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Testing Carlquistian hypotheses on the functional significance of vessel element length

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Summary – While total vessel length is widely recognized as being of fundamental functional significance, opinion is more divided regarding the potential functional importance of vessel element length, a variable that Sherwin Carlquist regarded as functionally significant. We show that vessel element length can, as Carlquist predicted, affect vessel resistance to deformation. Perforation plates are locally thickened annuli, so tubes with annuli resist deformation better than those without, and tubes with closely-spaced annuli resist deformation better than those with distantly spaced ones. However, there is a tradeoff between deformation resistance and conductance. With a comparative analysis across more than 1000 species of angiosperms, we show that both vessel element length and the areas of individual inter-conduit pits scale positively with vessel diameter. Such covariation is expected if plants are to maintain conductance as they grow taller. Congruent with Carlquist's thinking, we found that species with vessel elements that are exceptionally short tend to grow in drylands, whereas those with vessel elements that are exceptionally long tend to grow in moist climates. Finally, we show evidence suggesting that selection on vessel element length is an important determinant of imperforate tracheary element length. Conversely, the evidence for selection on imperforate tracheary element length affecting vessel element length appears to be weaker. These results seem sufficient to establish that, whatever the functional importance of total vessel length, vessel element length is a variable of functional significance in its own right, congruent with Sherwin Carlquist's long-held views.

Keywords – adaptation, allometry, ecological wood anatomy, intervessel pits, natural selection, perforation plates, Sherwin Carlquist, xylem structure–function relations.

Introduction

Vessel length is likely to be a major target of natural selection. From vessel terminus to vessel terminus (elements with single perforation plates) and including the many elements that make up a vessel, vessels can vary from millimeters to meters in length (Zimmermann & Jeje 1981; Ewers & Fisher 1989; Nijsse 2004; Jacobsen *et al.* 2012). For a given diameter, longer vessels impose less resistance to water movement (Comstock & Sperry 2000). Because

vessels are mostly made up of cells with perforation plates at either end (vessel elements), resistance to flow cell-to-cell within vessels is low, compared to movement between vessels. Toward their termini, vessels narrow and are capped, at each end, with an element with a single perforation plate. In passing from vessel to vessel, water must make its way through the pits piercing the vessel walls and through the membranes that envelop individual vessels (Jansen *et al.* 2009; Li *et al.* 2016). If embolisms propagate freely across perforation plates, then selection should act strongly on the distribution of vessel lengths depending on the likelihood of embolism. This expectation is congruent with the observation of very short vessels (even referred to as "megatracheids", i.e., vessels of just a few elements in length; Carlquist 2012) in lianas (Brodersen *et al.* 2013) and in other plants, which are expected to have highly embolism resistant conductive pathways in addition to the very vulnerable ones represented by their very wide and presumably long vessels (Rosell & Olson 2014). For their part, authors such as Vinya *et al.* (2013) find empirical evidence for selection altering vessel length by virtue of its effect on hydraulic resistance. All else being equal, long vessels offer more opportunities for air-seeding, so longer vessels are likely more vulnerable to embolism than shorter ones (Markesteijn *et al.* 2011; Jacobsen *et al.* 2019). There is every reason, therefore, to regard vessel length as being of prime functional significance and a very important feature shaped by natural selection in the context of climate, habitat, and plant life form. Less clear, however, is the functional significance of vessel element length.

There are many reasons to regard vessel element length as being a variable of functional importance, but there are varying takes in the literature on its potential significance. On the one hand, there is abundant experimental and modeling evidence to suggest that vessel element length can strongly influence hydraulic resistance (Cao *et al.* 2020). For example, Ellerby & Ennos (1998) constructed tubes with structures that mimicked perforation plates. Shorter tubes, with closer spacing of perforation plates, were associated with greater resistance to water movement. Likewise, perforation plates represent rings or annuli of wall material. Because a tube with annuli has more wall material, it seems intuitively obvious that a tube with annuli should resist deformation under negative pressure better than a tube lacking annuli but otherwise of similar lumen diameter and wall thickness (Niklas 1992; Spatz & Niklas 2013). Along the same lines, a tube with more annuli should resist better than one with fewer annuli. Yet authors routinely question the potential adaptive value of vessel element length. For example, in *Xylem Structure and the Ascent of Sap* (Tyree & Zimmermann 2002), the authors note (on p. 221): "I cannot hold with Carlquist's (Carlquist 1975) argument that shorter vessel elements are an adaptation to greater tensions because they make vessels mechanically stronger and thus prevent collapse. A little added wall thickness does this too well, and there are too many other factors involved. Vessel-element length, it seems to me, is merely a by-product, e.g., the result of cambial initial length, which might be under some other control, perhaps fiber length, although fibers can also elongate by intrusive growth. The variation in vessel-element length seems to me a perfect example of what Baas (1982) calls 'functionless trends imposed by correlative restraints'". Baas (1976: p. 164) concurs, noting that "I find any adaptive explanation of the positive correlations between ecological factors and vessel member length unconvincing. I would favor indirect effects of these different ecologies on vessel element length through genetically fixed differences in morphogenetic processes controlled by, for instance, differences in phytohormone metabolism". Other authors downplay the functional importance of vessel element length by stating that total vessel length is more functionally important, but this statement might confuse different hypotheses. For example, authors such as Scholz *et al.* (2013: p. 4) background the importance of vessel element length because "total vessel length plays a more important role in determining hydraulic resistance than the vessel element length" (see also Lens *et al.* 2011). Given this ambivalence in the literature, with some authors showing clear reasons to regard vessel element length as a functionally important variable and others discounting it, it is opportune to focus on vessel element length and potential functional significance in the light of the emphasis that Sherwin Carlquist put on vessel element length as a functionally significant variable. We examine various potential ways that vessel element length could be functionally significant, with emphasis on Carlquist's hypotheses, here.

Resisting vessel deformation under negative pressure

Carlquist postulated that vessel element length should be subject to natural selection via its potential role in resisting mechanical deformation under negative pressure (Carlquist 1975). Sufficient deformation of vessels under negative pressure is almost certainly reliably associated with passage of gas bubbles into and rupture of the conductive stream of a functional vessel (Hacke & Sperry 2001). Therefore, it is no surprise that adaptations abound in the secondary xylem that counter such deformation. Vessel wall thickness is one, with, all else being equal, thicker walls of a pipe, tube, or vessel resisting deformation under mechanical loading as opposed to thinner walls (Gere & Timoshenko 1999). Also, the thickness-to-span ratio is involved, because, for a given wall thickness, a narrower pipe will resist deformation better under the same mechanical load as compared to a wider one. Selection therefore shapes the relationship between the negative pressures experienced by a given species, wall thickness, and vessel diameter, in fundamental ways. Another example of an adaptation that helps vessels resist deformation is the binding of vessels to adjacent cells via the middle lamella, distributing mechanical loads from vessels to surrounding cells (Jacobsen *et al.* 2005). These examples illustrate that a major aspect of selection shaping the conductive system is clearly the avoidance of deformation of conduits under negative pressure.

Vessel element length is another potential player in the suite of features favored by natural selection in resisting vessel deformation under negative pressure. To our knowledge, this hypothesis was first proposed by Sherwin Carlquist (1975) in his *Ecological Strategies of Xylem Evolution*. He based his reasoning on the morphology of the perforation plate (Fig. 1). Vessel elements are often described as hollow cylinders, stacked end-to-end to make up a tube that is the vessel. However, vessel elements are not perfectly cylindrical. Instead, the perforation itself is narrower than the vessel lumen (Fig. 1). Between the perforation proper and the vessel wall is a stretch of material, labeled in Fig. 1 as the perforation plate "rim", e.g., as in Bolton & Robson (1988). The fact that the perforation plate is narrower than the lumen of the vessel element that bears it means that, rather than vessels being uniform tubes, they are instead characterized by periodic constrictions at each perforation plate.

Carlquist hypothesized that these constrictions or rims could help resist vessel deformation. A pipe of uniform wall thickness (Fig. 2A), subject to negative pressure on the inside, resists deformation by virtue of the diameter of the pipe and the thickness of the pipe wall. In Fig. 1, it can be seen that the rims of adjacent perforation plates of successive vessel elements make up constrictions within the vessel. These constrictions represent annular zones of locally thicker walls. If vessel elements are shorter, then there are more annuli per unit length (Fig. 2B), and if vessel elements are longer, then the annuli are spaced farther apart (Fig. 2C). Because additional wall material increases the second moment of area and therefore increases resistance to deformation, Carlquist reasoned that, all else being equal, such as negative pressure, vessel diameter, and lumen wall thickness, vessels with closely-spaced annuli would resist deformation better than those with distantly-spaced or no annuli. Thus, Carlquist (1975) hypothesized that, in addition to features such as lumen wall thickness, thickness-to-span ratio, and the characteristics of the cells surrounding vessels, another variable that could be acted upon by selection in resistance of deformation under negative pressure is vessel element length, by virtue of its influence on the spacing of the rims of perforation plates. Using Carlquist's hypothesis as a starting point, we examined the potential functional significance of vessel element length using a variety of approaches, which we will briefly summarize now.

The mechanical role of perforation plates

We first examined the notion that shorter vessel elements could have any effect on resisting deformation under negative pressure. Authors such as Tyree and Zimmermann are skeptical that closer spacing of perforation plates could render vessels more resistant to deformation under negative pressures. To examine the potential mechanical contribution of perforation plates, we used a finite element modeling approach. We modeled the three situations depicted in Fig. 2, and estimated their resistance to deformation under negative pressure. If it is true that vessel element length has no effect at all on resistance to deformation under negative pressure, then tubes with the "annuli"
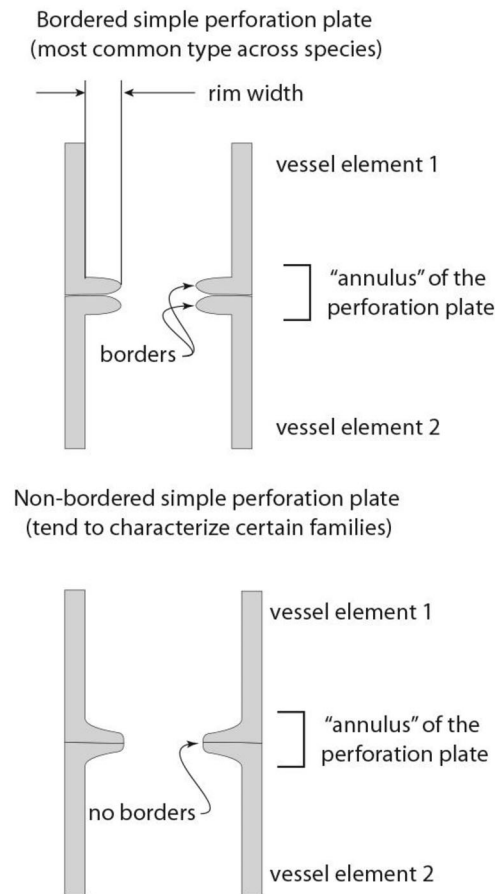
Fig. 1. Simple perforation plate morphology. This figure shows schematics of two simple perforation plates in longitudinal section. Each plate is made up of parts of two vessel elements, part of each of which are shown here. Perforation plates, even simple ones, represent constrictions with respect to the vessel lumen. As such, they also represent areas with additional wall material as compared to the lumen. Areas with additional material can contribute to resisting deformation under negative pressure.

formed by perforation plates should resist deformation indistinguishably from the tube with no annuli. Similarly, tubes with closely spaced annuli should be just as subject to deformation as those with distantly spaced ones. We then used the results from our finite element modeling to examine the possibility of a mechanics-conductance tradeoff (Pratt & Jacobsen 2017).

Scaling of vessel element length and pit dimensions with vessel diameter

A further clue to determining whether vessel element length is of functional significance is to see whether there is a relationship between vessel element length and vessel diameter. If vessel element length has no functional relevance at all, then length should be free to vary in the context of vessel diameter. Carlquist (1975: p. 181) often emphasized that "control of vessel wideness is, of course, independent of the fusiform cambial initial control of length" and that "vessel element length, furthermore, is controlled by morphogenetic factors independent of those affecting diameter of vessels and number of vessels per sq. mm of transection" (Carlquist 1977: p. 888). By these statements, Carlquist meant that a very wide range of lengths could be found in the context of any given diameter. Given this very wide field of possible proportions, selection should be free to favor length–diameter combinations favored in a given situation.
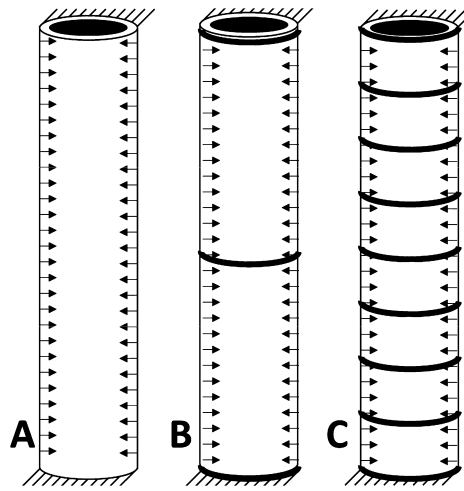
Fig. 2. Vessels modeled using finite elements. (A) A tube of uniform 5 μm wall thickness, without perforation plate "annuli". Small arrows indicate the forces exerted on the vessel walls by the negative pressure inside the vessels. (B) A tube otherwise identical to A with perforation plate annuli oriented at right angles to the lumen spaced every 200 μm. (C) A tube otherwise identical to A with perforation plate annuli oriented at right angles to the lumen spaced every 50 μm.

However, whether or not there is a relationship between vessel element length and diameter across species has not, to our knowledge, been extensively studied.

If there is a marked pattern of length–diameter association, then this pattern could potentially point to the functional significance of vessel element length. We test for the presence of a vessel element length-vessel diameter relationship across species. We place particular emphasis on any difference between species with scalariform versus simple perforation plates. Perforation plates are known to affect resistance to sap movement, with scalariform perforation plates imposing greater resistance than simple ones (Ellerby & Ennos 1998; Schulte 1999; Christman & Sperry 2010; Chen *et al.* 2012; Jansen & Nardini 2014). Plate spacing should therefore affect resistance to water flow. It seems unquestionable that natural selection shapes conductance, and therefore resistance, in vessels. If perforation plate spacing, and thus vessel element length, affect resistance, then it seems inevitable that selection would simultaneously shape both vessel element diameter and length. For example, for a given diameter and vessel element length, vessels with scalariform plates will experience higher resistance and lower conductance than otherwise identical vessels with simple plates. A key factor lowering resistance in species with scalariform plates is therefore longer elements for a given diameter, because longer elements lead to fewer passages of water through high-resistance scalariform plates. We test for the presence of a vessel element length–diameter relationship with a comparative dataset spanning 1681 species, including both simple and scalariform-plated species.

Moreover, there should be a relationship between the areas of individual inter-vessel pits and vessel element dimensions. Inter-vessel pits are the crucial sites of water movement into and out of vessels. In species with non-conductive imperforate tracheary elements and grouped vessels, water movement between vessels must necessarily occur through intervessel pits. In species with solitary vessels and true tracheids, vessel-tracheid pits are a key site of water movement between conduits (Carlquist 1984). Selection must lead to coordination between all of the sources of resistance along the entire length of the conductive path, such that water flow is maintained to the leaves as plants grow taller (Comstock & Sperry 2000; Koçillari *et al.* 2021). In conifers, pit resistance scales with lumen resistance along the entire length of the trunk (Lazzarin *et al.* 2016), and similar coordination must occur as well in angiosperms. If, as in conifers, wider pits lower resistance as lumen resistance becomes lower, then wider, and therefore longer, vessel elements should have larger individual pits.

"Developmental drag" among cambial products

We then examined the potential influence of developmental linkage between vessel elements and imperforate tracheary elements in determining vessel element length. All of the cells of the axial system — strands of axial parenchyma, imperforate tracheary elements, and vessel elements — are produced by fusiform cambial initials. Because they originate from the same mother cells, they all begin life at the same size. Selection favoring a given length in one cell type therefore has the potential to affect the sizes of the other cells originating from the same mother cell type. This is why Tyree and Zimmermann (Tyree & Zimmermann 2002: p. 221) come to the conclusion alluded to previously, that "Vessel-element length, it seems to me, is merely a by-product, e.g., the result of cambial initial length, which might be under some other control, perhaps fiber length." Carlquist (1975: p. 150) noted that "Longer cambial initials... produce longer imperforate elements, which might have a positive value in the woods of the more arborescent growth forms in the wet forest. Which of these two factors [selection on vessel element length or on imperforate tracheary element length] is more important in the evolution of the longer cambial initials is not easy to discern." Because both imperforate tracheary elements and vessel elements are produced from the same fusiform cambial initials, selection affecting either imperforate tracheary element or vessel element length in a way that alters fusiform cambial initial length would result in an evolutionary change in the length of the other cell type. We call the notion that vessel element length is a mere byproduct of selection on imperforate tracheary element length the "developmental drag" hypothesis. "Drag" denotes that selection on one cell type "drags" the length of the other cell type even though it is not subject to selection in that direction. We use patterns of association between vessel element length and imperforate tracheary element length to test the expectations of Tyree and Zimmermann and of Carlquist.

Shorter vessel elements for a given diameter in drylands

Finally, we examined Carlquist's adaptive hypothesis regarding natural selection on vessel element length in the context of water availability. Carlquist predicted that selection should favor shorter vessel elements in drought-prone plants. Consistent with this expectation, some authors have reported shorter vessel elements in drier habitats as compared to species living in moister habitats (Baas *et al.* 1983; Lindorf 1994; Villar-Salvador *et al.* 1997). Others find little evidence of such a relationship (Meyer *et al.* 2013). All of these studies share the problem that, to the extent that there might be a relationship between vessel element length and vessel element diameter, then this relationship could confound comparisons of vessel element length between species (Echeverría *et al.* 2022). Therefore, it would be ideal to take into account any possible relationship between vessel element length and diameter in making such comparisons across species and climates. We test the prediction that species in drier climates should have shorter vessel elements for a given vessel diameter with a comparative dataset of 2418 samples and 1801 species, drawn from Carlquist's own publications (Echeverría *et al.* 2022). This dataset can be accessed at 10.6084/m9.figshare.22656358. If Carlquist's hypothesis is correct, then, for a given vessel diameter, species with elements that are relatively long should be in moist locales or otherwise shielded from highly negative xylem pressures. Conversely, those with the shortest vessel elements for a given diameter should be in situations most subject to highly negative xylem pressures.

Material and methods

Perforation plate contribution to resisting deformation: finite element modeling

Because the very notion that such perforation plates can contribute to resistance of deformation has been questioned so emphatically by some authors, such as those mentioned above (Baas *et al.* 1983; Tyree & Zimmermann 2002), and because many authors downplay the functional significance of vessel element length relative to vessel length, it is important to show the potential mechanical contributions of perforation plate annuli quantitatively. Such

a demonstration would establish the plausibility of vessel element length as having the potential for contributing to resisting deformation of vessels, and therefore confirm it as a variable of functional study in its own right whatever the functional significance of vessel length.

Here, we used a finite element modeling approach. Using the program Solidworks Simulation (Dassault Systems, Vélizy-Villacoublay, France), we reproduced the conduit configurations in Fig. 2. We modeled conduits of 410 μm in length, and 100 μm in external diameter. All had wall thicknesses between perforation plates of 5 μm. We assumed that the wall materials were isotropic, with a Young's Modulus of 8890 N/mm², a Poisson coefficient of 0.354, and a yield strength of 28.30 N/mm² (approximating *Acer rubrum*). We modeled a conduit with uniform wall thickness with no annuli, as in Fig. 2A. We then modeled one with vessel elements of 200 μm in length, with annuli of 10 μm thickness and 10 μm in height (Fig. 2B). Finally, we modeled a conduit with vessel elements 50 μm in length (Fig. 2C). We subjected these conduits to negative pressure and quantified their stress and deformation by a static simulation.

For the simulations, we fixed the tubes at their tops and bottoms to avoid buckling along their longitudinal axes. We set the internal pressure to –5 MPa perpendicular to the tube wall. We expressed test results as von Mises stress, mean stress, the maximum deformation of each test, and the deformation and stress at a reference node. Von Mises stresses reflect the distribution of stress throughout the tube, thus giving an idea of the performance of the tubes as whole structures. Maximum deformation is the point with the highest deformation value in the entire conduit, wherever it fell. Mean stress was the sum of the stresses of all the elements modeled divided by the number of elements. Finally, we selected a point we designated as the "reference node," located between two annuli, allowing comparison of deformation and stress at functionally comparable points on each tube.

PERFORATION PLATE EFFECT ON HYDRAULIC CONDUCTANCE: IS THERE A TRADEOFF BETWEEN DEFORMATION RESISTANCE AND HYDRAULIC CONDUCTANCE?

To test Carlquist's hypothesis of a tradeoff between the increase in deformation resistance and the decrease in hydraulic conductance conferred by perforation plates, we estimated the hydraulic conductance of tubes with annuli with the same dimensions as those used for finite element modeling and compared the implosion resistance and hydraulic conductance of segments. We estimated the hydraulic conductance as the inverse of the sum of the hydraulic resistance (R) of individual segments using the Hagen–Poiseuille equation for ideal capillaries:

$$R = \frac{8\eta l}{\pi r^4}$$

where l is element length and r is element radius. Perforation plate resistance was modeled as a very short element with a lumen radius equal to the vessel lumen radius minus the perforation plate radius. The hydraulic conductance of the segments with dimensions described in the previous section were compared with the deformation results of the finite element modeling.

THE SCALING OF VESSEL ELEMENT LENGTH AND INTER-CONDUIT PIT AREA WITH VESSEL DIAMETER

If vessel element length has no functional significance, then there should be no difference in the vessel element length-vessel diameter relationship between species with low-resistance simple perforation plates and those with high-resistance scalariform perforation plates. To test for the relationship between vessel element length and diameter, we compiled the data from Sherwin Carlquist's publications 1957–2018 (this dataset can be accessed at 10.6084/m9.figshare.22656358). Our final vessel element length-vessel diameter dataset covered 1801 species in 158 families from 48 orders. We coded each species as having scalariform or simple plates, with the vessel diameter and scalariform/simple coding available in Echeverria *et al.* (2022). We explored the influence of self-supporting (trees,

shrubs) or non self-supporting (prostrate plants, climbers, etc.) status by fitting a linear regression predicting vessel element length based on vessel diameter plus the self/non-self supporting variable and the interaction between the predictors. We found a very weak relationship between vessel element length and vessel diameter for non self-supporting plants (Fig. A1 in the Appendix), so we removed them in further tests. We then fit a linear regression predicting vessel element length based on vessel diameter plus the scalariform/simple variable and the interaction term between the predictors. Finally, we tested the prediction of a relationship between individual inter-conduit pit area and vessel diameter by fitting a linear model predicting pit area based on vessel diameter.

ASSOCIATION BETWEEN VESSEL ELEMENT LENGTH AND IMPERFORATE TRACHEARY ELEMENT LENGTH AS A RESULT OF DEVELOPMENTAL DRAG?

To explore the possibility that natural selection on vessel element length (VEL) could developmentally "drag" imperforate tracheary element length (ITEL), we leveraged the marked differences in VEL for a given vessel diameter between species with simple and scalariform plates (Bailey & Tupper 1918). The greater resistance of scalariform perforation plates (Ellerby & Ennos 1998; Schulte 1999; Christman & Sperry 2010; Chen *et al.* 2012; Jansen & Nardini 2014) means that natural selection favors longer vessel elements in species with scalariform plates. Longer elements reduce the number of perforation plates per unit vessel length, and thus leads to markedly greater conductance than would be observed in a vessel with scalariform perforation plates and vessel elements as short as those in simple-plated species for a given vessel diameter. Thus, the different resistances imposed by simple versus scalariform perforation plates represent a situation in which selection unambiguously acts on vessel element length as a variable of key functional significance.

If, as Carlquist hypothesized, vessel element length is a variable of functional significance subject to natural selection, then it should be possible to find situations in which ITEL is clearly affected by selection acting on VEL (i.e., VEL can "developmentally drag" ITEL). Instead, if authors such as Tyree and Zimmermann and Baas, cited above, are correct, then variation in VEL should have no influence on ITEL. We fit two linear models, both predicting ITEL based on VEL plus a simple/scalariform binary variable and the interaction term. We fit one such model for species with true tracheids, which are conductive, and one for species with non-conductive ITEs (fiber-tracheids and libriform fibers). Because scalariform perforation plates tend to be associated with (conductive) true tracheids, and because selection tends to favor (conductive) true tracheids that are very long relative to non-conductive ITEs (Carlquist 1975; Echeverría *et al.* 2022), including both conductive and non-conductive ITEs in the same model would potentially confound the direction of developmental dragging. Fitting separate models for species with conductive and non-conductive ITEs therefore should reveal unambiguously if VEL variation can influence ITEL.

By the same token, selection on ITEL should be able to influence VEL. To explore this possibility, we fit two models analogous to the above, but designed to explore the effect of variation in ITEL on VEL. Again, because true tracheids are conductive, selection tends to favor much longer tracheid lengths for a given tracheid diameter as compared to non-conductive ITEs (fiber-tracheids and libriform fibers). So, to explore the possibility that natural selection on ITE length could developmentally drag VEL, we used the marked differences in ITEL for a given diameter between species with conductive (true tracheids) and non-conductive ITEs (libriform fibers and fiber-tracheids). True tracheids tend to be longer than non-conductive ITEs for similar ITE diameters, associated vessel diameters, and plant sizes. If ITEL can influence evolution of VEL, then VEL should be significantly longer in species with true tracheids as compared to those with non-conductive ITEs. Accordingly, we fit models predicting VEL based on ITEL + a conductive/non-conductive binary variable plus the interaction term. Again, because true tracheids tend to be associated with scalariform perforation plates, we fit two models, one for species with scalariform plates, and another for species with simple plates, in this way avoiding any potential confounding that the scalariform-tracheid association might introduce.

We then tested the hypothesis that selection should favor, for a given vessel diameter, shorter vessel element lengths in dry areas. To do so, we extracted geographical distribution data from species having the lowest and highest residual values from the linear models of vessel element length predicted by vessel diameter. Using residual values allowed us to identify the species with the shortest and longest vessel element lengths controlling for vessel diameter. We used residual values from two separate linear models, one for species with simple perforation plates (vel ∼ vd and simple plates) (Table A1 in the Appendix) and the other for species with scalariform perforation plates (vel ∼ vd and scalariform plates). To compare similar numbers of species with simple versus scalariform perforation plates we used different residual values in each model (shortest simple plates < –0.38, longest simple plates > 0.35, shortest scalariform plates < –0.2 and longest scalariform plates < 0.2).

To extract georeferenced distribution data we used the BIEN_species_distribution function from the BIEN R package (Maitner *et al.* 2018) (v 1.2.5). BIEN curates GBIF coordinate points by removing coordinates with high probabilities of being erroneous. We used the cleanGbifCoords.1.0.py script (Edwards *et al.* 2015) to dismiss additional erroneous/suspicious points. To compare the climate preferences of species with the shortest and longest vessel element lengths, we harvested data from the Worldclim database using the raster (v3.5-29) and sp (v 1.5) R packages (Hijmans *et al.* 2021; Pebesma *et al.* 2021). To examine the prediction that there should be ecological differences between species with different vessel element lengths for a given diameter, and to test for the potential influence of perforation plate type, we fit linear models comparing climate variables across four categories of vessel element length and perforation plate type. To unravel differences between each factor we used pairwise comparisons using estimated marginal means from the emmeans R package (Lenth *et al.* 2021) and we summarize results using the compact letter display method (Piepho 2004).

Results

PERFORATION PLATE CONTRIBUTION TO RESISTING DEFORMATION: FINITE ELEMENT MODELING

Our finite element modeling bore out the prediction that, all else being equal (lumen wall thickness, internal vessel diameter, negative pressure), tubes with annuli better resist deformation than those without. Moreover, tubes with closely-spaced annuli deform less than those with distantly spaced ones. These differences are shown in Table 1, which gives the results of 3 different tubes, including the tube of uniform wall thickness (without annuli), a tube with annuli each 200 µm along the tube, and a tube with annuli each 50 µm. Results are represented graphically in Fig. 3, which shows the distribution of stresses and deformations along the tube walls. Maximum and mean stresses, maximum deformation, node stress, and node deformation were all lowest in the tube with closely-spaced annuli, congruent with the prediction that shorter vessel elements help resist deformation under negative pressure.

Table 1. Results of finite element models, showing that even shorter vessel elements increase resistance to deformation of vessels under 5 MPa negative pressure.

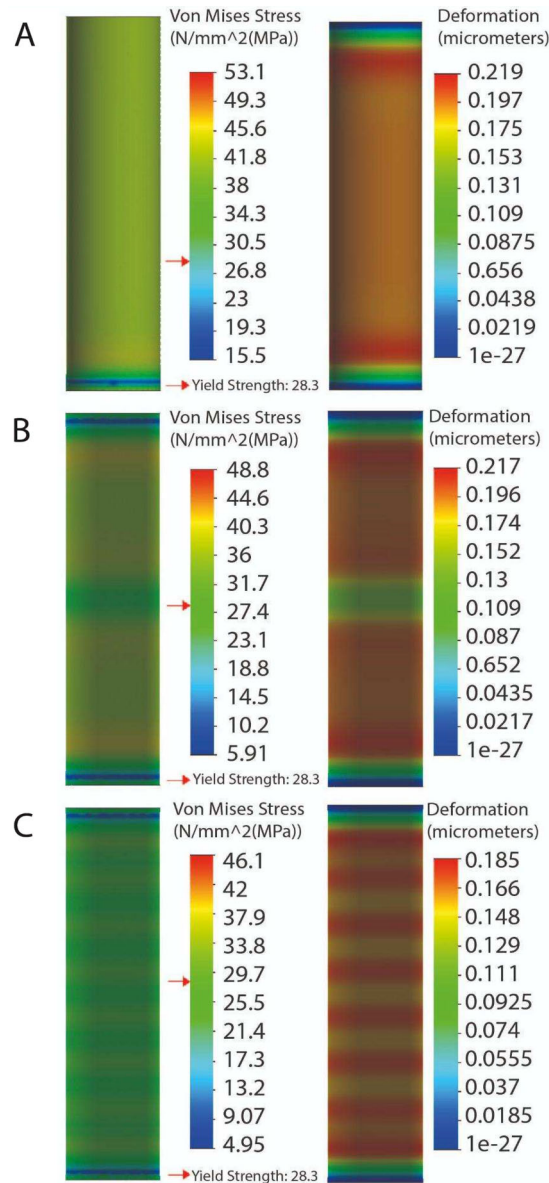| Specimen	Max. stress (MPa)	Max. deformation (µm)	Reference node stress (MPa)	Reference node deformation (µm)	Mean stress (MPa)	Mean deformation (µm)
Uniform walls	53.1	0.219	46.1	0.203	39.9	0.182
Annuli each 200 µm	48.8	0.217	45.8	0.201	38.9	0.176
Annuli each 50 µm	46.1	0.185	40.5	0.181	34.3	0.155

Fig. 3. Finite element analysis exploring the influence of annulus presence and spacing on the deformation of tubes by negative pressure. Internal view of the distribution of Von Mises stresses (left) and deformations (right) (A) In uniform tubes (without annuli) stresses and deformations are distributed uniformly along the wall of the tube. (B) In tubes with annuli every 200 μm deformation is greatly reduced in the vicinity of annuli. (C) In tubes with annuli every 50 μm, deformation is reduced to an even larger extent in the vicinity of annuli. With shorter vessel elements and more annuli per length of vessel, total deformation under negative pressure is lower in the tube with closely spaced annuli (C) than in the tube without annuli (A) or the tube with distantly spaced annuli (B). The location of the yield strength, which was specified in the model, is provided for reference.
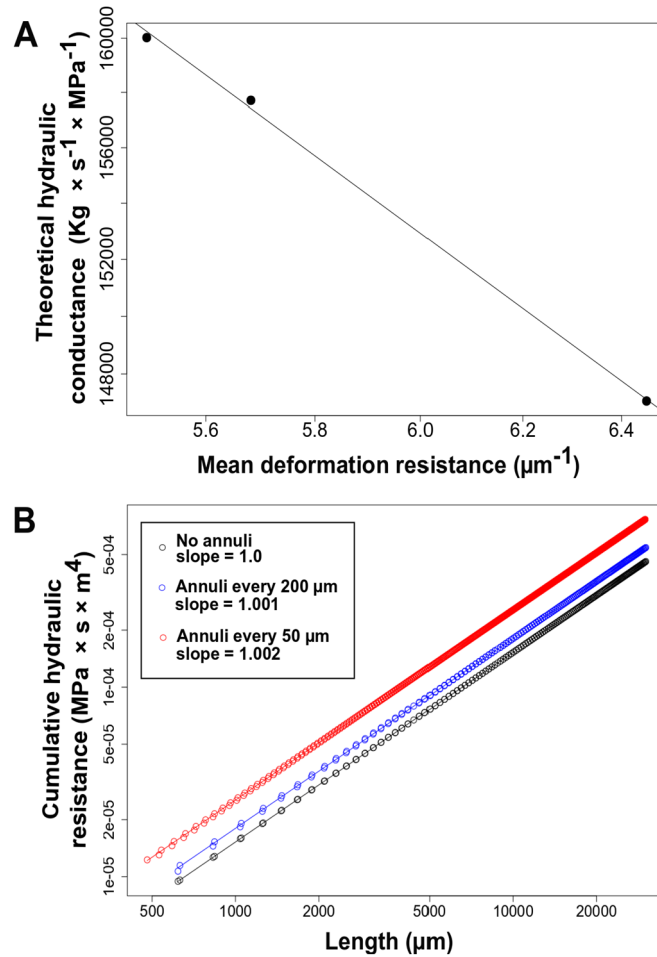
Fig. 4. The conductive efficiency-mechanical resistance tradeoff and perforation plate spacing. (A) Decreasing spacing between perforation plates increases resistance to vessel deformation under negative pressure but also causes an increase in resistance to water flow, leading to a predictable negative relationship (slope = −0.53) between deformation resistance and conductance. (B) Tubes with closely spaced annuli (red points) have higher cumulative hydraulic resistance per unit length than tubes with distantly spaced annuli (blue points) or tubes with no annuli (black points).

Perforation plate effect on hydraulic conductance: is there a tradeoff between implosion resistance and hydraulic conductance?

Our results showed a clear negative relationship between hydraulic conductance and deformation resistance (Fig. 4). Hydraulic conductance of tubes with closely spaced annuli (50 μm) was lower than the conductance of tubes with distantly spaced annuli (200 μm) and both were lower than that of tubes without annuli (Fig. 4). Vessels with higher conductance had lower resistance to deformation (Fig. 4A). Our conductance analysis also showed that presence of annuli caused the cumulative resistance of tubes to increase slightly superlinearly for a given diameter (Fig. 4B), and that the magnitude of the deviation was affected by annuli lumen diameter and the spacing of annuli within tubes (not shown).
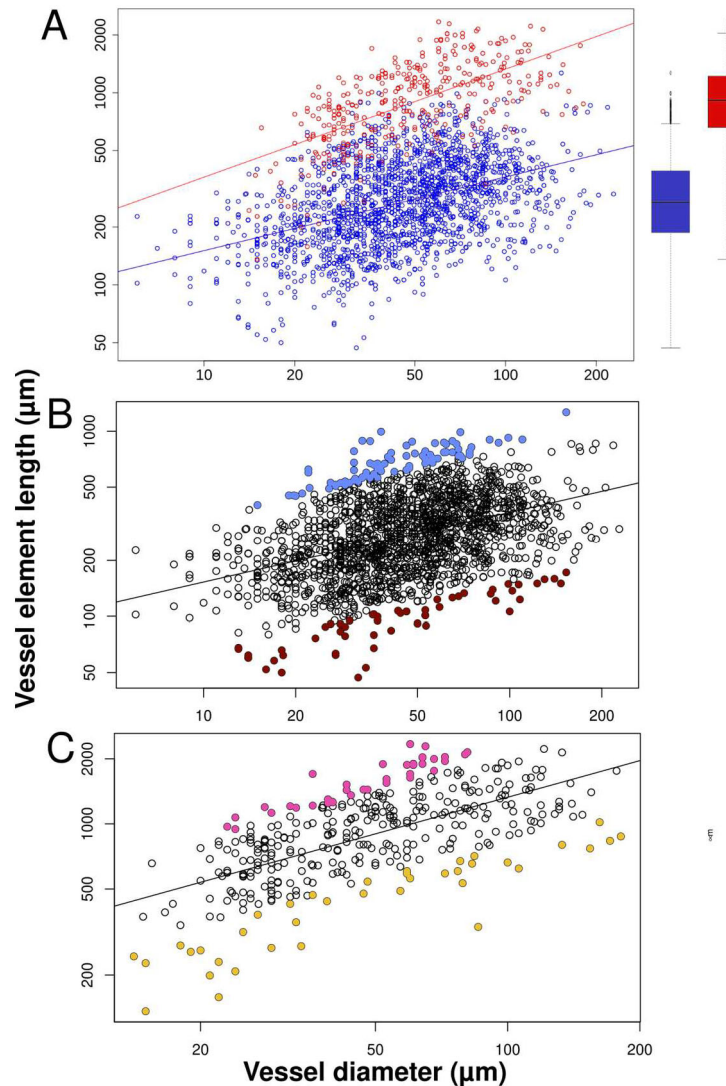
Fig. 5. Vessel element length–vessel diameter relationship across species with different perforation plate types. (A) Vessel element length scales predictably with vessel diameter across self-supporting plants (simple perforation plates (blue) slope = 0.38, $P < 0.001$; scalariform perforation plates (red) slope 0.56, $P < 0.001$). (B) Vessel element length–vessel diameter-vessel diameter relationship in species with simple perforation plates and in (C) species with scalariform perforation plates. Blue points and brown points in (B), and pink points and yellow points in (C) represent species having longer and shorter vessel elements for a given diameter and have the largest absolute residuals from the linear models. Because vessel element length scales with vessel diameter, it is helpful to standardize comparisons of vessel element length by diameter. Otherwise, we could risk saying that species X has longer vessel elements than species Y solely by virtue of species X having wider vessels. Standardizing by element length, as via the residuals of a length–diameter regression, achieves such comparability.

THE SCALING OF VESSEL ELEMENT LENGTH AND PIT AREA WITH VESSEL DIAMETER

Across self-supporting plants, vessel element length scaled predictably with vessel diameter (Fig. 5), with differing relationships between species with simple and scalariform perforation plates ($R^2 = 0.55$, $P < 0.001$). Vessel elements with scalariform perforation plates were longer for a given vessel diameter than vessel elements with simple plates.
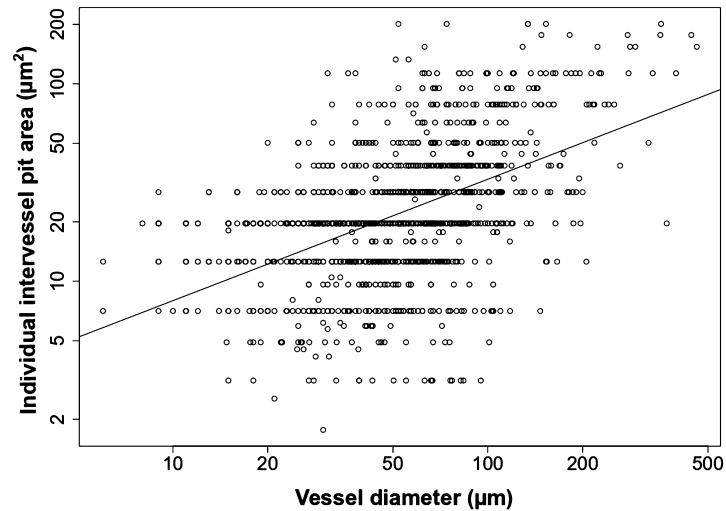
Fig. 6. Inter-vessel pit area-vessel diameter scaling across angiosperms. We recovered a moderate (R^2 = 0.24, see Table 2) ability of vessel element diameter to predict the areas of individual inter-conduit pit areas, which scaled with a slope of about 0.6.

The slope of the relationship between vessel element length and vessel diameter differed between species with simple and scalariform perforation plates (Fig. 5A). Vessel element length scaled with vessel diameter with a slope of 0.38 for plants with simple plates, and with a slope of 0.56 for plants with scalariform plates. Individual intervessel pit area also scaled predictably with vessel diameter with a slope of 0.61 (Fig. 6, R^2 = 0.24, P < 0.001). We identified the samples with the longest and shortest vessel elements given diameter for species with simple (Fig. 5B) and scalariform (Fig. 5C) perforation plates, for use in analyses of the association between vessel element length and climate.

Association between vessel element length and imperforate tracheary element length as a result of developmental drag?

Our results reject notions such as that of Tyree & Zimmermann (2002) or Baas (1976) that vessel element length has no functional significance and is the mere result of factors such as selection on imperforate tracheary element length. Our models predicting ITEL based on VEL, separating by ITE type (Table 2), confirmed the ability of VEL to influence ITEL. The two models predicting ITEL show that, both in species with conductive ITEs (true tracheids, Fig. 7A) and non-conductive ITEs (fiber-tracheids and libriform fibers, Fig. 7B), species with scalariform perforation plates tend to have longer ITEs for a given VEL, and that even the slope of the ITEL–VEL relationship differs between species with scalariform and simple perforation plates, with the slope being significantly shallower in the case of simple perforation plates than scalariform (see the two models predicting ITEL in Table 2). In contrast, ITEL had a weaker effect on VEL (see the two final models in Table 2). In the case of species with scalariform perforation plates, there was no difference in VEL between species with conductive versus non-conductive ITEs (Fig. 7C). With regard to species with simple perforation plates, for the most part the entire range of variation in VEL given ITEL overlapped between species with conductive versus non-conductive ITEs, though the slope of the VEL-ITEL relationship was significantly shallower in species with true tracheids (Fig. 7D).

Vessel element length and climate

We tested the notion that species with vessel elements that are relatively long for a given diameter should be in moist areas, and those with vessel elements that are relatively short for a given diameter should be in dry areas. We did so by examining the species with the greatest absolute residual values extracted from linear models predicting

Table 2. Linear regression models fit exploring the relationship between vessel element length and other variables.

Model	N	R^2	Model ANOVA	Slope equality test	Intercept equality test	Slope (CI)	Intercept (CI)
Vessel element length $\sim$ Vessel diameter $*$ self non-self	2418	0.14	$F_{3,2414} = 137.2$	$P < 0.001$	$P = 0.007$	Self = 0.48 (0.4, 0.49) Non-self = 0.16 (0.06, 0.27)	Self = 1.77 (1.69, 1.84) Non-self = 2.09 (1.88, 2.3)
Vessel element length $\sim$ Vessel diameter + Perforation plate type + Vessel diameter $*$ Perforation plate	2278	0.55	$F_{3,2274} = 929.2$	$P < 0.001$	–	Simple = 0.38 (0.34, 0.41) Scalariform = 0.56 (0.48, 0.65)	Simple = 1.8 (1.59, 2.05) Scalariform = 2 (1.86, 2.14)
Individual intervessel pit area $\sim$ Vessel diameter	1111	0.24	$F_{1,1110} = 351.3$	–	–	0.61 (0.55, 0.68)	0.29 (0.18, 0.40)
Species with conductive ITEs (true tracheids): ITE length $\sim$ Vessel element length $*$ perforation plate type	396	0.73	$F_{3,1110} = 355.3$	$P < 0.01$	–	Simple = 0.56 (0.43, 0.70) Scalariform = 0.98 (0.89, 1.06)	Simple = 1.35 (1.01, 1.68) Scalariform = 0.18 (−0.07, 0.43)
Species with non-conductive ITEs (fiber-tracheids, libriform fibers): ITE length $\sim$ Vessel element length $*$ perforation plate type	1490	0.75	$F_{3,1486} = 1519$	$P < 0.001$	–	Simple = 0.74 (0.71, 0.77) Scalariform = 0.85 (0.72, 0.99)	Simple = 0.91 (0.83, 0.98) Scalariform = 0.58 (0.27, 0.88)
Scalariform-plated species only: Vessel element length – ITE length $*$ ITE conductive status	339	0.86	$F_{3,336} = 689$	$P = 0.11$	$P = 0.677$	Conductive ITEs = 0.89 (0.85, 0.94) Non-conductive ITEs = 0.81 ()	Conductive ITEs = 0.2 (0.05, 0.34) Non-conductive ITEs = 0.45 (−0.05, 0.56)

vessel element length by vessel diameter (VEL = b_0 + m(VD)) for simple and scalariform perforation plates separately (colored points in Fig. 5). We found different climate preferences for species with the longest and shortest vessel element lengths given diameter (Figs 8 and 9; Table 3). Species with the shortest vessel elements tend to grow in places with lower annual precipitation than species with the longest vessel elements, with slight differences between woods with simple and scalariform perforation plates (Figs 9 and 10). Species with the shortest vessel element lengths and simple perforation plates tend to grow in drier and hotter places. In contrast, species with

Table 2. (Continued.)

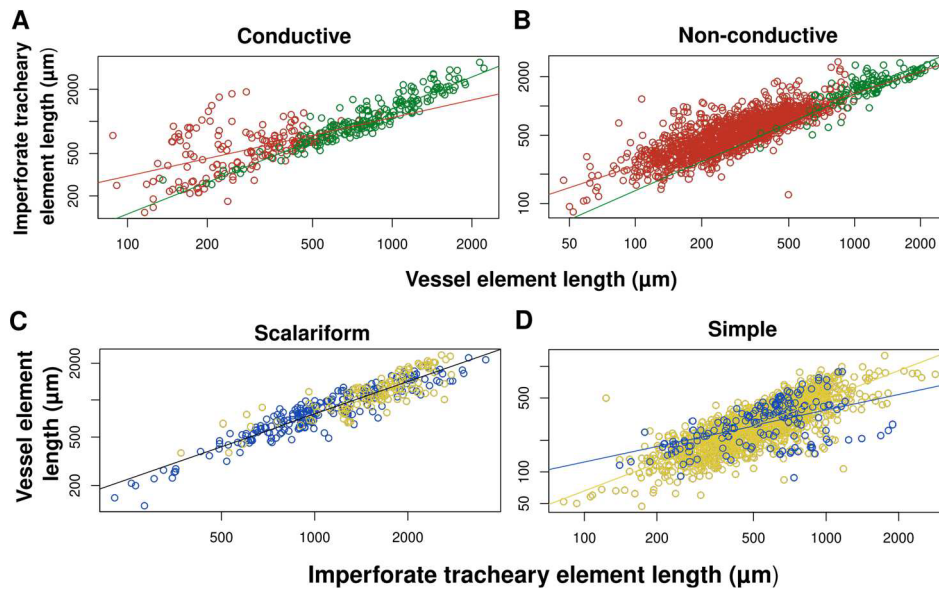| Model	N	R^2	Model ANOVA	Slope equality test	Intercept equality test	Slope (CI)	Intercept (CI)
Simple-plated species only: Vessel element length – ITE length * ITE conductive status	1536	0.61	$F_{3,1533} = 815.4$	$P < 0.001$	–	Conductive ITEs = 0.50 (0.38, 0.62) Non-conductive ITEs = 0.88 (0.84, 0.91)	Conductive ITEs = 1.09 (0.76, 1.42) Non-conductive ITEs = 0.06 (−0.03, 0.16)

Variables were $\log_{10}$-transformed.

Fig. 7. Vessel element length (VEL) clearly affects imperforate tracheary element length (ITEL), but it is less clear that imperforate tracheary element length affects vessel element length. (A,B) ITE-VEL models (models given in Table 2). Both in the case of species with (conductive) true tracheids (A), as well as those with (non-conductive) fiber-tracheids and libriform fibers (B), species with scalariform perforation plates (red data points), which tend to have longer VEL, also tend to have longer ITEL, showing that selection on VEL can affect ITEL. (C,D) VEL-ITE models. (C) In species with scalariform perforation plates, there was no difference in the ITEL-VEL relationship between species with conductive versus non-conductive ITEs. (D) In species with simple perforation plates, those with conductive ITEs scaled with a lower ITEL-VEL slope, but for the most part the data overlapped markedly. These results show clear evidence that VEL can "developmentally drag" ITEL, but less evidence (though in principle it must be possible) for ITEL to drag VEL. Red points (simple plates), green points (scalariform plates), blue points (non-conductive), yellow points (conductive).

the shortest vessel element lengths and scalariform perforation plates tend to grow in colder places characterized by high temperature seasonality and temperatures below zero during the coldest month of the year (Fig. 8C). Also, species with short vessels and scalariform perforation plates grow in places with lower precipitation during the driest month in comparison with species having longer vessel element lengths. Species with the longest vessel element lengths and scalariform plates tend to grow in places having the lowest temperature seasonality, the lowest annual range temperature and the highest mean annual precipitation, such as highland tropical cloud forests (Figs 8C and 9). Mean number of bars per plate in short scalariform vessel elements was 8.8, in contrast with 89 bars in the
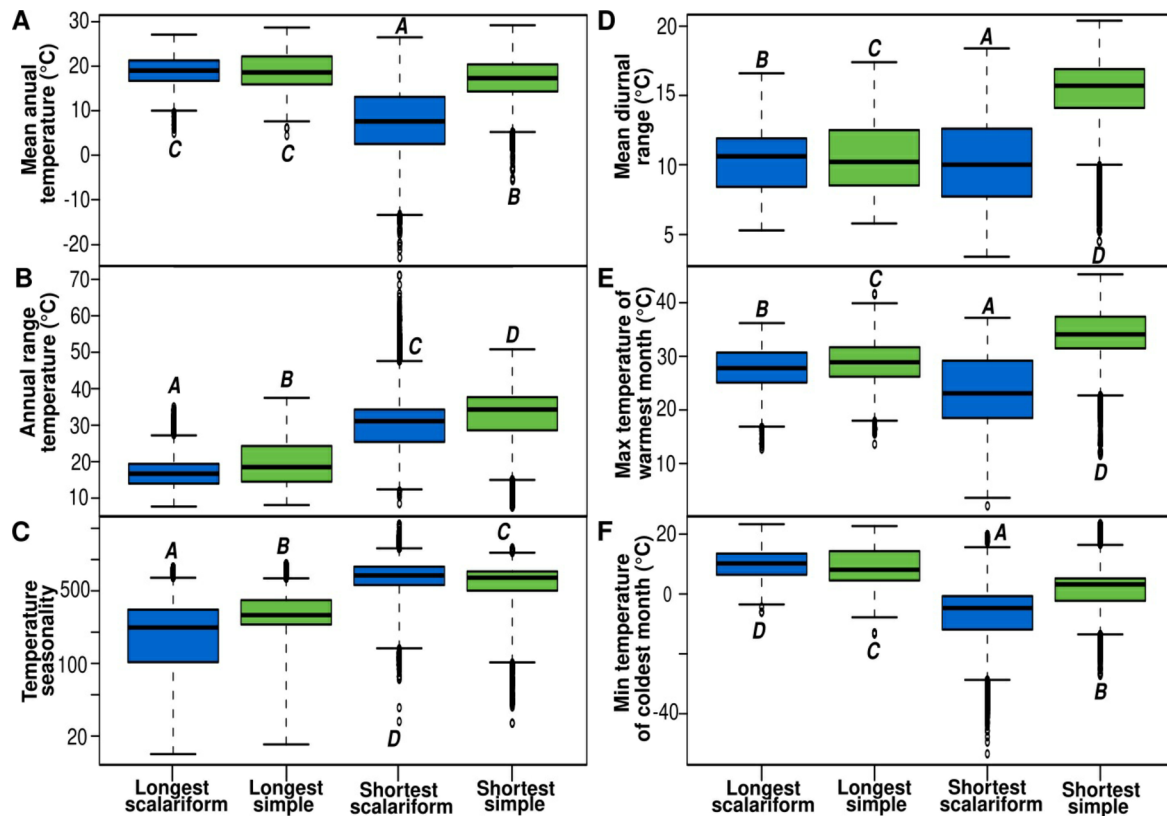
Fig. 8. Species with the shortest and longest vessel element lengths with different perforation plate types grow in places with different temperature regimes. Controlling for vessel diameter, species with the shortest vessel element lengths and scalariform perforation plates grow in places with the lowest (A) mean annual temperature, the lowest (E) maximum temperature of the warmest month, and the lowest (F) minimum temperature of the coldest month. Species with the longest vessel element length and scalariform perforation plates grow in places with the lowest (B) annual range temperature and the lowest (C) temperature seasonality (standard deviation of the mean monthly temperature). Species with the shortest vessel element lengths and simple plates grow in places with the highest (B) annual range temperature, the highest (D) mean diurnal range and (E) the maximum temperature of the warmest month. Blue boxes, scalariform plates; green boxes, simple plates. Letters in the graphs denote statistically significant differences among perforation plate type-vessel element length categories.

longest scalariform vessel elements, highlighting the likely differing roles of short vessel elements with few bars per scalariform plate in freezing environments in contrast to long vessel elements with many bars per scalariform plates in environments with stable, moderate temperatures and high precipitation.

Discussion

Vessel element length as a variable of functional significance in its own right

Congruent with Carlquist's hypotheses, our analyses suggest that vessel element length is indeed a functionally relevant variable in its own right, whatever the functional significance of vessel length. Authors who affirm the importance of vessel length are no doubt correct (Lens *et al.* 2011; Scholz *et al.* 2013). The distribution of vessel lengths determines how many high-resistance passages there are from vessel to vessel along the root-to-leaf path. Shorter vessels mean more such high-resistance passages (Comstock & Sperry 2000). Because of the relationship between
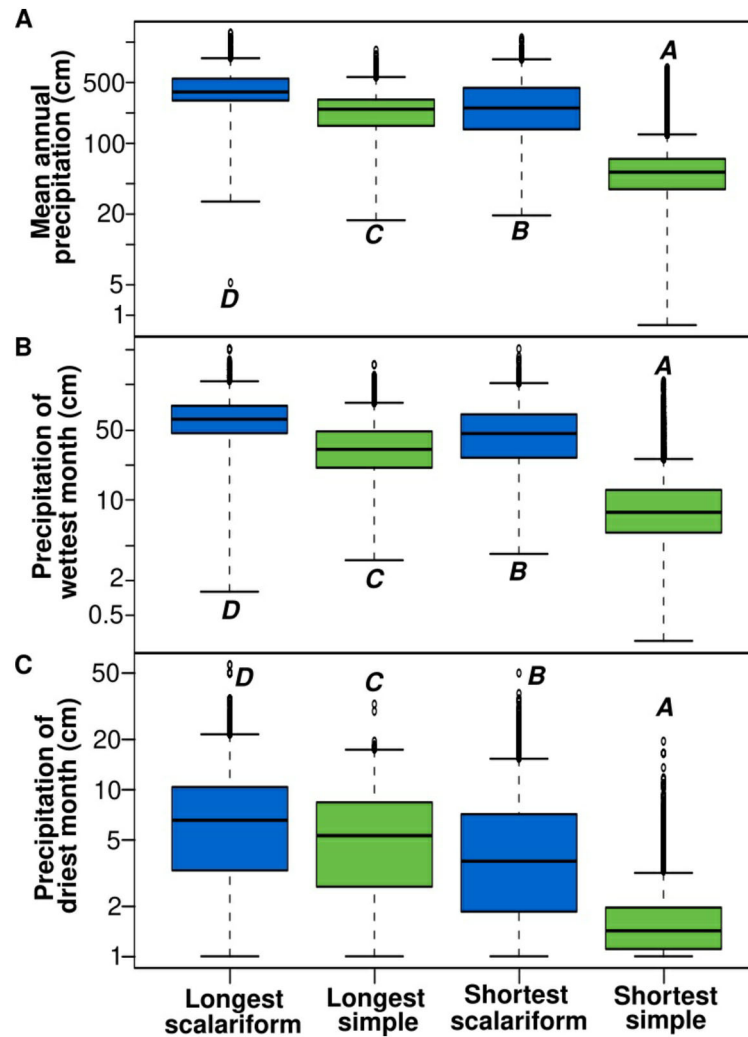
Fig. 9. Species with the shortest and longest vessel element lengths with different perforation plate types grow in places with different precipitation regimes. Controlling for vessel diameter, species with the shortest vessel element lengths and simple perforation plates grow in places with the lowest (A) mean annual precipitation, the lowest (B) precipitation of the wettest month, and with the lowest (C) precipitation of the driest month. Blue boxes, scalariform plates; green boxes, simple plates. Letters in the graphs denote statistically significant differences among perforation plate type-vessel element length categories.

vessel diameter and hydraulic resistance, on average, vessel length must scale with lumen resistance, such that vessels are on average longer in the roots and base of the plant, becoming shorter toward the tips (Koçillari *et al.* 2021). For these and other reasons, there is little question that total *vessel* length is unquestionably a functionally relevant variable (Jacobsen *et al.* 2012).

However, Carlquist's hypotheses regarding vessel element length are not at all obviated by the observation that total vessel length likely affects conductance. For one, his hypotheses regarding mechanical resistance to stresses that should cause inward deflection of vessel walls are not directly related to the scaling of axial resistance. Therefore, dismissing the potential adaptive value of vessel element length in resisting deformation because total vessel length better reflects the scaling of hydraulic resistance conflates two largely separable issues. Moreover, it seems clear that even simple perforation plates impose resistance to sap movement (Ellerby & Ennos 1998). It remains to be seen what

Table 3. Estimated marginal means from linear models predicting climate variables as a function of the factors vessel element length class and vessel element perforation plate type.

Climate variable	Longest vessel elements with simple plates	Longest vessel elements with scalariform plates	Shortest vessel element with simple plates	Shortest vessel element with scalariform plates
Mean annual temperature	19.15 (19.01, 19.3)	19.04 (18.79, 19.28)	16.65 (16.58, 16.73)	7.99 (7.88, 8.11)
Temperature annual range	19.69 (19.51, 19.88)	17.99 (17.69, 18.3)	33.03 (32.94, 33.12)	30.68 (30.53, 30.82)
Temperature seasonality ($\log_{10}$)	2.42 (2.41, 2.42)	2.26 (2.25, 2.27)	2.78 (2.77, 2.78)	2.81 (2.807, 2.815)
Mean diurnal range	10.59 (10.53, 10.66)	10.3 (10.19, 10.41)	15.31 (15.28, 15.34)	10.5 (10.45, 10.55)
Max temperature of the warmest month	28.92 (28.79, 29.05)	27.7 (27.48, 27.91)	34.17 (34.11, 34.24)	24.06 (23.96, 24.16)
Min temperature of the coldest month	9.22 (9.03, 9.42)	9.73 (9.4, 10.06)	1.14 (1.04, 1.24)	−6.62 (−6.67, −6.46)
Annual precipitation ($\log_{10}$)	2.03 (2.02, 2.04)	2.21 (2.2, 2.22)	1.4 (1.396, 1.4)	1.96 (1.955, 1.965)
Precipitation in the wettest month ($\log_{10}$)	1.243 (1.24, 1.25)	1.39 (1.38, 1.4)	0.61 (0.6, 0.612)	1.11 (1.108, 1.12)
Precipitation in the driest month	0.544 (0.538, 0.55)	0.74 (0.73, 0.75)	0.183 (0.18, 0.186)	0.58 (0.579, 0.589)

proportion of total pathway resistance is contributed by perforation plates, but because they are so numerous with respect to vessel–vessel passage, it is likely to be significant. Therefore, whatever the functional importance of vessel length, vessel element length is a viable candidate for being a variable of functional significance in its own right. We now touch on some of the aspects of functional significance we examined in our analyses, and some of the questions they raise.

Vessel element length can influence resistance to deformation under negative pressure

Our finite element analysis is sufficient to illustrate the point that vessel element length can clearly influence the resistance of vessels to deformation under negative pressure. Vessels with long vessel elements, and thus more widely spaced perforation plates, were more resistant to deformation than vessels with uniform wall thickness (Table 1). Vessels with shorter vessel elements and more closely spaced perforation plates had the highest resistance to deformation of all. This result is expected given that mechanical stress is expressed as mechanical load divided by the area or volume bearing the load. The *ceteris paribus* situation in all the models was the same lumen wall thickness, the same internal vessel diameter, the same tube length, and the same negative pressure inside the vessels. The only difference was the presence and spacing of the perforation plate annuli. Given that tubes with perforation plate annuli have more material than those without, and that those with more plates have more material than those with fewer plates, stress is necessarily lower in the presence of more plates. Moreover, the deformations encountered, for example up to 0.2 µm in the case of the vessels without annuli, make it seem plausible that in real conduits the range of deformations could be sufficient to cause distension of the intervessel pit membranes in the absence of sufficient reinforcement. The inevitability of lower mechanical stress in tubes with shorter vessel elements *ceteris paribus* makes the skepticism of authors such as Tyree & Zimmermann (2002) on the potential significance of vessel element length in resisting deformation especially puzzling. While much remains to be explored, including
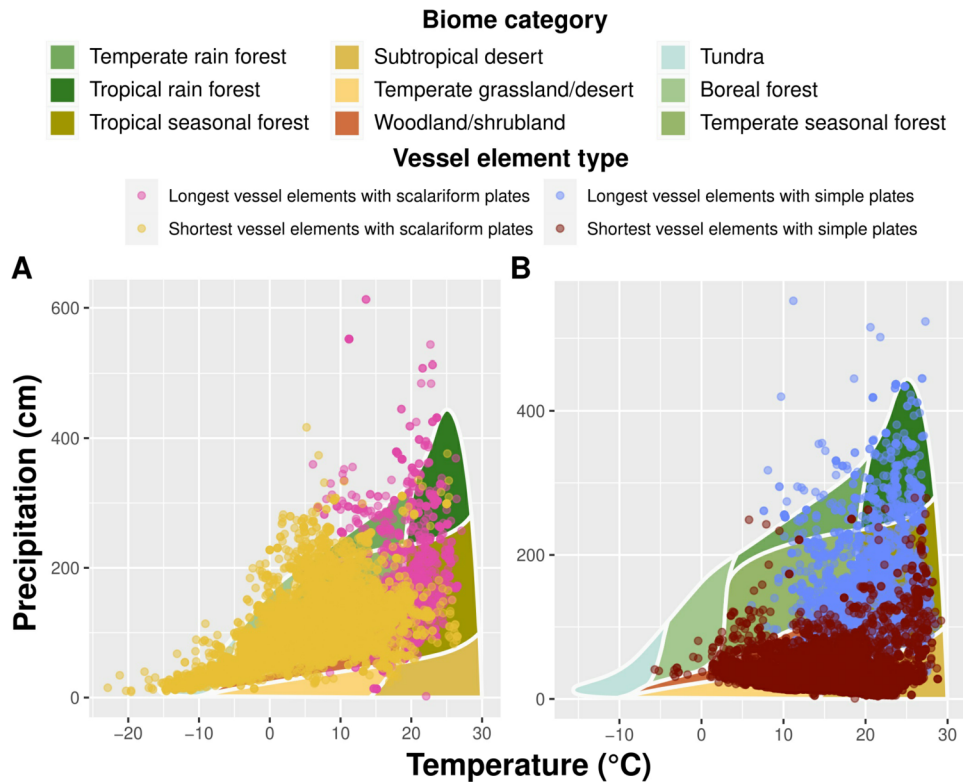
Fig. 10. Whittaker plots showing distribution across biomes of some of the species with the shortest and longest vessel element lengths for a given diameter with scalariform (A) and simple (B) perforation plates. Species with the shortest vessel element lengths and scalariform plates (yellow points) grow in the tundra and boreal forests while species with the shortest vessel element lengths and simple plates grow in temperate grasslands and subtropical deserts.

the influence of perforation plate angle, the mechanical behavior of vessels with scalariform perforation plates, and the relative carbon costs between vessels with perforation plates and simple tubes of similar mechanical resistance, our finite element analyses illustrate Carlquist's postulate that vessel element length can in principle be a target of selection in the context of resistance of vessel deformation under negative pressure.

Perforation plate effect on hydraulic conductance: is there a tradeoff between implosion resistance and hydraulic conductance?

Our analysis of the contribution of the perforation plate to the decrease in hydraulic conductance was consistent with Carlquist's hypothesis of a tradeoff between implosion resistance and hydraulic conductance. Shorter vessel elements, which were more resistant to mechanical deformation under negative pressure, had lower conductance. This finding makes it plausible that there is a balance between natural selection favoring shorter vessel elements by virtue of the resistance to deformation that they confer, longer vessel elements by virtue of the lowered resistance to conduction that they confer, together with other features, such as vessel wall thickness or total vessel length.

Vessel element length–diameter scaling across species and the possible role of individual pit area

Our finding that longer vessel element length VEL for a given vessel diameter VD in species with scalariform plates is strongly congruent with the notion that VEL is functionally significant. Scalariform perforation plates impose much

more resistance than do simple plates (Christman & Sperry 2010; Jansen & Nardini 2014). Therefore, for a similar per-vessel conductance, perforation plates must be widely spaced if plates are scalariform and can be closely spaced if they are simple (Ellerby & Ennos 1998; Gao *et al.* 2020). This is a clear case in which selection should strongly favor differing vessel element lengths for a given diameter. Our evidence shows that, consistent with these expectations, for a given vessel diameter, vessel elements with scalariform plates are significantly longer than those with simple plates (Fig. 5), showing that vessel element length can be a subject of selection in its own right.

The scaling of pit dimensions with lumen resistance could potentially be involved in selection favoring a given vessel element length. In our dataset, wider elements tend to correspond to larger individuals. Within-individual data from Lazzarin *et al.* (2016) and Williams *et al.* (2019) show that wider, more basal, conduits have much lower individual pit resistance, scaling with lumen resistance. They showed that in tracheids along tall conifers, the number of pits scales as D^1 (cell length scales isometrically with cell diameter D), the area of the pits scales as D^1, thus the total area of the pits increases as D^2. Together, total conductance of the pits scaled with VD^4, exactly balancing the increase in the potential flow due to the increase of tracheid diameter.

Something similar must likewise occur in vessel-bearing plants. Broadly speaking, selection should favor wider individual pits in wider vessels, exactly the relationship that emerges in our data (Fig. 6). Because packing a certain number of larger pits requires more total space than the same number of small pits, individual vessel elements, plausibly, must be longer. Given that the perforation plate subtracts wall space on which pits can be arranged, elements that are too short for a given diameter would not provide enough wall area on which to deploy the larger pits favored in wider vessels. This scenario implies that selection tends to favor individual elements that are longer when vessels are wider. This idea suggests that the distance between pits, not just the pit border but the distance between pit apertures, is an important variable to measure. Consistent with these expectations, Silva *et al.* (2021) found that the diameter of the pit chamber was positively correlated with both theoretical conductivity as well as Carlquist's vulnerability index. To the extent that pit dimensions and packing could affect the relationship between pit and vessel lumen resistance, then individual pit dimension is another potential vector of natural selection involving vessel element length.

Developmental drag

Our analyses shed light on the "difficult to discern" evolutionary negotiation that Carlquist highlighted (e.g., Carlquist 1975: p. 150) between selection acting on vessel element length and imperforate tracheary element length. Given that both vessel elements and imperforate tracheary elements are derived from the same mother cells, they are bound in a shared developmental cascade, such that selection favoring greater or lesser length in one cell type should be countered by selection in the opposite direction in the other cell type (Montes-Cartas *et al.* 2017). Our analyses of the "developmental drag" hypothesis actually appear to show that selection on vessel element length VEL has a greater influence on imperforate tracheary element length ITEL than the reverse (i.e. our data suggest less influence of ITE on VEL). Here, we take the "developmental drag hypothesis" in the sense used by Tyree & Zimmermann (2002) that vessel element length has no functional relevance and is only a reflection of some other factor, such as selection on imperforate tracheary element length. Our analyses clearly show that selection on vessel element length can affect ITE length, but are much more ambiguous with regard to the possibility that selection on ITE length affects vessel element length. We take these two categories of results in turn.

First, it is clear that selection on VEL can affect (i.e., "developmentally drag") ITEL. Our models (the two models predicting ITEL based on VEL in Table 2) showed that there are statistically significantly different ITEL-VEL relationships between species with simple and scalariform perforation plates. In particular, species with scalariform perforation plates, which have longer VEL for a given vessel diameter than do species with simple perforation plates (Fig. 5, Table A2 in the Appendix) tend to have longer ITEL. This result was observed both in species with true tracheids, which are conductive (Fig. 9A) as well as in species with non-conductive imperforate tracheary elements

(fiber-tracheids and libriform fibers) (Fig. 9B). Recall that we performed separate analyses for conductive and non-conductive ITEs because selection tends to favor long ITEs when they are conductive. Because scalariform plates tend to be associated with true tracheids, which are conductive (Echeverría *et al.* 2022), if we had fit just one model pooling all ITE types, then the relationship longer VEL–longer ITEL would have emerged as the sheer result of the scalariform-tracheid association and the longer lengths of tracheids as compared to non-conductive ITEs. Instead, by fitting two ITEL-VEL models separating by conductive and non-conductive ITE type, we maximized the chance of finding a lack of developmental drag of ITEs by selection on VEL. In this way, our results confidently point to the phenomenon of natural selection acting on vessel element length as a functionally significant variable in its own right affecting ("developmentally dragging") imperforate tracheary element length.

Second, and in surprising contrast, our results are much more ambiguous with regard to the possibility that selection on ITEL can developmentally drag VEL, which was the preferred hypothesis of Tyree & Zimmermann (2002). In the case of species with scalariform perforation plates, we found no difference at all in VEL given ITEL between species with conductive versus non-conductive ITEs (Fig. 7C). In the case of species with simple perforation plates, the range of VEL given ITEL overlapped almost entirely (Fig. 7D). While the VEL-ITEL slopes were significantly different in species with simple plates between those with conductive versus non-conductive ITEs, the overlap was so extensive that it seems possible that further sampling will reveal that, as is the case in species with scalariform plates, there is in fact no discernible difference. As a result, the evidence is less compelling that selection on ITEL can strongly affect VEL, in contrast to the clear pattern of selection on VEL affecting ITEL.

In principle "dragging" can likely go in any direction. Dragging is here understood to mean that selection acting in one direction on one cell type will alter fusiform cambial initial length and be countered by selection in an opposite direction in another cell type. Even the rays are likely involved in this web of opposing vectors of natural selection. Ray cells are produced by ray initials. Ray initials, in turn, are produced from subdivision of fusiform initials. Therefore, the sizes of the bundles of ray initials that can be produced are to an extent limited by the sizes of the fusiform initials. Moreover, if selection favors very tall ray cells, then this could limit the minimum sizes of ray, and therefore fusiform, cambial initials. As with selection favoring long ITEs, selection favoring axially tall ray cells could also "drag" axial cell length. "Drag" in this context implies leading to a longer length that would otherwise be observed given only selection on the axial cells. Along these lines, rayless species bear examination with regard to their ITE and vessel element lengths. Rayless species are presumably freed from any selection pressure on ray cell vertical dimensions. As a result, they should reflect only the effects of selection on the axial system cells. In our sampling, most of the samples of *Pentaphragma* are rayless, and were unusual in that the fiber tracheids, which are thought to be non-conductive, are somewhat shorter than the vessel elements (Carlquist 1997). This unusual proportionality (ITEs are usually longer than vessel elements) is a potential consequence of freedom from selection favoring very short ray cells. Taken together, these examples suggest that the lengths of secondary xylem cells are the result of evolutionary tugs-of-war between vectors of selection favoring different lengths of different cell types, and that these networks of "negotiation" can potentially even include not just the axial but also the radial system.

Vessel element length and climate

Controlling for vessel diameter, species having the shortest and longest vessel element lengths have clearly distinct climate preferences, consistent with Carlquist's hypothesis regarding the adaptive role of vessel element length. Species with the shortest vessel element lengths for a given diameter grow in places with greater ranges in annual temperature and greater temperature seasonality than do species with the longest vessel element lengths (Fig. 8B, C). Furthermore, by taking into consideration vessel perforation plate type, we identify different plausible selective roles for the interaction of vessel element lengths and perforation plates having differing numbers of bars, from 0 (simple plates) to more than 100 (Fig. 10; Tables A3 and A4 in the Appendix). Species with the shortest vessel elements and simple perforation plates grow in places with the highest mean diurnal temperature ranges, the highest

maximum temperatures of the warmest month, and the lowest mean annual precipitation, conditions that are more prone to highly negative water potentials (Figs. 8E and 9C). In contrast, species with the shortest vessel elements and scalariform perforation plates grow in places with the lowest annual temperatures and the lowest temperatures of the coldest month, conditions that are more prone to embolism induced by freeze-thaw cycles (Figs. 8F, 10). Carlquist recently proposed what we regard as the first truly compelling explanation for the presence of long scalariform perforation plates (more than 10 bars or so). In brief, they are favored because they help prevent or recover from drought-induced embolism. However, the hydraulic resistance they impose outweighs their embolism-resistance effect in all but the situations of highest resistance and slowest conductance. As a result, they are found chiefly in cloud forest plants and in distal twigs (for details on the hypothesis, see Carlquist 2018; Olson 2020).

IS THERE A RELATIONSHIP BETWEEN VESSEL LENGTH AND VESSEL ELEMENT LENGTH?

Some authors have wondered whether there is a relationship between vessel length and vessel element length (Silva *et al.* 2021). If so, this would almost certainly represent a correlation of significance for conductance and vessel mechanical resistance. Moreover, it would be very significant because it would provide a convenient basis for estimating vessel length from simple measurements. Certainly within a given stem, such a correlation seems very unlikely. The fusiform initials producing cells at any given point on a stem are the same length whether they are giving rise to an imperforate tracheary element cell, a strand of axial parenchyma, a vessel element participating in a short vessel, or a vessel element participating in a long vessel. Many authors have clearly shown that very short vessels routinely coexist with long ones in the same cross-section; the fact that all of the elements are produced by the same cambial cells shows that there is no necessary correlation between vessel length and element number (Brodersen *et al.* 2013; Wason *et al.* 2017; Jacobsen *et al.* 2019). Because long and short vessels are virtually always intermingled in the same sector of stem, and that all the secondary xylem cells are produced from the same initial cells, a relationship between vessel element length and vessel length within the same level of the same stem seems a clear developmental impossibility.

However, there could perhaps be a rough correlation across size classes. Taller plants have wider vessels, and, as we show here, vessel element length and diameter are positively correlated. Some evidence suggests that wider vessels tend to be longer (Ewers & Fisher 1989; Jacobsen *et al.* 2012). So, from short to tall species, or from tip-to-base along the stem of a plant, there could conceivably be a rough correlation between mean vessel element length and some index of vessel length. Such a correlation would involve selection lowering resistance via wide, long vessels with long elements. Presumably the lengths of ITEs would also be a factor of selective significance. Given the laboriousness of measuring vessel length, even a rough correlation across species would provide a useful proxy.

Conclusion

Our analyses are sufficient to show that, whatever the functional relevance of total vessel length, vessel *element* length is a variable of functional relevance in its own right. There clearly remains much to be explored regarding the empirical distribution of total vessel lengths across plant sizes, habits, and climates. Our analyses suggest that vessel element length should interact with total vessel length as a target of natural selection. This is because vessel element length, by virtue of its influence on total pathlength conductive resistance, influences conductance. To the extent that vessel element length is involved in resisting deformation under negative pressure, it can, in turn, influence veseel diameter and thereby total pathlength resistance and thus vessel length. As we show here, individual interconduit pit areas scale with vessel diameter, which in turn scales with vessel element length, likely as part of a suite of features that must be covarying — factors such as total vessel lumen resistance (including perforation plates) and vessel end/ pit resistances would seem necessarily to covary as plants maintain conductance to the leaves as they grow taller (Lazzarin *et al.* 2016). Very little is known regarding how all of these factors of resistance covary with one another

along the lengths of plants and how such covariation is involved in adaptation in the context of different plant habits and climatic conditions. However, because conductance must necessarily be the result of the coordinated variation in these traits, documenting how traits covary and why is essential for understanding how selection shapes plant conductive systems. Our results here seem sufficient to confirm that vessel element length is a variable of functional significance that needs to be taken into account in documenting these patterns of covariation.

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Appendix
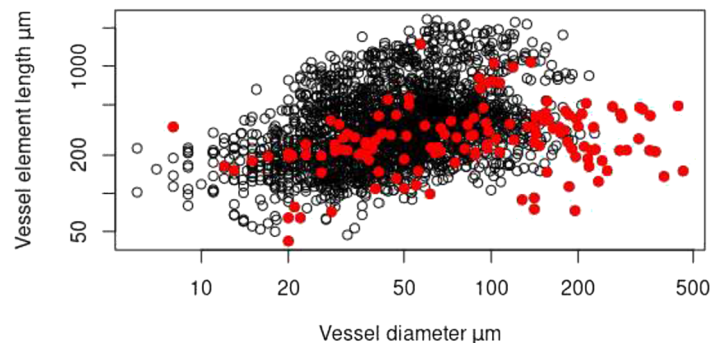
Fig. A1. Vessel element length–vessel diameter relationship across self supporting and non-self supporting species. Red points, non-self supporting species. There appear to be similar patterns in the relationship between vessel element length and diameter in non self-supporting species as compared to self-supporting ones. For example, the cloud of scalariform-plated species can be seen above that of the simple-plated species. Most lianas have simple plates, but the few scalariform-plated in our dataset can be seen falling in the expected area. As with all log-log relationships, abundant data at smaller sizes, in this case vessel diameters, are crucial for anchoring regressions. Relatively low sampling in key areas, such as narrow vessel diameters and the lack of scalariform-plated species, led us to exclude non self-supporting species for simplicity of analysis.

Table A1. Species with shortest vessel element lengths per vessel diameter and simple plates.

Family	Species	Vessel diameter	Vessel element length	Imperforate tracheary element type	Residuals
Zygophyllaceae	*Guaiacum sanctum*	32	47	Fiber tracheids	−0.7
Santalaceae	*Phoradendron leucarpum subsp. tomentosum*	33.9	53.1		−0.66
Brassicaceae	*Sinapidendron rupestre*	36	67.6	Libriform	−0.56
Brassicaceae	*Descurainia preauxiana var briquetii*	27	62.1	Libriform	−0.55
Brassicaceae	*Erysimum scoparium*	27	63.3	Libriform	−0.55
Misodendraceae	*Misodendrum angulatum*	16	52	Libriform	−0.54
Compositae	*Ambrosia salsola*	100	106	Libriform	−0.54
Zygophyllaceae	*Fagonia laevis*	53.3	89.3	Fiber tracheids	−0.51
Brassicaceae	*Lepidium serra*	36	79.6	Libriform	−0.49
Misodendraceae	*Misodendrum punctulatum*	16.2	59.2	Libriform	−0.49
Zygophyllaceae	*Viscainoa geniculata*	41	84	Fiber tracheids	−0.49
Amaranthaceae	*Krascheninnikovia lanata*	18.2	61.9		−0.49
Brassicaceae	*Sinapidendron angustifolium*	50	91.6	Libriform	−0.49
Compositae	*Encelia farinosa*	108	123	Libriform	−0.48
Brassicaceae	*Descurainia preauxiana*	29	78.5	Libriform	−0.46
Compositae	*Flourensia cernua*	90	121	Libriform	−0.46
Plumbaginaceae	*Limonium commune*	14	60	Libriform	−0.46
Boraginaceae	*Phacelia ramosissima*	53	101	Libriform	−0.45
Amaranthaceae	*Grayia spinosa*	23.2	76.4		−0.44
Amaranthaceae	*Maireana sedifolia*	28	83		−0.43
Compositae	*Bahiopsis laciniata*	102	137	Libriform	−0.44
Pedaliaceae	*Pedalium murex*	59	112	Libriform	−0.44
Aristolochiaceae	*Lactoris fernandeziana*	53	108	Libriform	−0.42
Convolvulaceae	*Ipomoea murucoides*	141	157.33	Fiber tracheids	−0.42
Amaranthaceae	*Rhagodia preissii*	37	95		−0.42
Bignoniaceae	*Chilopsis linearis*	86.7	132		−0.42
Convolvulaceae	*Ipomoea intrapilosa*	140	159	Fiber tracheids	−0.42
Zygophyllaceae	*Larrea tridentata*	29	88	Fiber tracheids	−0.41
Compositae	*Iva axillaris*	44	104	Libriform	−0.41
Onagraceae	*Gaura sinuata*	46	106	Libriform	−0.41
Convolvulaceae	*Ipomoea pauciflora*	130	158	Fiber tracheids	−0.41
Coriariaceae	*Coriaria ruscifolia subsp microphylla*	107	148	Libriform	−0.4
Plumbaginaceae	*Limonium californicum*	13	67	Libriform	−0.4
Brassicaceae	*Crambe fruticosa*	37	99.6	Libriform	−0.4
Convolvulaceae	*Ipomoea fistulosa*	88	139	Fiber tracheids	−0.4
Compositae	*Artemisia californica*	43.2	107	Libriform	−0.39
Misodendraceae	*Misodendrum gayanum*	13	68	Libriform	−0.39
Amaranthaceae	*Atriplex nummularia*	28	91		−0.39
Compositae	*Dimorphotheca cuneata*	68.1	128	Libriform	−0.39
Boraginaceae	*Echium nervosum*	65	126	Libriform	−0.39
Krameriaceae	*Krameria sonorae*	25	88	Tracheids	−0.39
Gyrostemonaceae	*Codonocarpus cotinifolius*	99	149	Tracheids	−0.39
Resedaceae	*Reseda alba*	30	95	Libriform	−0.39

Table A1. (Continued.)

Family	Species	Vessel diameter	Vessel element length	Imperforate tracheary element type	Residuals
Bataceae	*Batis maritima*	35.7	102	Fiber tracheids	−0.38
Brassicaceae	*Sinapidendron frutescens*	36	102.5	Libriform	−0.38
Solanaceae	*Lycium elongatum*	58	123	Fiber tracheids	−0.38
Compositae	*Deinandra minthornii*	71	133	Libriform	−0.38
Krameriaceae	*Krameria erecta*	26	91	Tracheids	−0.38

Table A2. Species with simple perforation plates and longest vessel element lengths per vessel diameter.

Family	Species	Vessel diameter	Vessel element length	Imperforate tracheary element type	Residuals
Pittosporaceae	*Pittosporum deplanchei*	38	995	Libriform	0.59
Penaeaceae	*Stylapterus ericoides*	31	781	Tracheids	0.52
Acanthaceae	*Sanchezia decora*	69	989	Libriform	0.49
Pittosporaceae	*Pittosporum mouanum*	46	838	Libriform	0.49
Pittosporaceae	*Pittosporum napaliense*	22	623	Libriform	0.48
Sabiaceae	*Ophiocaryon paradoxum*	153	1267	Fiber tracheids	0.47
Acanthaceae	*Sanchezia stenomacra*	57	863	Libriform	0.47
Picrodendraceae	*Tetracoccus dioicus*	22	599	Tracheids	0.46
Penaeaceae	*Penaea ruscifolia*	31	678	Tracheids	0.46
Pittosporaceae	*Pittosporum bracteolatum*	40	727	Libriform	0.45
Pittosporaceae	*Pittosporum glabrum*	41	733	Libriform	0.45
Penaeaceae	*Saltera sarcocolla*	34	691.4	Tracheids	0.44
Pittosporaceae	*Pittosporum pancheri*	35	672	Libriform	0.44
Pittosporaceae	*Hymenosporum flavum*	53	781	Libriform	0.43
Pittosporaceae	*Pittosporum rhombifolium*	66.5	843	Libriform	0.43
Fouquieriaceae	*Fouquieria columnaris*	34	663.5	Fiber tracheids	0.43
Achatocarpaceae	*Achatocarpus nigricans*	37	670	Libriform	0.43
Gentianaceae	*Tachia occidentalis*	36	661	Fiber tracheids	0.43
Penaeaceae	*Penaea cneorum*	50.5	742	Tracheids	0.42
Pittosporaceae	*Pittosporum gomonenense*	31	617	Libriform	0.42
Pittosporaceae	*Pittosporum rhytidocarpum*	65.67	820.67	Libriform	0.42
Acanthaceae	*Sanchezia williamsii*	86	901	Libriform	0.42
Campanulaceae	*Trematolobelia grandifolia*	56.75	759.75	Libriform	0.41
Pittosporaceae	*Pittosporum ferrugineum*	65	795	Libriform	0.41
Sabiaceae	*Meliosma occidentalis*	99	923	Libriform	0.4
Pittosporaceae	*Pittosporum cravenianum*	62	773	Libriform	0.4
Pittosporaceae	*Pittosporum napaulense*	75	821	Libriform	0.4
Pittosporaceae	*Pittosporum acuminatum*	37	627	Libriform	0.4
Pittosporaceae	*Pittosporum ramiflorum*	53	711	Libriform	0.39
Pittosporaceae	*Pittosporum viridiflorum*	46	670	Libriform	0.39
Pittosporaceae	*Pittosporum bicolor*	34	599	Libriform	0.39
Sabiaceae	*Meliosma alba*	92	868	Fiber tracheids	0.39

Table A2. (Continued.)

Family	Species	Vessel diameter	Vessel element length	Imperforate tracheary element type	Residuals
Goodeniaceae	*Scaevola beckii*	35.5	606	fiber tracheids	0.39
Campanulaceae	*Lobelia stuhlmannii*	56	717	Libriform	0.39
Boraginaceae	*Eriodictyon crassifolium*	38	610	Tracheids	0.38
Scrophulariaceae	*Buddleja colvilei*	26	528	Libriform	0.38
Compositae	*Faujasia* sp.	58.2	713	Libriform	0.38
Stilbaceae	*Euthystachys abbreviata*	22.2	495	Libriform	0.38
Lecythidaceae	*Scytopetalum tieghemii*	110	903	Libriform	0.38
Pittosporaceae	*Pittosporum pentandrum*	74	776	Libriform	0.38
Pittosporaceae	*Pittosporum glabratum*	34	578	Libriform	0.38
Pittosporaceae	*Pittosporum gayanum*	36	588	Libriform	0.38
Campanulaceae	*Cyanea pilosa subsp longipedunculata*	47	650	Libriform	0.38
Stilbaceae	*Retzia capensis*	31.3	556	Fiber tracheids	0.38
Pittosporaceae	*Pittosporum berberidoides*	55	688	Libriform	0.37
Penaeaceae	*Brachysiphon fucatus*	33	565	Tracheids	0.37
Pittosporaceae	*Pittosporum cornifolium*	28	526	Libriform	0.37
Stilbaceae	*Kogelbergia verticillata*	31	543	Libriform	0.36
Pittosporaceae	*Pittosporum divaricatum*	19	451	Libriform	0.36
Penaeaceae	*Stylapterus fruticulosus*	27	515	Tracheids	0.36
Campanulaceae	*Cyanea angustifolia var angustifolia*	56	677	Libriform	0.36
Pittosporaceae	*Pittosporum hematomallum*	47	633	Libriform	0.36
Pittosporaceae	*Pittosporum pronyense*	42	605	Libriform	0.36
Campanulaceae	*Cyanea leptostegia*	74	746	Libriform	0.36
Pittosporaceae	*Pittosporum ralphii*	35	561	Libriform	0.36
Rosaceae	*Lyonothamnus floribundus*	19.7	451	Tracheids	0.36
Fouquieriaceae	*Fouquieria fasciculata*	21	462	Fiber tracheids	0.36
Acanthaceae	*Pachystachys lutea*	27	507	Libriform	0.36
Myrtaceae	*Homalospermum firmum*	35	559		0.36
Penaeaceae	*Endonema lateriflora*	30	526	Fiber tracheids	0.36
Pittosporaceae	*Bursaria incana*	46	618	Libriform	0.36
Pittosporaceae	*Pittosporum erioloma*	32	535	Libriform	0.35
Stilbaceae	*Stilbe vestita*	25.7	491	Libriform	0.35
Berberidaceae	*Epimedium pinnatum*	15	400	Libriform	0.35
Campanulaceae	*Lobelia gibberroa*	70	716	Libriform	0.35
Violaceae	*Hybanthus floribundus*	29	512		0.35

Table A3. Species with shortest vessel element lengths per vessel diameter with scalariform perforation plates.

Family	Species	Vessel diameter	Vessel element length	Number of bars per perforation plate	ITE type	Residuals
Ericaceae	*Empetrum eamesii subsp eamesii*	22	158	4	Tracheids	−0.56
Cornaceae	*Cornus nuttallii*	85.7	334	35	Tracheids	−0.56

Table A3. (Continued.)

Family	Species	Vessel diameter	Vessel element length	Number of bars per perforation plate	ITE type	Residuals
Ericaceae	*Empetrum nigrum subsp hermaphroditum*	22.5	203.5	10.1	Tracheids	−0.46
Ericaceae	*Rhododendron occidentale*	33.9	272	4	Tracheids	−0.43
Ericaceae	*Empetrum atropurpureum*	29	267	5	Tracheids	−0.4
Ericaceae	*Corema album*	22	230	10.2	Tracheids	−0.4
Bruniaceae	*Brunia myrtoides*	79	532	30.8	Tracheids	−0.39
Sabiaceae	*Meliosma hachijoensis*	154	770	3.6	Libriform	−0.34
Sabiaceae	*Meliosma simplicifolia*	171	835	6.3	Libriform	−0.33
Ericaceae	*Empetrum rubrum*	17.5	243.5	5.95	Tracheids	−0.32
Sabiaceae	*Meliosma arnottiana*	181	875	3.3	Libriform	−0.32
Ericaceae	*Empetrum nigrum*	19	256	12	Tracheids	−0.32
Buxaceae	*Buxus glomerata*	33	351	9.5	Tracheids	−0.31
Sabiaceae	*Meliosma dilleniifolia*	91.5	613.5	10.15	Libriform	−0.31
Sabiaceae	*Meliosma parviflora*	100	663	3.2	Fiber tracheids	−0.3
Myricaceae	*Morella javanica*	133	800	6.6	Tracheids	−0.28
Ericaceae	*Corema conradii*	18	274	2	Tracheids	−0.28
Saururaceae	*Saururus cernuus*	72	590	14	Fiber tracheids	−0.28
Ericaceae	*Phyllodoce brewerii*	14.1	244	8	Tracheids	−0.27
Staphyleaceae	*Staphylea bolanderi*	54.33	518.33	14	Tracheids	−0.26
Sabiaceae	*Meliosma myriantha*	83	655	4.4	Libriform	−0.26
Stilbaceae	*Stilbe serrulata*	38.8	439	6.5	Fiber tracheids	−0.25
Myricaceae	*Myrica nagi*	60	560	6.6	Tracheids	−0.25
Sabiaceae	*Meliosma dentata*	78	673	9.5	Libriform	−0.23
Bruniaceae	*Thamnea massoniana*	27	380	15.4	Tracheids	−0.23
Myricaceae	*Morella salicifolia*	84	710	7.9	Tracheids	−0.23
Sabiaceae	*Meliosma lanceolata*	162	1018	6.8	Fiber tracheids	−0.23
Ericaceae	*Leucopogon australis*	32	427	0.4		−0.22
Myricaceae	*Morella pubescens*	59	605	9.2	Tracheids	−0.21
Myricaceae	*Morella kraussiana*	48	541	10.3	Tracheids	−0.21
Buxaceae	*Buxus macowanii*	36	469	5.8	Tracheids	−0.21
Myricaceae	*Myrica hartwegii*	35	466	12	Tracheids	−0.2
Platanaceae	*Platanus racemosa*	44	529	3	Tracheids	−0.2

Table A4. Species with longest vessel element lengths per vessel diameter with scalariform perforation plates.

Family	Species	Vessel diameter	Vessel element length	Number of bars per perforation plate	ITE type	Residuals
Paracryphiaceae	*Sphenostemon lobosporus*	36	1702	163	Tracheids	0.36
Chloranthaceae	*Ascarina maheshwarii*	62.5	2313	158	Fiber tracheids	0.36
Daphniphyllaceae	*Daphniphyllum borneense*	52	1890	65	Tracheids	0.31
Chloranthaceae	*Ascarina swamyana*	64	2034	105	Fiber tracheids	0.29
Chloranthaceae	*Ascarina rubricaulis*	62.5	1961.5	112.5	Fiber tracheids	0.28

Table A4. (Continued.)

Family	Species	Vessel diameter	Vessel element length	Number of bars per perforation plate	ITE type	Residuals
Chloranthaceae	*Hedyosmum scaberrimum*	68	1997	116	Fiber tracheids	0.27
Chloranthaceae	*Hedyosmum bondplandianum*	72	2039	120	Fiber tracheids	0.27
Bruniaceae	*Berzelia squarrosa*	28	1196	30.2	Tracheids	0.27
Schisandraceae	*Illicium ridleyanum*	43	1520	111	Tracheids	0.26
Chloranthaceae	*Ascarina subfalcata*	64	1894	142	Fiber tracheids	0.26
Chloranthaceae	*Hedyosmum arborescens*	70.5	1984	127	Fiber tracheids	0.26
Chloranthaceae	*Ascarina philippinensis*	81	2146	146	Fiber tracheids	0.26
Bruniaceae	*Berzelia burchellii*	24	1071	33	Tracheids	0.26
Chloranthaceae	*Ascarina polystachya*	72	1952	144	Fiber tracheids	0.25
Daphniphyllaceae	*Daphniphyllum gracile*	59.5	1755.5	84.5	Tracheids	0.25
Schisandraceae	*Illicium anisatum*	43	1434	83	Tracheids	0.24
Paracryphiaceae	*Paracryphia alticola*	53	1612	159	Libriform	0.24
Chloranthaceae	*Chloranthus elatior*	32	1207	78	Fiber tracheids	0.24
Schisandraceae	*Illicium tenuifolium*	60	1702	77	Tracheids	0.23
Buxaceae	*Sarcococca confusa*	29	1125	45.3	Tracheids	0.23
Bruniaceae	*Berzelia alopecuroides*	23	971	18.9	Tracheids	0.22
Chloranthaceae	*Chloranthus japonicus*	33	1188	76	Fiber tracheids	0.22
Daphniphyllaceae	*Daphniphyllum pentandrum*	53	1547	45	Tracheids	0.22
Balanopaceae	*Balanops sparsifolia*	39	1300	25.3	Fiber tracheids	0.22
Daphniphyllaceae	*Daphniphyllum scortechinii*	47	1441	46	Tracheids	0.22
Chloranthaceae	*Hedyosmum racemosum*	68	1761	112	Fiber tracheids	0.22
Chloranthaceae	*Hedyosmum brasiliense*	48	1440	79	Fiber tracheids	0.21
Daphniphyllaceae	*Daphniphyllum himalayense*	36	1215	43	Tracheids	0.21
Aextoxicaceae	*Aextoxicon punctatum*	44	1357	66	Tracheids	0.21
Grubbiaceae	*Grubbia rourkei*	39.9	1267	26.3	Tracheids	0.20
Chloranthaceae	*Chloranthus multistachys*	39	1248	72	Fiber tracheids	0.20
Bruniaceae	*Brunia microphylla*	24	946	34.8	Tracheids	0.20
Schisandraceae	*Illicium floridanum*	40	1258	69	Tracheids	0.20