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THE EVALUATION OF MURRAY'S LAW IN *PSILOTUM NUDUM* (PSILOACEAE), AN ANALOGUE OF ANCESTRAL VASCULAR PLANTS¹

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Previous work has shown that the xylem of seed plants follows Murray's law when conduits do not provide structural support to the plant. Here, compliance with Murray's law was tested in the stem photosynthesizer *Psilotum nudum*, a seedless vascular plant. *Psilotum nudum* was chosen because the central stele does not provide structural support, which means that Murray's law is applicable, and because its simple shoot structure resembles the earliest vascular plants. Murray's law predicts that the sum of the conduit radii cubed (Σr^3) should decrease in direct proportion with the volume flow rate (Q) to maximize the hydraulic conductance per unit vascular investment. Agreement with Murray's law was assessed by estimating the transpiration rate distal to a cross-section, which should determine Q under steady state conditions, and comparing that with the Σr^3 of that cross-section. As predicted, regressions between the Σr^3 of the cross-section and Q resulted in a linear relationship with a y-intercept that was not different from zero. Two more rigorous statistical tests were also unable to reject Murray's law. *Psilotum nudum* plants also increased their conductance per investment by having more conduits distally than proximally, which is more efficient hydraulically than equal or declining conduit numbers distally.

Key words: conduit furcation number; hydraulic architecture; Murray's law; *Psilotum nudum*; xylem flow rate.

In the 420 million years of vascular plant evolution, natural selection has presumably improved plant transport efficiency. However, we still know relatively little about what specific tradeoffs and constraints are important in transport networks. Plants should benefit from a xylem network that maximizes hydraulic conductance per investment in vascular tissue volume, given that CO₂ fixation is dependent on transport (Meinzer and Grantz, 1990; Sperry et al., 1993; Sperry and Pockman, 1993; Hubbard et al., 2001; Meinzer et al., 2001). A useful starting place for assessing the efficiency of the plant vascular system is Murray's law. This law predicts the conduit taper required to maximize hydraulic conductance for a given investment in vascular volume and a given branching topography (Murray, 1926).

Although Murray's law was originally derived for animal vascular systems, it has recently been extended to plant xylem (McCulloh et al., 2003, 2004). For Murray's law to be applicable to the xylem, the hydraulic conductance must be proportional to the Hagen-Poiseuille predicted value, meaning it must be proportional to the sum of the inner radii of the conduit lumens each raised to the fourth power (Σr^4). Xylem conductivity is substantially less than the Hagen-Poiseuille value because of the added obstruction of conduit end-walls; Murray's law only requires the reduction to be proportional across a given branch network. Further conditions are that the conduit wall volume must be proportional to the total conduit volume, the conduit branching topography must be fixed, and the conduits cannot provide the plant with structural support. When these conditions are met, the Murray's law solution occurs when the sum of the conduit radii cubed (Σr^3) at every level

is proportional to the volume flow rate, Q (Murray, 1926; McCulloh et al., 2003).

Previous work has focused on evaluating Murray's law in circumstances where Q remained constant across branch ranks. In this case, the law predicts that the Σr^3 will be constant. This work showed that when the xylem conduits were functioning only to transport water, as in compound leaves, vines, and young ring-porous wood, compliance with Murray's law was generally observed (McCulloh et al., 2003, 2004). However, when the xylem conduits provided the additional function of structural support, such as in diffuse-porous and conifer wood, they did not comply with Murray's law, suggesting that some other optimality criterion was selected for in these cases (McCulloh et al., 2004).

The application of Murray's law to xylem is complicated because the number of conduits running in parallel can change across branch ranks. This change is referred to as the conduit furcation number (F) defined specifically as the number of functional conduits in cross-section in a distal rank, divided by the number in an adjacent, proximal rank. The large number of relatively short conduits running both in parallel and in series means that F can be any positive value in plants. An earlier analysis has shown that the total hydraulic conductance per volume increases with F , implying that plants would benefit from increasing the number of conduits in cross-section moving from trunk to twig (McCulloh et al., 2003). The largest values for F were found in compound leaves ($F \approx 1.3$) and vines ($F \approx 1.15$) (McCulloh et al., 2003). The smallest values have been found in freestanding trees with values ranging from 1.1 in a ring-porous species, to 0.96 in a diffuse-porous species, and 0.95 in a conifer species (McCulloh et al., 2004).

In this paper, we test agreement with Murray's law in the seedless vascular plant *Psilotum nudum* (L.) Griseb., which was selected for two reasons. First, the simple morphology of *P. nudum* closely resembles the earliest vascular plants known only from fossil rhyniophytes such as *Cooksonia* and *Rhynia*,

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which lived during the Devonian (360–408 mya; Judd et al., 2002). Although *P. nudum* is now known to be most closely related to ferns, its centrally located stele, lack of true roots, and upright bifurcating structure led early researchers to incorrectly associate *P. nudum* with these extinct vascular plants (Beirhorst, 1977; Pryer et al., 2001; Judd et al., 2002). Studying the vascular network of *P. nudum* provides insight into whether evolution has optimized this simplest of vascular networks, which is analogous to the ancestral pattern.

The second reason for examining correspondence with Murray's law in *P. nudum* is the central location of the vascular stele, which is far from the highest mechanical stresses near the outside of the shoot (Niklas, 1992). This suggests that the xylem conduits are not providing the plant with structural support, fulfilling an important assumption of Murray's law (McCulloh et al., 2003, 2004). Furthermore, we predict that because the conduits are not restricted by the mechanical requirements of supporting the plant, the conduit furcation number should be greater than one to exploit the increased efficiency of these networks (McCulloh et al., 2003).

The application of Murray's law is complicated in *P. nudum* because it is a stem photosynthesizer. Unlike previous work, where Q was constant across branch ranks (McCulloh et al., 2003, 2004), Q declines distally in *P. nudum* as water is lost from the stem surface. In this case, Murray's law predicts that the Σr^3 should not be constant, but should decline from the base to the top of the plant in proportion with the drop in Q :

$$Q = c \sum r^3, \quad (1)$$

where c is the proportionality constant between Q and Σr^3 . Although c is constant for a shoot at a given time, it can vary between shoots and with environmental conditions such as vapor pressure deficit. However, its value is unimportant for evaluating Murray's law. The prediction of Murray's law that must be assessed is the direct proportionality between Q and Σr^3 .

MATERIALS AND METHODS

Plant material—*Psilotum nudum* plants were grown in a greenhouse at the University of Utah. In December 2002, 12 shoots of *P. nudum* sporophytes were separated from large clumps of plants and repotted individually. These individuals were allowed to grow for 3 mo without competition for light, water, or nutrients. Transpiration rates were then measured in three individuals, and anatomical characters measured for five individuals.

Estimate of volume flow rate (Q) in branch ranks—Under steady-state conditions, Q at any branch level will be equal to the transpiration rate from the entire stem surface area above that level. Thus, to comply with Murray's law, the Σr^3 of a branch level should be directly proportional to the total transpiration distal to that level. Transpiration from three plants was measured in the greenhouse on a sunny day in March 2003 using a Li-Cor 100–07 cylindrical chamber with a Li-Cor Li1600 steady state porometer (Li-Cor, Inc., Lincoln, Nebraska, USA). One segment of the shoot was measured at a time using a closed-top chamber. Surface areas were estimated by multiplying the length of the segment in the chamber (50 mm) by the perimeter of the shoot. Perimeters were measured in the middle of the measured length with Image-Pro software (Media Cybernetics, Inc., Silver Spring, Maryland, USA) on digital micrographs of cross-sections taken with a Nikon COOLPIX 990 attached to a Nikon BH2 light microscope (Nikon USA, Melville, New York, USA). For simplicity, the measured perimeter was assumed to be constant for the entire length and tapering was ignored. The relationship between transpiration rate per area (y-axis) and shoot perimeter (x-axis) was determined by a model I linear regression (Fig. 1). This type of regression has been shown

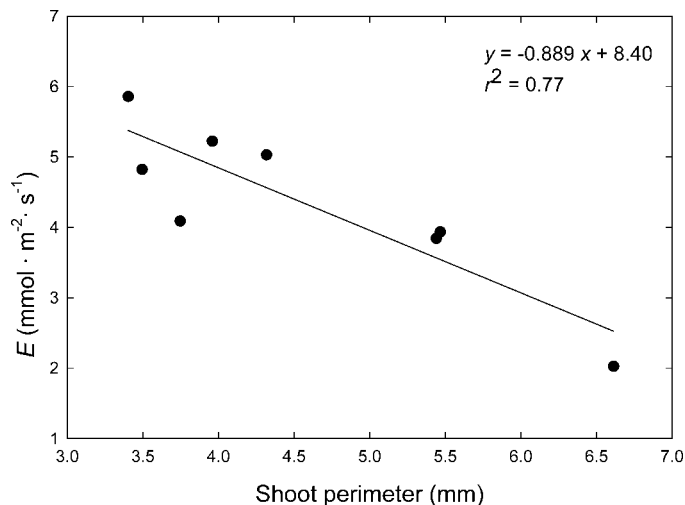


Fig. 1. The relationship between *Psilotum nudum* shoot perimeter and transpiration rate (E). The perimeter was measured at the middle of the shoot on which transpiration measurements were made. The regression was used to predict how xylem flow rate declined with increasing branch rank. Data are from three individuals. Average measurement conditions were ambient temperature of 27°C, relative humidity 18%, and photosynthetic photon flux density of 900 $\mu\text{mol} \cdot \text{s}^{-1} \cdot \text{m}^{-2}$.

to be sufficient to predict values of y based on x (Quinn and Keough, 2002). Although this regression is auto-correlated, the relationship between the transpiration rate and the perimeter was not known a priori. The regression was used to predict the value for Q at each cross-section for which Σr^3 was measured (see *Conduit measurements* section). The rate of transpiration per unit surface area of each branch distal to the cross-section was determined from its perimeter using the regression, and the total branch surface area was estimated as the product of perimeter and total branch length. The product of surface area and transpiration per area gave the total branch water loss rate, which was summed for all branches above the cross-section to give Q .

Although the intercept of the regression between transpiration rate per unit surface area and perimeter may vary between plants and environmental conditions, we assumed that the slope of the regression, and hence the relative change in Q with perimeter, was the same for all the plants in which Σr^3 was measured. Variation in the absolute value of the transpiration rate and Q would alter c in equation 1, but not the proportionality between Σr^3 and Q expected from Murray's law. By using one relationship for E vs. perimeter, we effectively eliminated environmental effects on c . The remaining variation in c between plants depends on variable allometry between perimeter, length, and Σr^3 .

Conduit measurements—Conduit radii (r) were measured in five individuals to determine the Σr^3 at each rank. For all functional conduits, the radii were calculated for a circle with the same area as the lumen, measured using ImagePro software on digital images of freehandsectioned cross-sections. Functional conduits were identified by dye perfusions of 0.5% basic fuchsin. Ranks were defined by branch points, with rank 1 being most proximal (Fig. 2). At each rank, conduits were measured in all segments (i.e., at rank 2, two segments were measured, and at rank 4, eight segments were measured).

The conduit furcation number (F) for adjacent ranks was determined for the same five plants. The conduit furcation numbers were not standardized for differences in mother-to-daughter branch number as in previous work (McCulloh et al., 2003, 2004), because the branching topography was identical (bifurcating) between all ranks and individuals.

Statistical analyses—Qualitative compliance of *P. nudum* xylem conduits with Murray's law was determined by testing for significance of a linear regression in a plot of Σr^3 vs. Q and determining if the y-intercept was different from zero for each individual. If the y-intercept was not statistically

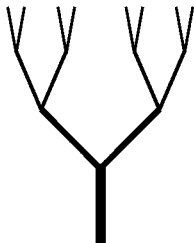
<i>Psilotum nudum</i>	Rank (abbreviation)	Mean r (μm)	CN
	Rank 4 (r_4)	10.3 (1.9) c	1.73 (.3)
	Rank 3 (r_3)	10.8 (2) bc	1.29 (.3)
	Rank 2 (r_2)	12.1 (1.7) ab	1.07 (.2)
	Rank 1 (r_1)	13.5 (1.8) a	1 (0)

Fig. 2. Definition of branch ranks in *Psilotum nudum* and corresponding anatomical values. The average mean conduit radius (r , μm) and the mean increase in conduit number (CN) relative to rank 1 are shown for the ranks that were used for statistical analyses. Identical letters in the mean conduit radius column indicate values that were statistically indistinguishable between ranks. The standard deviations from the mean are shown in parentheses. Means are derived from five individuals.

different from zero, we could more rigorously test agreement with Murray’s law by log transforming the data. Murray’s law then predicts:

$$\log Q = \log \sum r^3 + \log c. \tag{2}$$

This transformation defines the slope predicted by Murray’s law as 1 in a regression between $\log \sum r^3$ and $\log Q$.

To test whether the slope in equation 2 equaled 1, we developed a linear mixed-effects model for each value of x with $\log \sum r^x$ as the random effect, $\log Q$ as the fixed effect, and the data clustered by individual (S-Plus 6.0r2, Insightful Corp., Seattle, Washington, USA). The value of x was varied from 2 to 4 at 0.25 increments to test the Murray’s law value of $x = 3$ against other exponents. The linear mixed-effects model did not provide a method for determining whether the slope pooled for all individuals was statistically different from 1, so the pooled slope was determined to be different when one standard error did not include the Murray’s law predicted value of 1.

The linear mixed-effects model was based on a model I regression, which does not account for error in the x -axis. Unfortunately, to our knowledge, a linear mixed-effects model that incorporates a model II regression is unavailable. To assess the importance of the regression type on determining agreement with Murray’s law, model II linear regressions were calculated between $\log Q$ and $\log \sum r^x$ for each individual. For values of x between 2 and 4 at 0.25 increments, the value of the slopes from the five plants were compared with the predicted value of 1 using Student’s t -test. If the P -value of the t -test >0.05 when $x = 3$, Murray’s law could not be rejected. This statistical approach was weak, because in the absence of the linear mixed-effects model, the sample size collapsed from the number of $\sum r^3$ measurements ($N = 57$) to the number of individuals ($N = 5$).

RESULTS

There was a two-fold decline in transpiration per rank surface area from the thin shoots of the highest rank to the thick “trunk” of rank 1 (Fig. 1). The change in stem perimeter explained 77% of the difference in water loss rates across branch ranks. The lower transpiration rate towards the base of the plant meant that the relative Q increased more from the bottom to the top rank than did the total shoot surface area (data not shown).

The $\sum r^3$ increased exponentially as shoot perimeter increased from shoot tip to trunk (Fig. 3). Plotting this increase in $\sum r^3$ from top to bottom with the corresponding increase in Q for each plant yielded the expected direct proportionality (Fig. 4). The y -intercept of Q vs. $\sum r^3$ was not different from zero in any individual (Fig. 4), which allowed us to log trans-

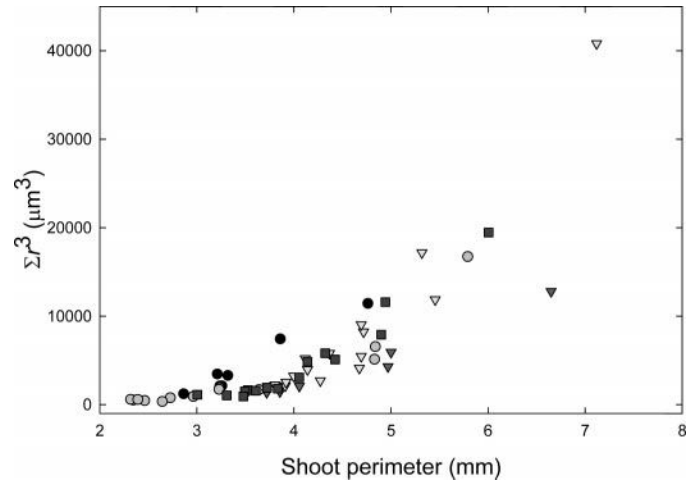


Fig. 3. The relationship between the sum of the conduit radii cubed ($\sum r^3$) of a cross-section and its shoot perimeter in *Psilotum nudum*. There is an exponential rise in $\sum r^3$ as perimeter increases. The different symbols represent the five individuals used for conduit measurements.

form the values of $\sum r^3$ and Q and test Murray’s law more rigorously.

As hypothesized, the results from both statistical tests showed that *P. nudum* generally complied with Murray’s law. For the more powerful linear mixed-effects model, the slope between $\log \sum r^3$ and $\log Q$ when $x = 3$ was 1.01 (Fig. 5A), which is extremely close to the Murray’s law predicted value of 1. This test was also able to distinguish between the slope of the regression and 1 when $x = 2$ and 4 (Fig. 5B). For the less powerful t -tests between the Murray’s law predicted slope of 1 and the model II regression slopes, the entire range of x values tested resulted in p -values >0.05 (data not shown). The peak p -value occurred when $x = 3.75$, rather than the Murray’s law predicted value of 3. Although this meant that Murray’s

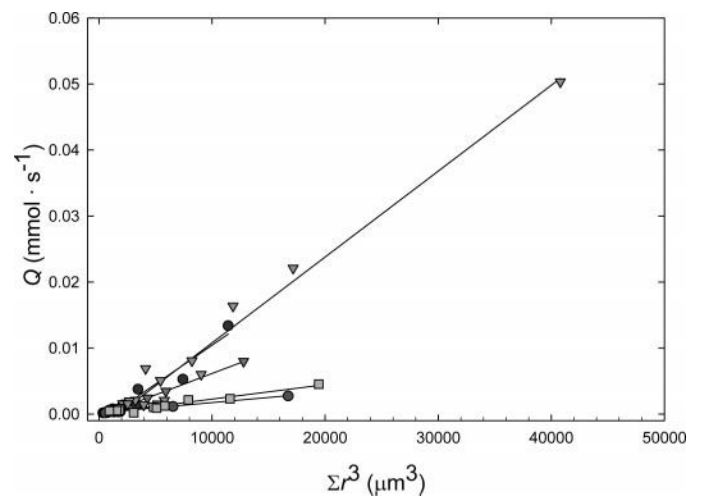


Fig. 4. The relationship between the sum of the conduit radii cubed ($\sum r^3$) and estimated volume flow rate (Q) for five individuals of *Psilotum nudum*. For each plant, the relationship between $\sum r^3$ and Q was linear and the y -intercept was not different from 0, as predicted by Murray’s law. Differences in slope (c , Equation 1) resulted from different allometry between $\sum r^3$, perimeter, and branch length between plants. Different symbols represent the five individuals analyzed.

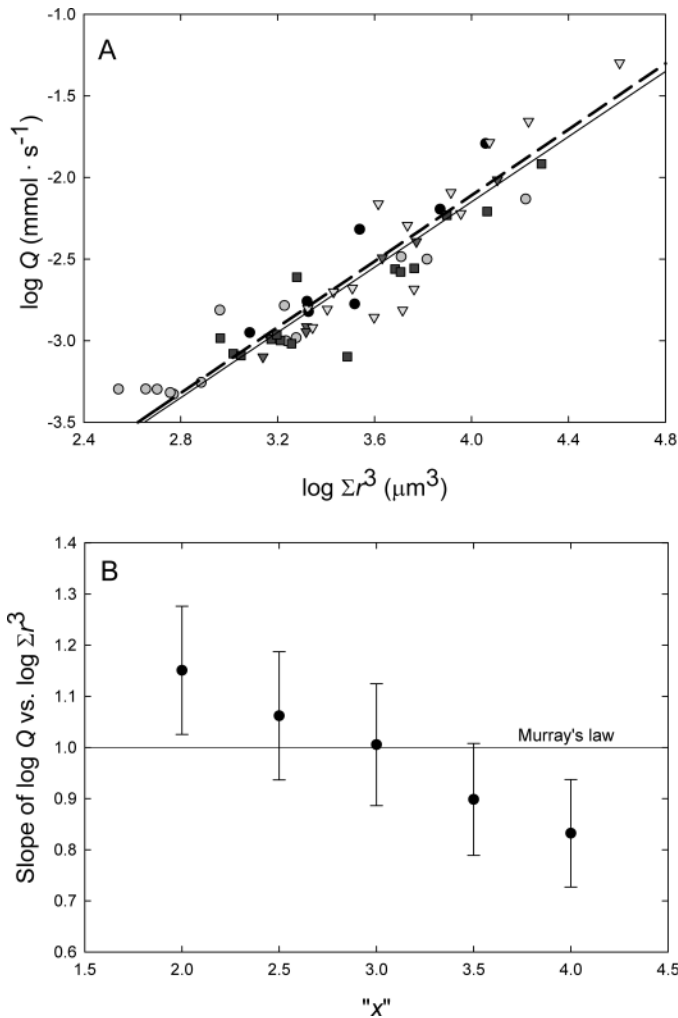


Fig. 5. The results from the linear mixed-effects model in *Psilotum nudum*. A. The relationship between log of the sum of the conduit radii cubed (Σr^3) of a cross-section and the log of the flow rate (Q) through the section. The solid line shows the slope of 1 predicted by Murray's law. The dashed line shows the pooled slope from the linear mixed-effects model. Different symbols represent the five individuals analyzed. B. The slope of $\log Q$ vs. $\log \Sigma r^3$ vs. the exponent x determined from a linear fixed effects model. The error bars show SE = 1. The slope when $x = 3$ was closest to the Murray's law predicted value of 1. When $x = 2$ and 4, the slope was >1 SE from the predicted value.

law could not be rejected, the poor statistical power of the test made it otherwise uninformative.

The number of conduits in cross-section increased from the trunk to the highest rank, and the mean conduit radius declined (Fig. 2). The increase in conduit number was reflected in average conduit furcation numbers for adjacent ranks above 1 (Fig. 6). The mean furcation number increased distally to a maximum of nearly 1.4 (Fig. 6).

DISCUSSION

The results showed that the Σr^3 diminished distally in direct proportion to the decline in Q as predicted by Murray's law (Figs. 5, 6A). Qualitatively, the decline in Σr^3 was consistent with Murray's law because the regression between Σr^3 and Q resulted in a linear relationship with a y-intercept not different

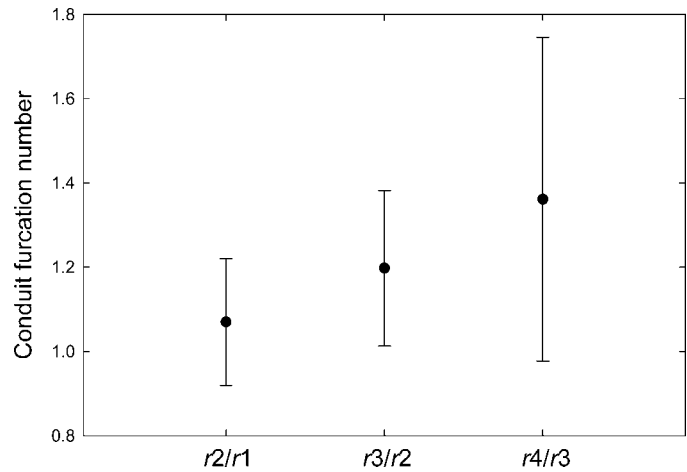


Fig. 6. The conduit furcation numbers for adjacent ranks (r) in *Psilotum nudum*. All mean values are >1, as predicted. Networks with high conduit furcation numbers exploit the increased efficiency of a few large conduits proximally. The error bars show SD = 1.

from zero (Fig. 5). Quantitatively, the slope of 1.01 when $x = 3$ for the linear mixed-effects model meant that Murray's law could not be rejected (Fig. 6B).

The fact that the conduits in *P. nudum* are tracheids rather than vessels means that tracheid-bearing species can follow Murray's law as faithfully as the previously studied vessel-bearing angiosperms (McCulloh et al., 2003). This reinforces our earlier conclusion that the strong deviation from the law seen in tracheid-bearing conifers was not a consequence of their having tracheids, but rather that the tracheids were functioning in a structural support role (McCulloh et al., 2004). Although tracheids are much shorter than vessels, in both conduit types the end walls reduce conductivity by a similar proportion (Sperry et al., in press).

In addition to following Murray's law, the high conduit furcation numbers observed in *P. nudum* shoots further increase efficiency because fewer, larger conduits proximally are supplying a given number of conduits distally (McCulloh et al., 2003, 2004). The conduit furcation numbers obtained were similar to the 1.15–1.3 range in vines and the compound leaves of angiosperms (Fig. 3; McCulloh et al., 2003). It is not surprising that *P. nudum* conduit furcation numbers are similar to those in compound leaves, because the current morphological interpretation is that *P. nudum* shoots are actually reduced leaves (Bierhorst, 1977a, b; Bold et al., 1987). From a functional standpoint, however, we would expect similarly high F values in any organ as long as the xylem network was not involved in supporting the plant. When conduits do provide support, F would be limited because high values of F , accompanied by compliance with Murray's law, result in conduit area increasing distally, which tends to create top-heavy structures.

The conduit furcation numbers from this study also agree with previously measured values in *P. nudum* (Fig. 6; Schulte et al., 1987). The values calculated from Schulte et al. (1987) agree well with those measured in this study, except at the base of the plant, which was much higher in the work of Schulte and coworkers. One explanation for this difference is that Schulte et al. (1987) may have measured at the base of branch junctions, whereas we measured at branch midpoints because junctions may have hydraulic constrictions. For ex-

ample, we observed a local drop in conduit number at the shoot–soil interface (data not shown).

Although all the individuals exhibited a linear relationship between Q (y -axis) and Σr^3 (x -axis), proportionality factors (c) ranged from $1.5E-7$ to $1.2E-6$ $\text{mmol} \cdot \mu\text{m}^{-3} \cdot \text{s}^{-1}$ (Fig. 4). This variation was not due to environmental conditions, because we used one relationship between E and perimeter to estimate the relative change in Q from the top to the bottom of the plant (Fig. 1). Instead, the range in c resulted from variation in Σr^3 per perimeter between individuals (e.g., Fig. 4) and in branch length per perimeter (data not shown). A larger proportionality factor would result from less Σr^3 per perimeter and/or greater branch length per perimeter. Variations in these properties may be related to the growth environment the shoots experienced prior to transplantation.

The results from this work show that agreement with Murray's law has evolved not just in the seed plants, but also in the simplest of seedless vascular plants. Did the extinct seedless vascular plants that so closely resemble *P. nudum* also follow Murray's law? The similarity in external morphology between these extinct plants and *P. nudum* is extraordinary, and like *P. nudum*, both *Rhynia* and *Cooksonia* had centrally located steles. This suggests that their conduits did not function to support the plant and that Murray's law should apply. If these early vascular plants did have architecture consistent with Murray's law, then selective advantage must have been very strong for compliance to have evolved nearly simultaneously with vascular tissue.

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