Figure S4 Modelled relationships between photosynthetic rate and D_v under three different atmospheric CO₂ concentrations.

Table S1 Systematics, collection details, ecology, and vein density values for the 318 species of angiosperms.

Table S2 Phylogenies used in determining the evolution of vein density evolution in angiosperms.

Table S3 Data sources for molecular divergence estimates and minimum fossil ages for dating nodes.

Appendix S1 Modelling photosynthetic capacity (P_c) in living and fossil leaves.

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