Inferring climate from angiosperm leaf venation networks

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Summary

- Leaf venation networks provide an integrative linkage between plant form, function and climate niche, because leaf water transport underlies variation in plant performance.
- Here, we develop theory based on leaf physiology that uses community-mean vein density to predict growing season temperature and atmospheric CO2 concentration. The key assumption is that leaf water supply is matched to water demand in the local environment. We test model predictions using leaves from 17 temperate and tropical sites that span broad climatic gradients.
- We find quantitative agreement between predicted and observed climate values. We also highlight additional leaf traits that may improve predictions.
- Our study provides a novel approach for understanding the functional linkages between functional traits and climate that may improve the reconstruction of paleoclimate from fossil assemblages.

Introduction

Identifying the mechanisms underlying the distribution of species across broad climatic gradients remains a central question in ecology (Westoby & Wright, 2006). Species’ traits determine performance, which then should influence fitness in a given environment (McGill et al., 2006). This central assumption in trait-based ecology predicts that species with certain traits should be associated with specific environments in which the traits are adaptive. While this framework is conceptually simple, identifying these trait-environment linkages has remained elusive (Weiser et al., 1999; Díaz et al., 2004; Soudzilovskaia et al., 2013). In plants, leaf hydraulic traits may often mediate these linkages, because water transport between a plant and its environment determines many aspects of structure and growth (Tyree & Ewers, 1991; Sack & Holbrook, 2006).

Here, we test the hypothesis that leaf venation networks provide a mechanistic link between plant physiological functioning and climate. Because allocation to the venation network results in a trade-off between transport (Brodribb et al., 2007) and the cost of constructing a leaf (Sack & Scoffoni, 2013), different venation geometries should be adaptive in climates with differing hydraulic environments (Brodribb et al., 2010; de Boer et al., 2012). Some data support a linkage between climate and the density of minor veins (VD, length of minor veins per unit leaf area, units mm⁻¹). In observational studies, variation in VD has been linked to climate, including temperature, precipitation and moisture availability. Species from warmer and drier sites tend to have higher VD (Sack & Scoffoni, 2013), although sometimes contradictory or weak relationships are found (Dunbar-Co et al., 2009; Blonder et al., 2013; Jordan et al., 2013). Additionally, in experimental manipulations over developmental timescales, VD changes with light availability (Tumanow, 1927; Carins Murphy et al., 2012) and humidity (Lebedincev, 1927). In paleoecological studies, VD appears to have increased over evolutionary time (Boyce et al., 2009), especially during the Late Cretaceous (Feild & Brodribb, 2013), possibly driven by falling atmospheric CO2 concentrations (Brodribb et al., 2009; Feild et al., 2011; but see Boyce & Zwieniecki, 2012). Similarly, VD appears to vary adaptively in response to climate within some clades (Carlquist, 1959; Jordan et al., 2013).

Our primarily empirical understanding of the linkage between vein traits and climate has made it difficult to predict and isolate the relationship between each vein trait and each dimension of climate and to make predictions for climate based on community-scale distributions of venation network traits. For these reasons, vein traits have not yet been widely adopted as paleoclimate proxies or climate indicators (Manze, 1967; Uhl & Mosbrugger, 1999) despite recent interest (Sack & Scoffoni, 2013). Leaves do offer a potentially powerful way to reconstruct climate, with extant methods based on statistical correlations between leaf size and shape (Wolfe, 1993; Royer et al., 2005). The physiological justification for such correlations has been limited (Royer & Wilf, 2006). There is a case for building more predictive theory. Recent physiological models have established quantitative linkages between VD and other leaf traits (Blonder et al., 2011, 2013; Sack et al., 2012) or between vein traits and atmospheric CO2 concentrations (Brodribb & Feild, 2010; de Boer et al., 2012; Boyce & Zwieniecki, 2012). However, some of these models are controversial (Sack et al., 2013; Blonder et al., 2014) and others are not yet able to predict how other dimensions of climate, such as temperature and moisture availability, modulate...
this trait–climate linkage. Articulating the linkages between leaf traits and climate could provide a useful approach for predicting community composition and species’ climate niches.

Here, we develop theory for how variation in climate selects for communities with species with certain venation network geometries. By extending extant physiological models, we provide equations that couple variation in leaf physiology to variation in ambient temperature and atmospheric concentrations of CO₂.

Our central hypothesis is that the maximum rate of water supply to the leaf (transpiration rate; \(E\)) is coupled to the maximum potential water demand of the environment (potential evapotranspiration; \(PET\)) as \(E = \alpha \cdot PET\), where \(\alpha\) is a dimensionless coefficient reflecting potential differences in plant water-use efficiency and site hydrology. The model defines \(E\), \(\alpha\) and \(PET\) in terms of minor vein density (VD), growing season temperature (\(T_c\)), atmospheric CO₂ pressure (\(C_a\)), latitude (\(\theta\)) and several other minor parameters. The model can then be solved analytically to predict that VD is positively linked to \(T_c\) and negatively linked to \(C_a\) (Fig. 4, Supporting Information Fig. S1), consistent with other theories (Brodribb & Feild, 2010; de Boer et al., 2012). A key assumption of the theory is that the appropriate community-mean VD can be achieved either through evolutionary lability or species sorting, such that trait values reflect an adaptive response to local climate rather than biogeographic history.

We test the theory’s predictions and assumptions using data from modern plant communities. We measure VD on 1048 leaves of 186 nonmonocot angiosperm species at 17 sites ranging across a 2480–3370 m temperate elevation gradient and a 60–3250 m tropical elevation gradient. [Correction added after online publication 29 May 2014. The number of species measured has been changed from 187 to 186. A single leaf specimen (‘ManuelAntonio.1.23._’) of Clitoria javitensis (Fabaceae) was incorrectly labeled as Calophyllum longifolium (Clusiaceae). Voucher specimens are deposited at the University of Arizona Herbarium in Tucson, Arizona, USA. This change propagates as a negligible change in all reported statistics (no deviation appearing at two significant figures, except Blomberg’s \(K\), which changes from 0.38 to 0.39. The figures and supplementary data files have been updated, and all conclusions and discussion points remain unchanged.] We first assess empirical correlations between community-mean VD and elevation.

We then compare the model’s predictions for growing season temperature and atmospheric CO₂ concentration to values predicted from each community’s species-mean vein density.

**Description**

**Theory**

We develop a model to approximate the physiology of C₃ angiosperm species with abaxial stomata. C₃ species dominate terrestrial ecosystem productivity, comprise the largest fraction of contemporary plant diversity, as well as the paleodiversity after the late-Mesozoic angiosperm radiation (Stewart & Rothwell, 1993). In principle, the model could be expanded to include the specific anatomies and physiologies of other groups (e.g. gymnosperms, ferns, bryophytes or monocots). All model parameters are summarized in Table 1.

A key assumption is that, under ‘ideal conditions’, leaf water supply (\(E\); transpiration rate; mmol m⁻² s⁻¹) is proportional (\(\alpha\); dimensionless) to environmental water demand (\(PET\); potential evapotranspiration; mmol m⁻² s⁻¹) where:

\[
E = \alpha \cdot PET
\]

Eqn 1

Here, ‘ideal conditions’ means that the model will be most valid when leaves are functioning at low levels of physiological stress (e.g. open stomata and favorable leaf–stem water potentials). As a result, the venation network, which is constructed early during leaf development (Sack et al., 2012), should have a structure that matches, but does not exceed, the environmental demands that the leaf is likely to experience over a typical lifespan. This hypothesis is consistent with the coordination of leaf hydraulics with environmental conditions in several species (Brodribb & Jordan, 2011; Carins Murphy et al., 2012, 2014; Blonder et al., 2013). Moreover, variation in leaf minor vein density matches variation in environmental water demand (Uhl & Mosbrugger, 1999; Givnish et al., 2005; Sack & Frole, 2006; Brodribb & Feild, 2010; Brodribb & Jordan, 2011) at both the developmental and evolutionary timescales (but see Feild et al., 2011).

Next, we assume that leaf transpiration rate, \(E\), will be related to the leaf–stem water potential, \(\Delta \Psi_{ls}\) (MPa); and the leaf

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**Table 1 Summary of model parameters**

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Name</th>
<th>Units</th>
<th>Central value</th>
<th>Half-width</th>
</tr>
</thead>
<tbody>
<tr>
<td>(T_c)</td>
<td>Growing season temperature</td>
<td>°C</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>(C_a)</td>
<td>Atmospheric carbon dioxide pressure</td>
<td>Pa</td>
<td>40</td>
<td>10</td>
</tr>
<tr>
<td>(\theta)</td>
<td>Latitude</td>
<td>°</td>
<td>9 (tropical)</td>
<td>1</td>
</tr>
<tr>
<td>(h)</td>
<td>Atmospheric humidity</td>
<td>%</td>
<td>60</td>
<td>10</td>
</tr>
<tr>
<td>(D)</td>
<td>Vapor pressure deficit</td>
<td>kPa</td>
<td>1</td>
<td>0.5</td>
</tr>
<tr>
<td>(g_l)</td>
<td>Stomatal conductance coefficient</td>
<td>MPa</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>(\Delta \Psi_{ls})</td>
<td>Leaf–stem water potential</td>
<td>MPa</td>
<td>0.10</td>
<td>0.05</td>
</tr>
<tr>
<td>(d_y)</td>
<td>Leaf half-thickness</td>
<td>µm</td>
<td>80</td>
<td>30</td>
</tr>
<tr>
<td>(s)</td>
<td>Insolation factor</td>
<td></td>
<td>1</td>
<td>0.1</td>
</tr>
</tbody>
</table>

The central value was used to parameterize the model. Uncertainty was explored by sampling values from a uniform distribution with center and half-width given here.
hydraulic path length $d_m$ (µm). We formally link $E$, $\Delta \Psi_b$, and $d_m$ using an empirical relationship reported by Brodribb et al. (2007):

$$ E = 12 670 \cdot \Delta \Psi_b \cdot d_m^{1.27} \quad \text{Eqn 2} $$

Further, we can write $d_m$, the hydraulic path length (µm), as

$$ d_m = \frac{\pi}{2} \left( d^2_{sv} + d^2_{ij} \right)^{1/2} \quad \text{Eqn 3} $$

where $d_i$ is the leaf half-thickness (µm), representing the characteristic distance from vein to abaxial stomata, and $d_{sv}$ is the distance between minor veins, which is empirically related to vein density (VD; mm$^{-1}$) as

$$ d_{sv} = \frac{650}{VD} \quad \text{Eqn 4} $$

Note that this transpiration model may lose some accuracy depending on the mode of water transport but deviations are important only at very high VD values (de Boer et al., 2012).

The proportionality factor $\alpha$ empirically relates actual evapotranspiration to potential evapotranspiration (Trabuco & Zomer, 2010). This coefficient is used in the context of agricultural crop coefficients (Allen et al., 1998) and is similar to the Horton index, which is defined as the ratio of water vaporization through any means to catchment wetting at the landscape scale (Troch et al., 2009). We define $\alpha$ as the Priestley–Taylor coefficient, the fraction of surface moisture available for evaporation (Priestley & Taylor, 1972), because it can be empirically linked to stomatal conductance (eqn 3 of Komatsu, 2005):

$$ \alpha = 1.26 \cdot \left( 1 - e^{-g_s/5} \right) \quad \text{Eqn 5} $$

($g_s$, stomatal conductance to water vapor (mm s$^{-1}$)).

We assume that, across differing climates, leaves maximize carbon gain per unit water loss by regulating stomatal conductance (Cowan & Farquhar, 1977). Based on a recent optimality model (Medlyn et al., 2011) we use:

$$ g_s = 1.6 \cdot 10^{-3} R T_0 \cdot \frac{A}{C_a} \left( 1 + \frac{g_l}{D^{1/2}} \right), \quad \text{Eqn 6} $$

where $10^{-3} R T_0$ is a unit conversion factor from mol m$^{-2}$ s$^{-1}$ to mm d$^{-1}$, $R = 8.31447$ J mol$^{-1}$ K$^{-1}$ and $T_0 = 288.15$ K. 1.6 converts from conductance of CO$_2$ to that of H$_2$O ($A$, the peak photosynthetic rate (µmol m$^{-2}$ s$^{-1}$); $C_a$, atmospheric CO$_2$ pressure (Pa); $g_l$, a constant (kPa$^{1/2}$) reflecting the marginal water cost of carbon and the CO$_2$ compensation point for photosynthesis; $D$, vapor pressure deficit (kPa)). For analytic tractability, we linearize Eqn 6 in terms of $C_a$ by performing a first-order Taylor approximation around a central value, $C_{a*}$:

$$ g_s = 1.6 \cdot 10^{-3} R T_0 \cdot \frac{A}{D^{1/2}} \cdot \left( g_1 + D^{1/2} \right) \cdot (2 C_{a*} - C_a) \quad \text{Eqn 7} $$

here choosing $C_{a*} = 40$ (the modern atmospheric value).

We make a further assumption that venation networks are coupled to photosynthesis (Brodribb et al., 2007). Primarily for analytic tractability, we make the linear approximation that

$$ A = \eta \cdot VD. \quad \text{Eqn 8} $$

We use $\eta = 1$ (mm µmol m$^{-2}$ s$^{-1}$) based on an approximate fit to the data of (Brodribb et al., 2007).

PET is modeled using a modified version of the Hargreaves–Samani (Hargreaves & Samani, 1982) model. This version was chosen because it requires relatively few parameters:

$$ \text{PET} = \frac{625}{972} \cdot \frac{75}{10 000} \cdot R_a \cdot C_t \cdot T_f \cdot \Delta T^{-1/2}, \quad \text{Eqn 9} $$

where the 625/972 prefactor converts from mm d$^{-1}$ to mmol m$^{-2}$ s$^{-1}$ ($R_a$ total incident solar radiation; $C_t$, a humidity factor; $T_f$, temperature (°F); $\Delta T$, average temperature range). We convert $T_f$ to a Celsius temperature ($T_c$; °C) as $T_f = 1.8 \cdot T_c + 32$, where again, $T_c$ is the growing season temperature, with $R_a$ defined as

$$ R_a = s \cdot 15.392 \cdot d_r \cdot (\omega - \varphi \cdot \sin \delta + \cos \varphi \cdot \cos \delta \cdot \sin \omega) \quad \text{Eqn 10} $$

and where $s$ is an insolation factor (dimensionless), and orbital parameters $d_r$, $\omega$, and $\delta$ are given as

$$ d_r = 1 + 0.033 \cdot \cos \left( \frac{2 \pi J}{365} \right) \quad \text{Eqn 11} $$

$$ \omega = \cos^{-1} (-\tan \varphi \cdot \tan \delta) $$

$$ \delta = 0.4093 \cdot \sin \left( \frac{2 \pi J}{365} - 1.405 \right) $$

($J$, day of year (dimensionless); $\theta$, latitude (°)). The humidity factor is empirically defined (Hargreaves & Samani, 1982) as

$$ C_t = \begin{cases} 0.035 \cdot (100 - b)^{1/3} & b > 54 \\ 0.125 & \text{otherwise} \end{cases} \quad \text{Eqn 12} $$

where $b$ is the relative humidity (%). The isothermality factor $\Delta T$ can be empirically written (Hargreaves & Samani, 1982) as

$$ \Delta T = 10 + 0.32 \cdot (100 - b) \quad \text{Eqn 13} $$

In order to reduce the number of free parameters in the model, we use the assumption that the model applies only during the ‘ideal’ conditions previously described. Under these conditions, we can further parameterize the model with $J = 180$ (midsummer), $D = 1$ and $g_s = 3$ (typical for the species being modeled) (Medlyn et al., 2011) and $\Delta \Psi_{sw} = 0.10$ (typical under low water-stress conditions across diverse species) (Sack & Holbrook, 2006).
Lastly, we assume that leaf thickness is a constant. This simplifying assumption reduces the dimensionality of the model and makes it possible to use the model for paleoclimate applications. Thickness cannot be easily measured on fossil leaves. We choose \( d_r = 80 \) because it represents many common species and is commonly used in other paleoecological models (de Boer et al., 2012). Modern insolation intensities are by definition characterized by \( s = 1 \). Across paleotime, the value of \( s \) may be variable, depending on orbital variation, solar output (Laskar et al., 2011) and mean cloudiness.

We solve the above equations analytically to predict atmospheric carbon dioxide \( C_o \) (or \( T \)) based on measured values of leaf venation, VD, the growing season temperature \( T_c \) (or \( C_o \)), latitude \( \theta \) and relative humidity \( h \). While the solutions are too large to present here, they are shown in full in the Supporting Information (Eqns S1–S3 in Notes 1). We also provide a Mathematica notebook (Notes S2) for direct manipulation and parameterization of the model. In general, higher values of VD are predicted to yield higher values of \( T_c \) or lower values of \( C_o \) if all other parameters are held constant. The analytic form of these equations also makes it possible to explore the consequences of variation in traits that we assumed to be constant (e.g. leaf thickness, water potential).

Field collections

We tested predictions of our model by collecting leaves from species found within 17 sites along a temperate and tropical elevation gradient (Table 2). At each site we measured latitude and longitude using a GPS unit. For tropical sites, elevation was obtained directly from satellites; for temperate sites, elevation was obtained from a digital elevation model (USGS, National Elevation Dataset).

The temperate gradient was located in the Gunnison Valley of western Colorado in the United States (39°N) and included 11 sites (each \( 1 \text{ m}^2 \)) ranging in elevation from \( 2440 \) to \( 3370 \) m asl. These sites span a continuum from arid montane riparian areas to alpine meadow and include both woody and herbaceous species. During the 2010 growing season we sampled the more common nonmonocot angiosperm species (\( n = 6 \pm 3 \) SD), taking several (9 \( \pm 1 \) SD) mature undamaged leaves from individuals of each species.

The tropical gradient was located in the Savegre River drainage of western Costa Rica (9°N) and included six sites ranging in elevation from \( 65 \) to \( 3250 \) m. These sites span a continuum from tropical moist forest to tropical wet montane forest and include only woody species. For each site we set up a 0.1-ha ‘Gentry transect’ (Phillips & Miller, 2002), identifying every individual with dbh \( \geq 2.5 \) cm. We then sampled at least one leaf from at least one individual of every observed species. At each site, we then chose a random subset of fifty leaves, each from a different individual for venation analysis. We took this random sampling approach because the high diversity at these tropical sites (mean richness = 68 \( \pm 31 \) SD species) made a full analysis of all leaves time-prohibitive.

Vein density measurements

All leaves were pressed flat and dried at 60°C for at least 3 d. We then cleared each leaf to expose its venation using established protocols (Pérez-Harguindeguy et al., 2013). We cut a 1-cm\(^2\) section from each leaf, selecting a region of the lamina that did not include any primary veins. We immersed the leaf sample in a solution of 5% w/v sodium hydroxide : water heated to a temperature of 50°C for up to 7 d, until the leaf became transparent. We then rinsed the leaf in water and transferred it to a 2.5% w/v sodium hypochlorite : water solution for up to 5 min, until the leaf became white. We then rinsed the leaf in water and transferred it to 50% v/v ethanol : water solution for 5 min, and then to a staining solution of 0.1% w/v safranin : ethanol for 30 min. We then transferred the leaf to a destaining solution of 100% ethanol for 1 h before transferring to 50% v/v ethanol : toluene for 30 s and then to 100% toluene. We then mounted each leaf on a glass slide using the toluene-based Permount medium (Fisher Scientific, Waltham, MA, USA). We allowed slides to dry for 3 d during which the clearing process continued. Some samples were inadvertently destroyed by this chemical process. The final dataset included 225 tropical leaves and 529 temperate leaves from 186 nonmonocot angiosperm species.

We then imaged each leaf using a dissecting microscope (SZX-12; Olympus) coupled to a digital camera (T2i; Canon, Japan). Slides were back-illuminated using a light box. Images were obtained at a final resolution of 430 pixels per millimeter with a full extent of \( 10 \text{ mm} \times 7 \text{ mm} \). We then retained only the green channel of each image and applied a contrast-limited adaptive histogram equalization procedure to improve image quality.

We estimated vein density on each image using a stochastic line-intersection technique. The distance between veins is known to strongly correlate with the density of veins (Uhl & Mosbrugger, 1999; Brodribb et al., 2007). Distance can be rapidly estimated by counting the number of veins crossed by a line of a known length (cartooned in Fig. S1). To calibrate this approach, we first used a collection of previously traced leaves from 25 morphologically diverse species (Blonder et al., 2011) on which we simulated the placement of a number of randomly oriented line segments. We then compared the known vein density of the leaf to the mean distance between veins, as estimated as the total length of all line segments divided by the total number of vein intersections.

For as few as 10 random line segments (c. 7 cm total length) there was a very strong correlation \( (r^2 = 0.89, P < 10^{-15}) \) between vein density (VD, mm\(^{-1}\)) and distance (d, mm):

\[
VD = 0.629 \cdot \left( \frac{1}{d} \right) + 1.073 
\]

Eqn 14

We then pooled leaf-level measurements to calculate species-at-site mean vein densities and used these to then estimate site-mean vein density. We used species-at-site means because some species occurred at multiple sites, potentially obscuring trait variation due to between-site climate variation.
### Table 2 Summary of collections at each location (the number of leaves collected follows each species name)

<table>
<thead>
<tr>
<th>Elevation (m asl)</th>
<th>Taxa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tropical</td>
<td></td>
</tr>
<tr>
<td>65</td>
<td>Annonaceae/Oxandra venezuelana (1), Apocynaceae/Aspidosperma desmanthus (3), Bignoniaceae/Arrabidae sp. 2 (1), Bignoniaceae/Callichlamys latifolia (1), Boraginaceae/Cordia sp. 2 (1), Chrysobalanaceae/Licania operculipetala (1), Connareaceae/Rourea glabra (1), Erythroxylaceae/Erythroxylum macrophyllum (3), Fabaceae/Clitorea javitensis (2), Fabaceae/Machaerium kogelli (1), Fabaceae/Machaerium salvadorensis (1), Fabaceae/Swartzia ochracea (1), Icacinaceae/Discophora guianensis (1), Lactistemonaceae/Lactistema aggregatum (2), Lauraceae/Nectandra umbrosa (1), Lauraceae/Ocotea leucoxyon (1), Melastomataceae/Mouriri gleasoniana (2), Meliaceae/Ruagea glabra (2), Moraceae/Trophis involucrata (1), Myrtaceae/Eugenia acapulcensis (1), Myrtaceae/Myrciaria floribunda (1), Rubiaceae/Faramea occidentalis (1), Salicaceae/Tetrathyrium johansenii (1), Sapotaceae/Pouteria chiricana (3)</td>
</tr>
<tr>
<td>500</td>
<td>Brassicaceae/Capparis frondosa (1), Bursereae/Protium glabrum (1), Bursereae/Protium sp. 1 (1), Clusiaceae/Garcinia intermedia (1), Clusiaceae/Tovomita longifolia (1), Fabaceae/Inga caracasana (1), Fabaceae/Macropholium costaricense (1), Fabaceae/Swartzia ochracea (1), Lauraceae/Lactistema aggregatum (2), Lauraceae/Ruagea glabra (2), Moraceae/Trophis involucrata (1), Myrtaceae/Eugenia acapulcensis (1), Myrtaceae/Myrciaria floribunda (1), Rubiaceae/Faramea occidentalis (1), Rubiaceae/Linanica venezuelana (1), Salicaceae/Tetrathyrium johansenii (1), Sapotaceae/Pouteria chiricana (3)</td>
</tr>
<tr>
<td>1050</td>
<td>Annonaceae/Guatteria diospyroides (1), Apocynaceae/Lacmelia zamora (2), Bursereae/Protium sp. 3 (1), Bursereae/Protium sp. 4 (1), Fabaceae/Dichapetalum sp. 1 (1), Euphorbiaceae/Croton megistocarpus (1), Euphorbiaceae/Heronyma oblonga (1), Euphorbiaceae/Richeria dressleri (2), Fabaceae/Entada gigas (1), Fabaceae/Inga latipes (1), Fabaceae/Inga bhiudiana (1), Icacinaceae/Discophora guianensis (1), Lauraceae/Licaria sp. 1 (1), Lauraceae/Ocotea meziana (1), Lauraceae/Ocotea praeterrmis (1), Moraceae/Brosimum guianense (2), Moraceae/Pseudomedia glabra (1), Moraceae/Pseudomedia mollis (1), Myrtaceae/Ardisia dunlapiana (2), Myrtaceae/Mycia sp. 2 (1), Rubiaceae/Cousarea carolinia (2), Rubiaceae/Cousarea loftonii (2), Rubiaceae/Faramea sp. 1 (1), Rubiaceae/Posoqueria coriacea (1), Rubiaceae/unknown sp. 1 (1), Rubiaceae/Matayba apetala (1), Sapotaceae/Chrysophyllum sp. 2 (1), Sapotaceae/Chrysophyllum sp. 3 (1), Sapotaceae/Chrysophyllum sp. 4 (1), Sapotaceae/Pouteria chiricana (1), Verbenaceae/Aegiphila sp. 1 (1), Vochysiaceae/Vochysia allenii (1)</td>
</tr>
<tr>
<td>2050</td>
<td>Annonaceae/Guatteria oliviformis (1), Aquifoliaceae/Ilex turturiceps (1), Aquifoliaceae/Ilex sp. 2 (1), Araliaceae/Dendropanax quercetius (2), Araliaceae/Oreopanax xalapensis (6), Asteraceae/Verbena aethestiana (4), Bignoniaceae/Apocynacea sectissifolia (1), Brunelliaceae/Brunellia costaricensis (2), Fabaceae/Quercus copeyesis (1), Fabaceae/Quercus ruparahuensis (1), Fabaceae/Quercus seemannii (2), Lauraceae/Ocotea insularis (5), Lauraceae/Ocotea praeterrmisa (1), Lauraceae/Ocotea sp. 1 (1), Lauraceae/Ocotea valeriana (1), Magnoliaceae/Magnolia poasana (1), Myristicaceae/Ardisia sp. 3 (1), Myristicaceae/unknown sp. 2 (2), Rubiaceae/Palicourea sp. 2 (1), Rubiaceae/Rondeletia amoena (1), Rubiaceae/Rondeletia buddeioides (2), Sabiaceae/Meliosma vernicosa (5)</td>
</tr>
<tr>
<td>3250</td>
<td>Aquifoliaceae/Ilex sp. 2 (1), Aquifoliaceae/Dendropanax querceti (1), Asteraceae/Verbena aethestiana (1), Brunellaceae/Brunellia costaricensis (1), Caprifoliaceae/Viburnum stellatotomentosum (1), Cherantaceae/Hedyosmum goudotianum (2), Cornaceae/Cornus disciflora (2), Cunoniaceae/Weinmannia pinnata (2), Ericaceae/Satyrinia warszewiczii (1), Ericaceae/Vaccinium consanguineum (2), Fabaceae/Quercus copeyesis (1), Fabaceae/Quercus ruparahuensis (1), Fabaceae/Quercus seemannii (1), Juglandaceae/Alfaroa costaricensis (3), Lauraceae/Nectandra cufodontisii (1), Lauraceae/Ocotea pittieri (3), Magnoliaceae/Magnolia sororum (1), Malpighiaceae/Bunchiosia temata (2), Melastomataceae/Miconia sp. 1 (1), Meliaceae/Trichilia havanensis (3), Myrsinaceae/Ardisia glandulosomarginata (3), Myrsinaceae/Myrsine juergenseni (3), Rubiaceae/Palicourea salicifolia (1), Rutaceae/Zanthoxylum melanosistictum (1), Styracaceae/Styrax argentus (1), Symlocaceae/Symphlocos retusa (1)</td>
</tr>
<tr>
<td>4230</td>
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Table 2 (Continued)

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Phylogenetic analysis

We conducted a phylogenetic analysis of VD using species-mean values and a phylogeny constructed with Phylocom’s ‘phylogenetic’ tool (Webb et al., 2008) using the R2010 tree with branch lengths adjusted using the default Wikstrom ages file (Wikström et al., 2001). We then calculated Blomberg’s K statistic (Blomberg et al., 2003) using species-mean values of VD.

Model parameterization and test

The values of model parameters are listed in Table 1. Sites were assigned 0 values corresponding to their GPS-measured latitude (all temperate sites within 0.2° of 38.8°N; all tropical within 0.1° of 9.4°N). Site temperature ($T_s$) was defined as the 1950–2000 average of mean growing season temperature. This temperature was determined from 30 arcsecond-resolution Worldclim data (BIO10 variable) (Hijmans et al., 2005) using each site’s latitude/longitude coordinates. Site CO$_2$ pressure ($C_a$) was inferred based on a standard elevational lapse. We used site elevation $e$ (meters) to parameterize the barometric formula for an isothermal atmosphere:

$$C_a = C_a^* \cdot \frac{e g M}{R T_0} \quad \text{Eqn 15}$$

($g = 9.80665 \text{ m s}^{-2}$; $M = 0.0289644 \text{ kg mol}^{-1}$; and $C_a^* = 40 \text{ Pa}$). When $C_a$ was inferred, observed values of $T_s$ were used to parameterize the model. Conversely observed values of $C_a$ were used when inferring $T_s$.

Model uncertainty analysis

We measured the impact on $T_s$ and $C_a$ of two classes of uncertainty in the model: sampling error in VD and systemic error in all other model parameters. We first solved the model analytically for $T_s$ and $C_a$. To assess systemic error in all model parameters, we assumed that the remaining parameters ($D, \Delta \Psi, g, d_a, d_d$) were random variables uniformly distributed with a central value and a half-width reflecting to a physiologically relevant range (Table 1). When solving for $T_s$, we assumed that $C_a$ was uniformly distributed between 30 and 50 Pa; when solving for $C_a$, we assumed that $T_s$ was uniformly distributed between 5 and 15°C. We also allowed latitude to vary 1° in half-width around the observed value. To assess measurement error in VD, we assumed that VD was uniformly distributed between the 25% and 75% quantile of its distribution at each site. We sampled parameter values from each distribution and calculated the resulting $T_s$ ($C_a$) value.

We obtained parameter deviations by subtracting these parameter values from their central values, and prediction deviations by subtracting the $T_s$ ($C_a$) value from the value predicted when using central values for all parameters. Next, we repeated the resampling 1000 times per analysis. We calculated the middle quartile of each predicted deviation as a combined uncertainty estimate for each site. We also directly measured sampling uncertainty by solving the model, holding all parameters constant to their central values except VD, which was allowed to vary as above. All deviations are reported as interquartile ranges of these distributions.

We also measured the relative importance of each parameter to predictions of $C_a$ ($T_s$). We repeated the sensitivity analysis with the above parameter distributions, this time assuming VD to be uniformly distributed across its global range ($r = 1$–25 mm$^{-1}$; Boyce et al., 2009). We then constructed a linear model for deviations in $C_a$ ($T_s$) as a function of deviations in all parameters. For each parameter, we report the effect direction as the sign of the regression coefficient; and the overall effect size as the ratio of the parameter’s explained sum of squares divided by the total sum of squares in an ANOVA of the linear model (i.e. an $r^2$ value).

Results

Distribution of vein traits

Across sites, vein density varied by an order of magnitude both across all leaves (from 1.1 to 26.3 mm$^{-1}$) and across all sites 5.9–13.2 mm$^{-1}$ (Fig. 1). The full dataset is available in Notes S3 and S4. Within-site variation in VD ranged from 4.8 to 18.1 mm$^{-1}$ across sites. Site mean VD was negatively correlated with elevation within each gradient (tropical, $P = 0.02, r^2 = 0.79$; temperate, $P = 0.006, r^2 = 0.59$; Fig. 2). Similar relationships were observed with growing season temperature and other environmental variables correlated with elevation. The slope and intercept of this relationship varied with gradient; when pooling for both gradients, the site mean VD–elevation relationship was no longer significant ($P = 0.67$). The response of VD to
environment was potentially adaptive, with the trait showing evolutionary lability. Species-mean values of VD varied across the angiosperm tree more than expected based on a Brownian model of trait evolution (Blomberg’s K = 0.39, P = 0.001) (Fig. 3).

Model predictions

Using site-mean VD data, the model’s predictions for \( C_a \) were strongly correlated with observed values of \( C_a \) (SMA regression; \( r^2 = 0.65, P < 10^{-5} \) (Fig. 4a). The tropical dataset had a different slope than the temperate dataset (\( P = 0.005 \)). The model’s predictions for \( T_c \), also using site-mean VD data, were similarly correlated with observed values of \( T_c \) (SMA regression; \( r^2 = 0.76, P < 10^{-5} \) (Fig. 4b). The tropical slope was significantly different from than the temperate slope (\( P = 0.03 \)). We also show predictions and observations of \( T_c \) and \( C_a \) directly as functions of VD in Fig. S2.

Sensitivity analysis

We found that predictions for \( C_a \) were affected most strongly by VD (Fig. 5a). Increases in \( d_y \) or decreases in \( \Delta \Psi_t \) could bias \( C_a \) predictions upward but the overall magnitude of the effect was limited. For our predictions for \( T_c \), most parameters had minor effects (Fig. 5b). Two variables were notable exceptions: decreases in \( d_y \) or increases in \( \Delta \Psi_t \) could make an upward bias in our \( T_c \) predictions. As a result, our analyses indicate that if either \( d_y \) or \( \Delta \Psi_t \) directionally varies across environmental gradients then this could modulate the strength of the climate signal in VD. Similarly, systemic biases in constant values chosen for these variables could also reduce the inferred match between veins and climate.

Discussion

We have developed theory that links variation in the density of the leaf venation network to species’ climate niches. Specifically, the theory makes predictions for temperature and atmospheric CO\(_2\) concentration as a function of site-mean minor vein density. We examined leaves across temperate and tropical climate gradients, and found empirical correlations between vein density and elevation. Moreover, we found that the theory predicted local climate and atmospheric composition via values of \( T_c \) and \( C_a \) across both temperate and tropical sites, albeit with some error. We also found that appropriate community-mean VD values could be obtained, either through species sorting or evolutionary trait lability. These empirical results support the key assumptions and predictions of the theory, indicating that it provides a starting point for developing more quantitative linkages between plant form and climate.

Nevertheless, the model is not a complete description of reality. While the model has high predictability, its predictions are biased in certain environments. There are four potential reasons. First, biased predictions may be due to unmeasured variation in parameters of the model. While several model parameters are difficult to directly measure, our sensitivity analysis revealed that tissue density \( d_y \) and stem water potential \( \Delta \Psi_t \) are the other important parameters. In this study we were not able to measure either of these parameters and so directly measure their impacts on predictions. Leaf thickness is known to vary across environmental gradients (Niinemets, 2001; Hodgson et al., 2011) and could be correlated with other model parameters. Thickness may be related to VD via both functional (Noblin et al., 2008; Blonder et al., 2011) and developmental (Brodribb et al., 2013) mechanisms, though the relationship is often unclear (Sack et al., 2013). Leaf water potential, \( \Delta \Psi_t \), may be linked to leaf structural properties such as shrinkage (Blonder et al., 2012), but shows remarkable constancy under ‘ideal’ conditions (Sack & Holbrook, 2006). We are unaware of any studies measuring the

Fig. 1 Vein density is highly variable across species and sites. (a) Escallonia myrtilloides, from a cold tropical site (5.8 mm\(^{-1}\)); (b) Clitoria javitensis, from a warm tropical site (12.4 mm\(^{-1}\)). Bars, 500 \(\mu\)m.

Fig. 2 Vein density is negatively correlated with elevation within regions. (a) Temperate gradient (\( n = 11 \) sites); (b) tropical gradient (\( n = 6 \) sites). Each gray symbol represents an individual leaf. Colored circles represent site means (blue triangles, temperate; red squares, tropical), and colored lines are OLS regressions for site-mean data. Note that each panel has different axis scales.
The relationship between VD and $\Delta\Psi_L$ under appropriate conditions (but see Sack & Scoffoni, 2013).

Second, observed deviations from predictions may also arise from data quality issues. While the chemical clearing method we used is a community standard (Pérez-Harguindeguy et al., 2013), it may not completely clear poorly lignified leaves or those with thick palisade mesophyll or cuticular/hypodermal layers. Incomplete tissue clearing would potentially lead to underestimates of VD, lower predictions of $T_c$, and higher predictions of $C_a$ than expected. Such leaves and prediction biases are found for our high-elevation tropical sites, consistent with the operation of this effect. Imaging paradermal sections of stained leaves can produce less biased measurements, though this method is far more time-consuming and less suitable for large datasets.

Third, deviations may also arise from oversimplification in the model derivation. The equations were chosen primarily for analytic tractability to operationalize the hypothesis of leaf water supply matching environmental demand. More precise modeling...
The model is likely to perform less well for species with alternative strategies for coping with evaporative demand – for example, succulents, C₄/CAM species, or any species with amphistomatic or highly reflective leaves (Scoffoni et al., 2011; Sack & Scoffoni, 2013). Indeed, the large range of intra-site variation in VD seen here indicates that the predicted vein–climate coupling is not achieved in all species – variation in VD or other traits may occur to match other performance requirements (e.g. sequestration of secondary compounds, mechanical strength). Similarly, woody and herbaceous species may access different water-use strategies. Such an effect could also explain the differences in slope for the temperature gradient (woody + herbaceous plants) and the tropical gradient (woody plants only). We did not have enough data to separately test the model for each growth form. Nevertheless, it is clear that VD plays an important and understudied role in determining climate niches.

Fourth, other venation network traits may also be coupled to climate in ways not explored by this model. For example, globally, species can differ extensively in the geometry of their venation network, with the same VD obtained for either highly parallel or highly reticulate patterns (Ellis et al., 2009). This variation is not included in our model, as we were unable to formulate a quantitative hypothesis for the drivers of reticulation. While increasing reticulation is thought to be associated with more hydraulic redundancy (Mckown et al., 2010) or damage resistance (Katifori et al., 2010), there is limited available evidence for climate associations of this trait except with shady environments for monocots (Givnish et al., 2005). Similarly, the geometry or density of the major veins is currently not included in our model, though these structures may also reflect variation in climate (Sack & Scoffoni, 2013). Lastly, venation network traits are known to scale with leaf area (Sack et al., 2005). Similarly, the geometry or density of the major veins is currently not included in our model, though these structures may also reflect variation in climate (Sack & Scoffoni, 2013). Lastly, venation network traits are known to scale with leaf area (Sack et al., 2005). Similarly, the geometry or density of the major veins is currently not included in our model, though these structures may also reflect variation in climate (Sack & Scoffoni, 2013). Lastly, venation network traits are known to scale with leaf area (Sack et al., 2005). Similarly, the geometry or density of the major veins is currently not included in our model, though these structures may also reflect variation in climate (Sack & Scoffoni, 2013).

Paleoclimate reconstruction from fossil leaf assemblages is a key application of this model. By integrating information about multiple species, the model can make robust predictions at the community scale based on first principles of leaf water balance and physiology. By contrast, other methods such as leaf margin analysis (Wolfé, 1993) and stomatal index measurements (McElwain & Chaloner, 1995; Beerling & Royer, 2002) require empirical calibration and may suffer from extrapolation problems (Jordan, 2011) or are based on a trait showing strong phylogenetic niche conservatism (Little et al., 2010). Fossil leaf assemblages from applicable species with measurable venation...
networks are preserved from many critical periods of Earth’s history (Feild et al., 2011), suggesting that much may yet be inferred about past climate change.

Acknowledgements

T. Huxman, S. Saleska, J. Harte, G. Jordan, J. Sperry, K. Johnson, D. Royer and P. Wilf provided thoughtful feedback on the manuscript. B. Boyle led the tropical fieldwork. J. Bezançon, A. Henderson, N. Ioakem, D. Kahler, C. Lamanna, C. Magness, N. May, L. Parker, N. Prohaska, L. Sloat and E. Wollman assisted with data collection. B.B. was supported by a Rocky Mountain Biological Laboratory graduate research fellowship, a Geological Society of America student research grant, and a NSF pre-doctoral fellowship. B.J.E. was supported by a NSF ATB award and a NSF Macrosystems award.

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Supporting Information

Additional supporting information may be found in the online version of this article.

**Fig. S1** Cartoon of vein density estimation procedure.

**Fig. S2** Model predictions and observations as a function of site-mean vein density.

**Notes S1** Analytic solutions for $T_c$ and $C_a$ (includes Eqns S1–S3).

**Notes S2** Mathematica notebook implementing the above equations.

**Notes S3** Trait measurements for all leaves (CSV format).

**Notes S4** Site mean trait and climate value (CSV format).

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