Biomechanical and leaf–climate relationships: A comparison of ferns and seed plants[1]

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- *Premise of the study:* Relationships of leaf size and shape (physiognomy) with climate have been well characterized for woody non-monocotyledonous angiosperms (dicots), allowing the development of models for estimating paleoclimate from fossil leaves. More recently, petiole width of seed plants has been shown to scale closely with leaf mass. By measuring petiole width and leaf area in fossils, leaf mass per area (M_A) can be estimated and an approximate leaf life span inferred. However, little is known about these relationships in ferns, a clade with a deep fossil record and with the potential to greatly expand the applicability of these proxies.

- *Methods:* We measured the petiole width, M_A, and leaf physiognomic characters of 179 fern species from 188 locations across six continents. We applied biomechanical models and assessed the relationship between leaf physiognomy and climate using correlational approaches.

- *Key results:* The scaling relationship between area-normalized petiole width and M_A differs between fern fronds and pinnae. The scaling relationship is best modeled as an end-loaded cantilevered beam, which is different from the best-fit biomechanical model for seed plants. Fern leaf physiognomy is not influenced by climatic conditions.

- *Conclusions:* The cantilever beam model can be applied to fossil ferns. The lack of sensitivity of leaf physiognomy to climate in ferns argues against their use to reconstruct paleoclimate. Differences in climate sensitivity and biomechanical relationships between ferns and seed plants may be driven by differences in their hydraulic conductivity and/or their differing evolutionary histories of vein architecture and leaf morphology.

Key words: climate; ferns; leaf economic spectrum; leaf mass per area; leaf physiognomy; paleobotany; paleoecology.

A major recent advance in plant ecology has been the recognition that a suite of leaf structural and functional traits are correlated with each other and with environmental gradients. This covariation of leaf traits is known as the leaf economic spectrum (Wright et al., 2004, 2005). Plants with rapid resource acquisition, on the "fast-return" end of the spectrum, typically exhibit a short leaf life span (<12 mo), high photosynthetic and respiration rates, low leaf dry mass per area (M_A), high concentrations by mass of nitrogen and phosphorus, high palatability to herbivores, and fast growth rates

(Reich et al., 1997; Westoby et al., 2002; Diaz et al., 2004; Wright et al., 2004, 2005). Conversely, plants at the "slow-return" end of the spectrum exhibit the opposite suite of traits. Slow-return species that invest in a high M_A have low photosynthetic and respiration rates, resulting in a slow carbon uptake rate (Reich et al., 1997; Westoby et al., 2002; Diaz et al., 2004; Wright et al., 2004, 2005). These species compensate for their slower carbon uptake rate by having leaves with long life spans (>12 mo) (Reich et al., 1997; Westoby et al., 2002; Diaz et al., 2004; Wright et al., 2004, 2005). These interrelationships are largely independent of phylogeny in seed plants (Ackerly and Reich, 1999). Further, they likely reflect fundamental evolutionary trade-offs in leaf design that favor high performance in productive environments and resource conservation in harsher environments (Shipley et al., 2006). Thus, the leaf economic spectrum should be universally applicable to all vascular plants, including ferns (e.g., Karst and Lechowicz, 2007), and be applicable on macroevolutionary time scales.

Leaf economic traits cannot be directly measured from fossils. Recent work on woody and herbaceous non-monocotyledonous (referred to here as "dicot") angiosperms and woody gymnosperms has, however, documented a biomechanical scaling relationship between petiole width (PW) and M_A (Royer et al., 2007, 2010). The principle behind this relationship is that the geometry, stiffness, length, and transverse shape of the petiole all work together to support the leaf (Niklas, 1999), such that heavier leaves need larger and/or stouter petioles for support. This relationship can be used to estimate the mass of

fossil leaves and (normalizing by area) to predict M_A. In turn, the M_A of fossil leaves can be used as a rough proxy for leaf life span (Royer et al., 2007, 2010). The relationship between transpiration and leaf vein density seen in extant plants can also be used as a proxy for assessing leaf economics such as net carbon assimilation rate and potentially M_A in fossil plants (e.g., Uhl and Mosbrugger, 1999; Boyce et al., 2009; Blonder et al., 2011); however, we do not explore that approach here.

Up to this point, analyses of M_A–PW relationships have focused exclusively on seed plants, and primarily on angiosperms (Royer et al., 2007, 2010). Investigating these relationships in non-angiosperm and non-seed plant groups, such as ferns, is important because it could potentially provide a new paleoecological tool applicable to significantly more of the paleobotanical fossil record, including assessments of Paleozoic plants. Based on existing relationships from seed plants (Royer et al., 2007, 2010), it is reasonable to expect, based on biomechanics and physiology, that similar biomechanical scaling relationships exist across all vascular plant groups. From strictly a biomechanical standpoint, a heavier leaf of any type needs a wider and/or thicker petiole for additional support and to accommodate dynamic loading (e.g., Niklas, 1991, 1999). Physiologically, larger leaves have greater transpiration loads, which in turn probably require structural changes to the petiole to accommodate additional vascular tissue (e.g., Niinemets et al., 2007). Studies of fern leaf hydraulics suggest that hydraulic conductivity and tracheid diameter in terrestrial and ephiphytic ferns may influence petiole length and area (Watkins et al., 2010), providing evidence for a physiological influence on the relationship between petiole and leaf size in ferns. In addition to the biomechanical and physiological basis for the M_A–PW relationship, there is empirical evidence documenting allometric relationships between leaves and petioles in a variety of vascular plant groups, including ferns (Niklas, 1991; West et al., 1997; Enquist, 2002; Niinemets et al., 2007; Arcand et al., 2008; Creese et al., 2011), and to suggest that there is a convergence in petiolar mechanics to follow the design principle of uniform strength in both angiosperms and ferns (Niklas, 1993). Specifically, two studies of Hawaiian ferns indicate that fern petiole (stipe) diameters scale consistently with the whole leaf, leaf blade, pinnae lengths, and ultimate pinna width and that there is a geometric scaling relationship between frond and plant size (Arcand et al., 2008; Creese et al., 2011). Another study found similar allometric relationships between flexural stiffness and leaf size in ferns and angiosperms suggesting some convergence in the biomechanical structures of petioles in both groups (Niklas, 1991).

In woody dicots, leaf physiognomy (size and shape) correlates strongly with temperature and moisture, and there are functional explanations for these relationships (see Royer, 2012 and references therein). One of the strongest leaf–climate relationships is between leaf teeth and temperature. The percentage of woody dicots at a site with toothed leaves, and variables related to the number of teeth and tooth size, all negatively correlate with mean annual temperature (MAT) (see Royer, 2012 and references therein). The functional basis for these relationships is not well understood. One possibility is that leaf teeth may confer photosynthetic advantages early in the growing season via enhanced sap flow, which would be especially advantageous to plants living in cooler climates allowing them to maximize the duration of their growing season (Billings, 1905; Bailey and Sinnott, 1916; Baker-Brosh

and Peet, 1997; Royer and Wilf, 2006). In warmer climates the photosynthetic benefits of teeth are likely outweighed by the disadvantage of greater water use (e.g., Royer and Wilf, 2006). Leaf teeth may also help release excess root pressure through guttation, preventing the flooding of intercellular spaces in leaf lamina and freeze–thaw embolisms in cooler climates (Feild et al., 2005). This may be particularly important for herbaceous plants and for plants living in locally wet environments.

Although there are strong relationships between the presence of leaf teeth and MAT in woody dicots, there is no significant relationship in non-woody, herbaceous angiosperms (Royer et al., 2012). There is a strong height gradient in the strength of the relationship between leaf teeth and temperature (Royer et al., 2012), with canopy trees showing the strongest relationship, shrubs intermediate, and herbs the weakest. Fern fronds commonly have teeth, thus that aspect of fern leaf physiognomy may be related to climate. However, given that ferns are non-woody and commonly understory plants, it seems most likely that there will be no leaf–climate relationships. Additionally, growth chamber experiments and field measurements of five fern species did not reveal a predictable relationship between leaf margin state and climate (Benca et al., 2013), also suggesting limited climatic influences on fern leaf margin shape.

Here we quantified the biomechanical scaling relationship between PW and leaf lamina area in a geographically and phylogenetically diverse set of fern species (Fig. 1) with a view to developing a model for quantifying M_A in ferns. We compared the scaling relationship between PW and leaf area in fronds and all hierarchical levels of pinnae (primary, secondary, and tertiary) to determine whether there was a uniform scaling relationship. We then fit four different biomechanical models for estimating M_A using PW and leaf area (Royer et al., 2007) to observations from fern fronds and pinnae and compared the best-fit models for ferns and seed plants. Finally, we measured a variety of leaf physiognomic variables (Royer et al., 2005; Peppe et al., 2011) in a subset of toothed fern species to quantify the strength of correlation between leaf physiognomy and MAT and compared those results to results from woody and herbaceous dicots.

MATERIALS AND METHODS

In this study we followed the fern classification scheme of Smith et al. (2006) (Fig. 1C). We photographed sporophyte fronds and/or pinnae (primary, secondary, and tertiary) of 179 species from 188 geographically diverse extant sites from all continents except Antarctica (Fig. 1a). Fern specimens used in this study came from dried herbarium specimens from the Baylor University Herbarium (USA), Queensland Herbarium (Australia), Te Papa Herbarium (New Zealand), United States National Herbarium (USA), and University of Waikato Herbarium (NZ) and from fresh specimens collected in central Texas and dried in the Baylor Herbarium before analysis. When only a portion of the fern frond was preserved on the herbarium sheet, we measured the preserved portion of the frond (i.e., primary, secondary, and/or tertiary pinnae). We measured 90 fronds, 117 primary pinnae, 27 secondary pinnae, and 7 tertiary pinnae (see Table 1 for the distribution of the number of species measured in each fern frond hierarchical category, e.g., frond, primary, secondary, and tertiary pinnae). Mean annual temperature (MAT) at the fern collection sites ranged from −5.7° to 28.5°C, the mean annual range of temperature (MART) varied from 7.1° to 39.5°C, and the mean annual precipitation (MAP) from 28.5 to 605.6 cm/year (Fig. 1B). Mean monthly climate data were extracted from the global, interpolated, 1-km-resolution WORLDCLIM climate model (Hijmans et al., 2005). To allow direct comparisons between ferns and seed plants, in this paper we use the generalized terms "petiole" and
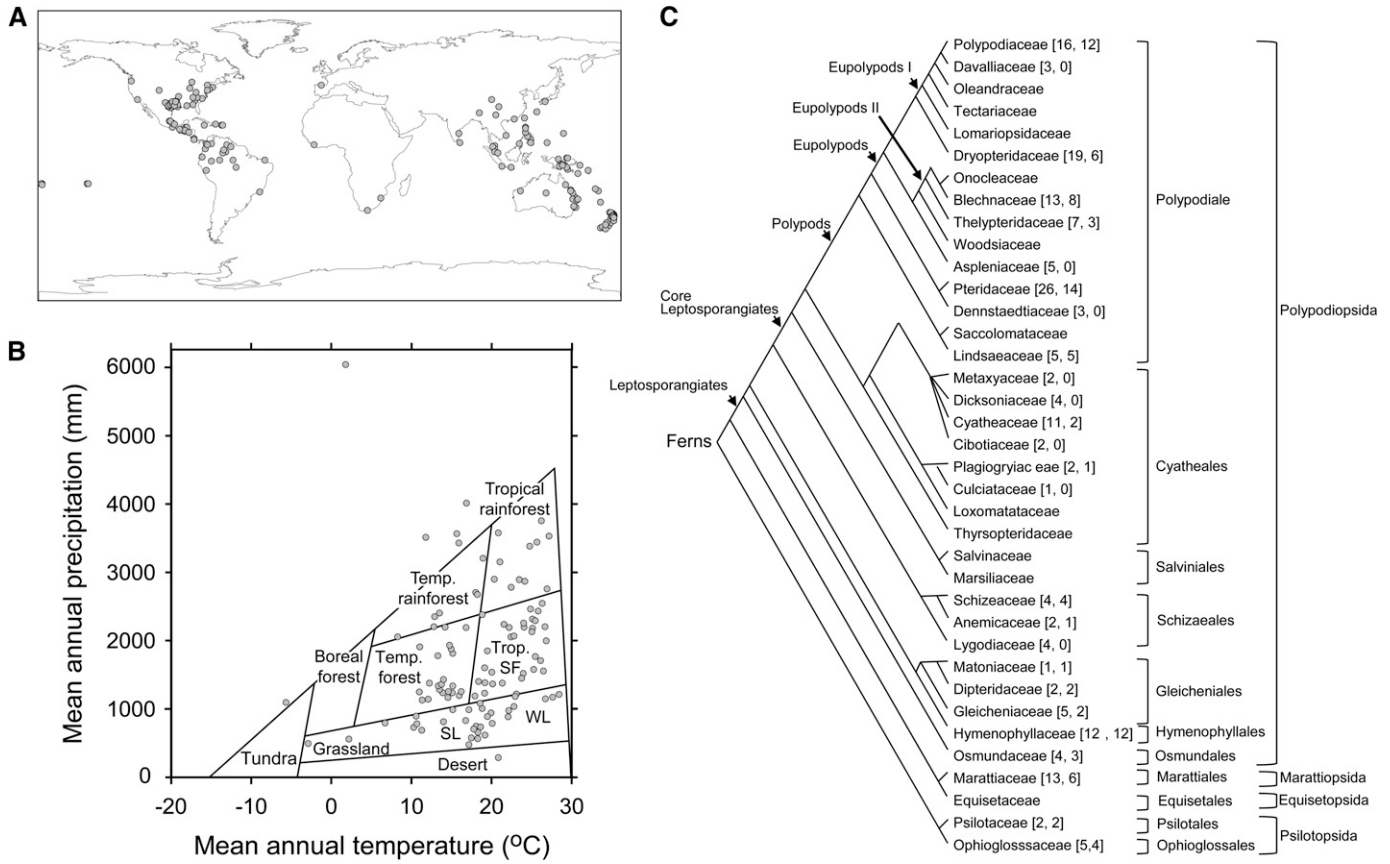
Fig. 1. Geographic, climatic, and phylogenetic distribution of data. (A) Geographic distribution of calibration sites. (B) Climatic distribution of calibration sites. Biomes follow Whittaker (1975); their boundaries are approximate. Temp. = temperate; Trop. SF = tropical seasonal forest; SL = shrubland; WL = woodland. (C) Phylogenetic distribution of calibration data modified from Smith et al. (2006). The first number in brackets is the number of species measured in each family; second number is number of species with frond measurements.

"petiolule" to refer to stipe and rachis and "leaf mass" to refer to frond mass and pinna mass.

Undamaged, average-size mature fronds with an intact petiole (or stipe) that were still attached to the blade were preferentially chosen for measurement. For leaf mass per area (i.e., frond mass per area or pinna mass per area) and petiole width (i.e., stipe width or rachis width) measurements, at least two fronds per species were photographed, weighed, and digitally processed (Appendix S1a, see Supplemental Data with the online version of this article). In compound fronds, when possible, two pinnae per frond at each hierarchical division (i.e., primary, secondary, and tertiary pinnae) were measured. For pinna measurements, the pinnae or pinnules were detached from the frond at the rachis, weighed, photographed, and digitally processed. More than two fronds or pinnae were used if there was a large variation in leaf form (e.g., compound fronds or species with both undissected and highly dissected pinna). In cases where a species occurred at more than one location, all measurements from all locations were combined for that species. Petiole width and leaf area were measured using ImageJ (http://rsbweb.nih.gov/ij/) following the protocols of Royer et al. (2007). The leaf mass per area data were assessed by category based on leaf hierarchy (fronds, primary pinnae, secondary pinnae, and tertiary pinnae). The petiole width–leaf mass per area relationships (PW–M_A) for fern fronds and all hierarchical levels of pinnae (primary, secondary, and tertiary) were fit to four different biomechanical models, described below, to determine a best-fit model. Once the best-fit model was chosen, the frond data set was also assessed by categorizing the species by (1) mechanical leaf structure (e.g., simple and palmately compound fronds vs. pinnately compound fronds), (2) growth form (e.g., epiphytic, climber/scrambler, terrestrial, tree fern, and rheophytic), and (3) phylogeny (see Table 2 for definition of mechanical leaf structure and growth forms). All images used in this study are available by request from the authors and from the Dryad Digital Repository (http://doi.org/10.5061/dryad.528td).

Physiognomic measurements were made on 25 fern species with teeth from 32 sites (Appendix S1b). For the physiognomic analyses, the data set was divided into compound and simple fronds. Compound fronds had primary pinnae and secondary and/or tertiary pinnae. Simple fronds represented all fronds without pinna, fronds with only primary pinnae, or specimens where only primary or secondary pinnae were photographed. We conducted measurements on both fronds and pinnae. Typically, two fronds per species from each site were used. If the frond was simple, the entire frond, including any primary pinnae, was processed as a single unit. If the frond was compound, measurements were made on the entire frond and on three secondary pinnae. In these instances, the secondary pinnae measurements were binned with the simple frond data. Frond images were manipulated in Adobe Photoshop (Adobe Systems, San

TABLE 1. Number of species (n) measured in each frond and pinnae category.

Category	n
Fronds	52
Primary pinnae	70
Secondary pinna	4
Tertiary pinna	2
Frond and primary pinna	30
Frond, primary, and secondary pinna	4
Frond, primary, secondary, and tertiary pinnae	2
Primary and secondary pinnae	12
Primary, secondary, and tertiary pinnae	0
Secondary and tertiary pinnae	3

Table 2.   Definitions for mechanical leaf structures (Niklas, 1991, 1999) and fern growth habits.

Type	n	Description	Type of frond
Mechanical leaf structure			
Structure A	40	Petiole acts as an untapered cantilever beam, anchored at one end by rhizomes and supporting a leaf mass at the other end.	Simple, palmately compound, dichotomously branched fronds
Structure B	51	Petiole acts as a tapered cantilever beam, anchored at one end by petiole and supporting a series of masses along its axis at the other end.	Pinnately compound fronds
Growth habit			
Ephiphytic	30	Leaves extend aerially outward and downward.	
Climber, Scrambler	2	Leaves climb outward and upward, not supporting themselves but using other surfaces for support.	
Terrestrial	58	Erect leaves extend upward.	
Tree fern	4	Long, thick petioles support large branch-like leaves.	
Rheophytic	1	Leaves extend out of deep, swift-flowing water.	

Notes: n = number of ferns measured. Some fern species are included in more than one category of growth habit due to variability in their growth habit.

Jose, California, USA) to separate the petiole (if present) and teeth from the laminar tissue following the protocols of Royer et al. (2005) and Peppe et al. (2011). Presence of teeth and number of teeth were determined visually. All other physiognomic characters were measured using the program ImageJ (National Institutes of Health, Bethesda, Maryland, USA). Physiognomic characters used in this study follow Royer et al. (2005) and Peppe et al. (2011). All 179 fern species were also scored for the presence or absence of teeth.

Petiole width (PW), leaf area (A), and leaf mass per area (M_A) measurements were log-transformed before conducting statistical analyses. In these analyses, PW and A units were in meters for comparison with M_A measurements, which are in g/m². For both fronds and pinnae, linear regression relationships between leaf mass per area and area-normalized petiole width were quantified using four biomechanical models, models A–D (Fig. 2, Tables 2–4; Niklas, 1999; Royer et al., 2007). Models A and D consider a leaf and its petiole as a cantilever beam. Model A assumes that petiole width is proportional to petiole length and that petiole length is proportional to flexural rigidity (Niklas, 1999). Model D assumes that petiole width and length are independent, that petiole shape is relatively invariant, and that leaf mass is proportional to the flexural rigidity of the entire petiole (Niklas, 1999). Model B models a leaf as a vertical petiole supporting a singular laminar mass. This model (model B) assumes that the cross-sectional area of the petiole is proportional to leaf mass (Royer et al., 2007). Model C models a leaf petiole to be analogous to an animal leg. Model C assumes that petiole length and petiole width are co-optimized to support the laminar mass and that they covary elastically (i.e., as length increases, so does width) (Royer et al., 2007).

The accuracy of each biomechanical model was tested using a jack-knife resampling approach based on the species in the frond and pinnae data set. We used the standard major axis (SMA) regression module in the program SMATR (http://bio.mq.edu.au/ecology/SMATR/support.html; Warton et al., 2006) to test for slope and intercept differences between regression lines. We then assessed factors (e.g., mechanical leaf structure, growth habit, and phylogeny) that could potentially influence the biomechanical scaling relationship in fronds and compared the PW–M_A relationship in ferns to seed plants.

Relationships between leaf physiognomic variables and MAT were quantified using ordinary least squares linear regression. Logistic regression was used to test for differences among ferns and other plant growth forms in the relationship between MAT and leaf margin state.

RESULTS

Comparison of biomechanical models—For each of the four models, the scaling relationships between area-normalized PW and M_A for whole fronds differed significantly from those for all hierarchical levels of pinnae (primary, secondary, and tertiary) (Fig. 2). When comparing the scaling relationships between different pinnae levels, the scaling relationships in primary and secondary pinnae for all four models were statistically indistinguishable, while in all models the scaling relationships in tertiary pinnae differed significantly from those in primary and secondary pinnae (Fig. 2).

When the entire data set was combined, models B, C, and D were statistically significant, with model D providing the best fit (Table 3). For fronds and primary pinnae, models B, C, and D were all statistically significant, and models C and D provided the best fits (Table 3). All models were significant for secondary pinnae, but models A, B, and C were the best fit (Table 3). For the combined primary and secondary pinnae data set (primary/secondary pinnae), models B, C, and D were significant and model D had the best fit. None of the models were significant for tertiary pinnae (Table 3). Therefore, tertiary pinnae were excluded from the remainder of our analyses.

Due to the different scaling relationships between fronds and pinnae, we examined the biomechanical models for fronds and pinnae separately. We first determined the best-fit model for the frond data. Because the relationship between area-normalized PW and M_A was statistically indistinguishable for primary and secondary pinnae, we then combined the observations for primary and secondary pinnae and examined the biomechanical models for the primary/secondary pinnae data set.

Leaf mass per area for fronds scaled most closely with area-normalized PW⁴ (i.e., model D). Regression parameters for models A–D are summarized in Table 4. The relationship between M_A and PW of fronds was not significant using biomechanical model A. Models B–D all indicated a relatively strong fit between leaf mass per area and petiole width (r^2 ranging from 0.14 to 0.44; Table 3). There were no significant differences in slopes of the regressions between models B and C (df = 1, P = 0.30), models B and D (df = 1, P = 0.92), or models C and D (df = 1, P = 0.19). Results of the jackknife-type approach and the results of SMA relationships forced through the origin, both indicated that model D most accurately described the scaling relationship between leaf mass per area and petiole width as it showed relatively low bias, the least dispersion in predicted values, and had the strongest correlation between predicted and actual values (Table 4). We therefore chose model D, although we acknowledge that it is not significantly better than models B and C.

Model D also best fit the area-normalized PW–M_A relationship for primary/secondary pinnae. Regression parameters for models A–D are summarized in Table 5. Biomechanical model A was not significant. Models B, C, and D all showed a significant relationship between area-normalized PW⁴ and pinna mass per area (M_A) (Table 5). Models A, B, and C all shared a common slope, but had significantly different intercepts (df = 1, P < 0.001 for all models) and were significantly shifted along the
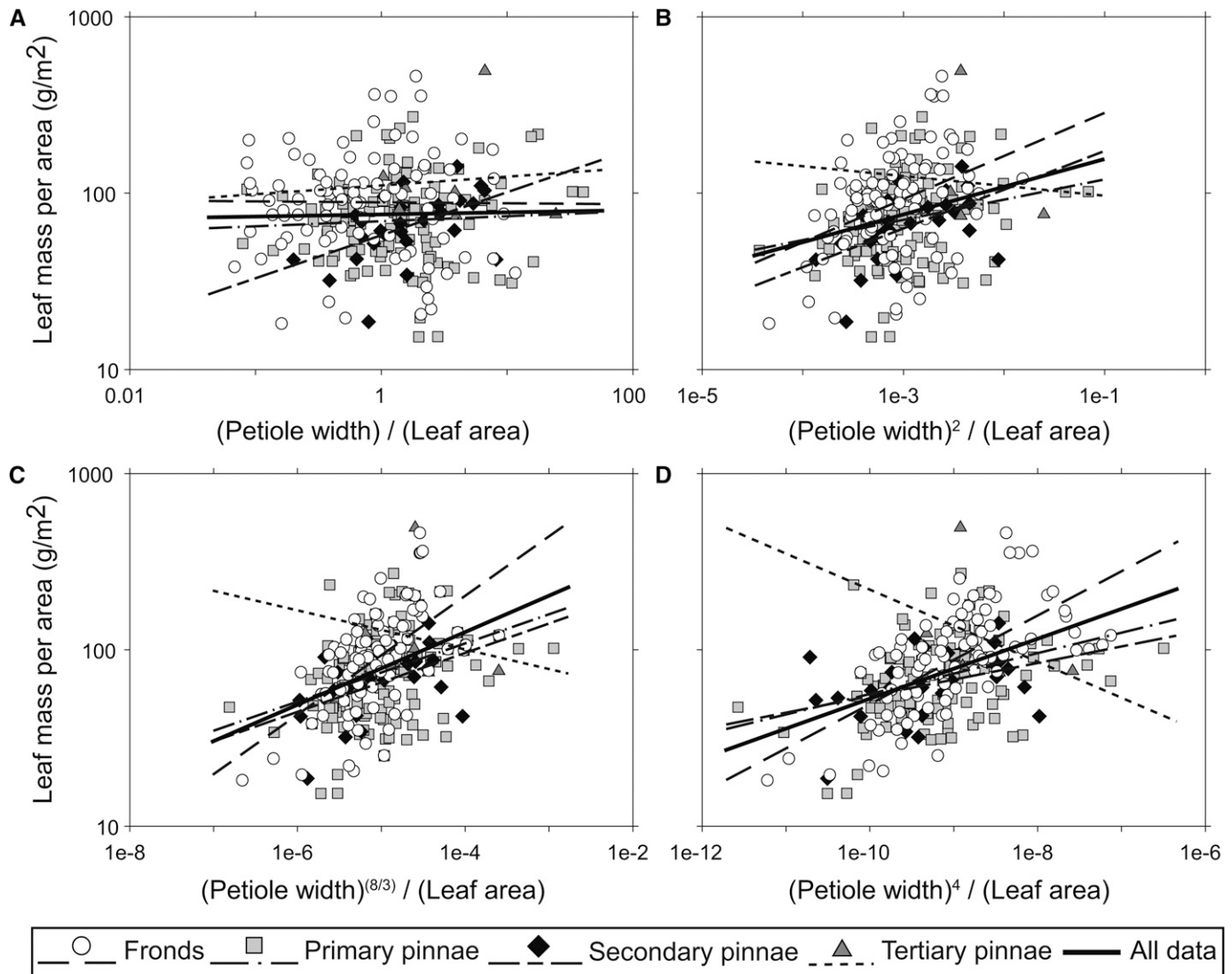
Fig. 2.   Relationship between petiole width (PW) normalized for leaf area. PW and A units in meters, M_A units in g/m^2. (*A*) (PW/*A*) and leaf dry mass per area (M_A) for all data, fronds, and primary, secondary, and tertiary pinnae. (A) Scaling relationships for biomechanical model A (PW/*A* vs. M_A). (B) Scaling relationship for biomechanical model B (PW^2/*A* vs. M_A). (C) Scaling relationship for biomechanical model C (PW$^{8/3}$/*A* vs. M_A). (D) Scaling relationship for biomechanical model D (PW^4/*A* vs. M_A). Correlation coefficients for each relationship in Table 3.

common slope (df = 1, $P < 0.001$ for all models). Model D had a significantly different slope than in models A, B, and C (df = 1, $P < 0.001$, df = 1, $P = 0.001$, and df = 1, $P = 0.004$, respectively). As with fronds, model D had the best fit relative to all other models ($r^2 = 0.12$ vs. 0.11, 0.07, and 0.01 for models A, B, and C, respectively, Table 5). The results of the SMA relationship forced through the origin showed the lowest bias for model D (slope of the plot for measured vs. estimated M_A is 0.994, Table 5). Based on these analyses, we chose model D. However, the difference in fit between models B, C, an D is relatively small, suggesting the relationship between area-normalized PW and M_A for primary/secondary pinnae may be explained equally well by all three models (models B, C, and D).

Using partial correlation, we found that the relationship between PW^4/A and M_A for fronds remained significant, and the correlation coefficients were largely unchanged after accounting for MAT, MAP, and MART (full correlation: $r = 0.66$,

correlation after accounting for MAT, MAP, and MART: $r = 0.64$, 0.65, and 0.65, respectively; df = 89, $P < 0.001$ for all three tests). As with fronds, after accounting for MAT, MAP,

TABLE 3.   Number of specimens (*n*) and correlation coefficients (*r*) for relationships between area-normalized petiole width and leaf mass per area for biomechanical scaling models A–C. Linear correlation plots shown in Fig. 2.

Measurement	*n*	A	B	C	D
All measurements	241	0.03	0.28**	0.41**	0.49**
Frond	90	−0.01	0.37**	0.57**	0.66**
Primary pinnae	117	0.06	0.23**	0.30*	0.35**
Secondary pinnae	27	0.51**	0.51**	0.47**	0.39*
Tertiary pinnae	7	0.08	−0.09	−0.2	−0.39
Primary + secondary pinnae	144	0.12	0.27**	0.33**	0.36**

Notes: *P < 0.05, **P < 0.01.

Table 4.   Models fitted for relationship between petiole width normalized for area (PW/A) and leaf mass per area (M_A) for fern fronds only. PW and A units in meters, M_A units in g/m^2. All models are based on species averages of fronds ($n = 90$).

Model	A [a]	b [a]	r^2	SE (log$_e$, g·m^{-2})	df	F	P	Slope [b]
A: log $M_A = a + b$·log (PW/A)	1.940 (1.877, 2.003)	−0.005 (−0.121, 0.110)	0.0001	0.29	1	0.009	0.92	0.939
B: log $M_A = a + b$·log (PW^2/A)	2.702 (2.297, 3.107)	0.245 (0.116, 0.374)	0.14	0.27	1	14.222	<0.001	0.973
C: log $M_A = a + b$·log (PW$^{8/3}$/A)	3.643 (3.116, 4.169)	0.332 (0.232, 0.438)	0.32	0.24	1	41.62	<0.001	0.978
D: log $M_A = a + b$·log (PW^4/A)	4.207 (3.659, 4.755)	0.252 (0.191, 0.312)	0.44	0.22	1	68.06	<0.001	0.982

[a] Lower and upper bounds of 95% confidence interval of coefficients are in parentheses.
[b] Slope of measured vs. estimated MA linear regression fixed through the origin.

and MART with partial correlation, the PW^4/A and M_A scaling relationships for primary/secondary pinnae remained significant, and the correlation coefficient did not change. This finding indicates that the scaling relationship between PW^4/A and M_A in fronds and primary/secondary pinnae is likely insensitive to these environmental parameters.

Potential confounding factors on biomechanical relationships—Given the wide range of frond structures, growth forms, and taxa of the fern species used in this study, we assessed the potential influence of these factors on the scaling between PW^4/A and M_A. Although model D best fits both the frond and primary/secondary pinnae data, the relationship is better explained for fronds (r^2 for fronds = 0.44 vs. r^2 = 0.12 for primary/secondary pinnae). For that reason, we focused these analyses only on fronds.

There was no significant difference in the scaling relationships of simple, palmately compound, or dichotomously branched fronds (structure A) and pinnately compound fronds (structure B) (df = 1, P = 0.102) (Fig. 3A). Thus, model D explains all fern frond structures equally well.

There was a significant difference in the slope of the scaling relationships between ferns with terrestrial and epiphytic growth habits (df = 1, P = 0.003) (Fig. 3B). Although we measured ferns with other growth habits, they were not included in this analysis because we measured fewer than five species that had tree fern, rheophytic, and climbing/scrambling growth habits.

In most cases, there was little difference in the scaling relationships among phylogenetic groups. The scaling relationships of all phylogenetic groups did not differ significantly (i.e., they shared a common slope) (Fig. 3C). That said, the Hymenophyllales showed a significant shift in the position along this common slope compared with all other phylogenetic groups because they tended to have smaller leaves and petioles. There was also a significant shift along the common slope between the Polypodiales and the Marratiales and the Schizaeales (df = 1, P = 0.003 for both) because the Polypodiales tended to have smaller leaves and petioles.

Comparisons with seed plants—Model B, which is the same model previously found to best apply to woody dicots, herbaceous dicots, and gymnosperms, showed a much weaker relationship in ferns than that observed for all other plant groups (r^2 = 0.14 for fern fronds and 0.07 for fern primary/secondary pinnae vs. r^2 = 0.55, 0.32, and 0.44 for woody dicots, herbs, and gymnosperms, respectively) (Fig. 4). The relationship between area-normalized PW (PW^2/A) and M_A using the frond data set and the primary/secondary pinnae data set differed significantly from that of woody dicots, herbs, and gymnosperms (df = 1, P = 0.001 in all cases).

Herbs have a different relationship between area-normalized PW and M_A than do woody dicots and gymnosperms (Royer et al., 2010). Herbaceous dicots and ferns are both non-woody, thus model D might better explain the PW vs. M_A relationship for non-woody plants. However, model D showed a significantly weaker fit for herbaceous dicots than for ferns (r^2 = 0.03 for herbs vs. 0.44 for fern fronds and 0.12 for primary and secondary pinnae). Additionally, herbs also had a much weaker relationships between area normalized PW and M_A with model D than model B (r^2 = 0.03 for model D vs. r^2 = 0.32 for model B).

Leaf physiognomy and climate—An assessment of the relationship between leaf physiognomy of ferns and MAT in 25 toothed species of ferns from 32 different geographic locations found very few significant relationships (Table 6). No significant relationship between leaf-margin state (i.e., presence or absence of teeth) and MAT was found in ferns, and the slope of the relationship was not distinguishable from zero (df = 1, P = 0.91) (Fig. 5).

DISCUSSION

Biomechanical scaling—The biomechanical scaling relationship between area-normalized petiole width and leaf mass per area differed significantly between fern fronds and all levels of pinnae (Table 3, Fig. 2). The different scaling relationships seen in fronds and pinnae are surprising given that, all else

Table 5.   Models fitted for relationship between petiole width normalized for area (PW/A) and leaf mass per area (M_A) based on combined primary and secondary pinnae data. PW and A units in meters, M_A units in g/m^2. All models are based on species averages of primary and secondary pinnae ($n = 144$).

Model	a [a]	b [a]	r^2	SE (log$_e$, g·m^{-2})	df	F	P	Slope [b]
A: log $M_A = a + b$·log (PW/A)	1.830 (1.790, 1.869)	0.058 (−0.024, 0.141)	0.01	0.24	1	1.98	0.16	0.992
B: log $M_A = a + b$·log (PW^2/A)	2.237 (2.000, 2.475)	0.134 (0.055, 0.213)	0.07	0.22	1	11.33	0.01	0.993
C: log $M_A = a + b$·log (PW$^{8/3}$/A)	2.561 (2.215. 2.907)	0.143 (0.075, 0.211)	0.11	0.22	1	17.23	<0.001	0.993
D: log $M_A = a + b$·log (PW^4/A)	2.863 (2.421, 3.305)	0.112 (0.064, 0.160)	0.12	0.22	1	21.15	<0.001	0.994

[a] Lower and upper bounds of 95% confidence interval of coefficients provided in parentheses.
[b] Slope of measured vs. estimated M_A linear regression fixed through the origin.
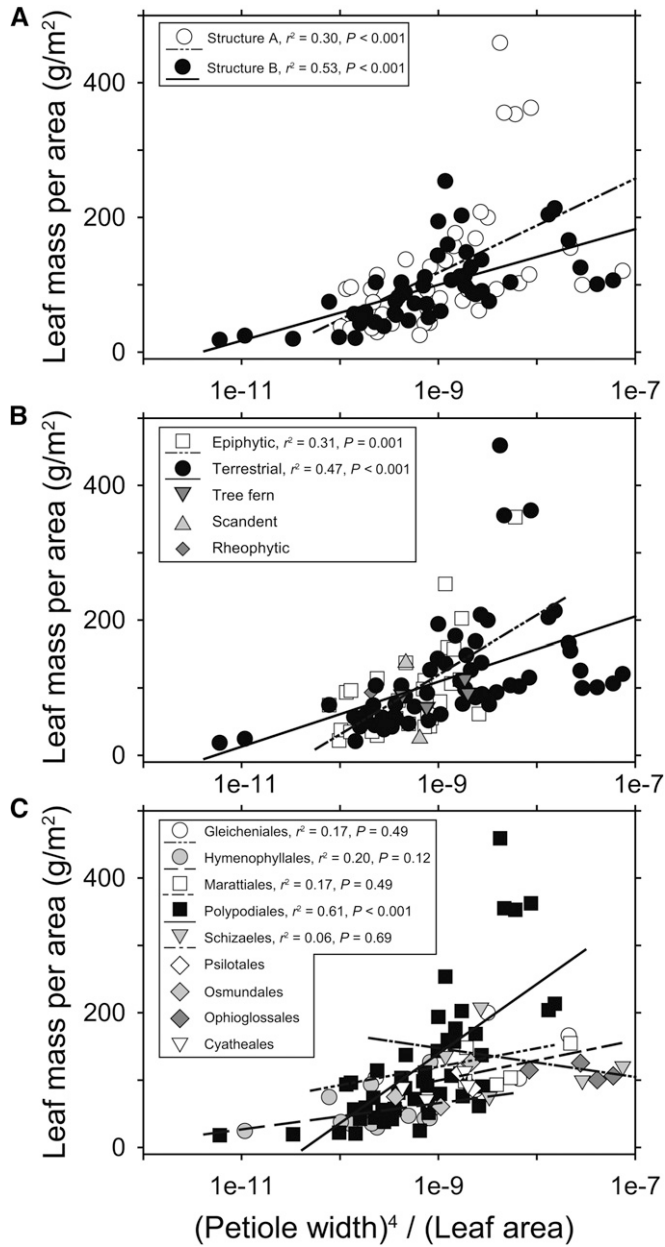
Fig. 3. Relationship between petiole width normalized for leaf area (PW^4/A) and leaf dry mass per area (M_A) for fern fronds. PW and A units in meters, M_A units in g/m^2. (A) Scaling relationship for different frond structures (Table 3). (B) Scaling relationships for different fern growth habits (Table 3). (C) Scaling relationships for different fern phylogenetic groups (Fig. 3, Appendix S1a). Phylogenetic groups based on Smith et al. (2006).

Watkins et al., 2010; Pittermann et al., 2011). One possible explanation for the difference may be that there are different strategies in petioles (stipes) and petiolules (rachises) for the co-optimization of biomechanical support and hydraulic capacity. As an example, in terrestrial ferns, the stipe must provide physical support to hold up the entire frond and a sufficient hydraulic supply for all pinnae and pinnules. On the other hand, in the petiolule of any individual pinnule, the co-optimization of hydraulic capacity and biomechanical support is probably less important to the plant than that of the stipe for an entire frond because a pinnule is more "disposable" than an entire frond. Given this, natural selection could favor lower mechanical safety margins in the rachis than in the stipe, as the potential adverse effects of the loss of a pinnule are minimal compared with those resulting from the loss of an entire frond. Alternatively, the differing relationships between fronds and pinnae may be related to differences in mechanical support load sharing. For example, in terrestrial ferns the stipe (petiole) provides mechanical support for the entire frond, while the pinnae are supported by both the rachis and the stipe. This partial displacement of load from the rachis to the stipe could explain the different scaling relationships between fronds and pinnae.

Model D (PW^4/A) best explained the scaling relationship between area-normalized PW and M_A in fern fronds and the combined primary/secondary pinnae data set (Tables 4, 5). This model considers petioles (stipes) as end-loaded cantilever beams (Niklas, 1999), in which leaves are anchored at just one end and support laminar tissue at the other and leaf mass is proportional to the flexural rigidity of the petiole. The slope of the relationship of model D for fern fronds modeled as either an
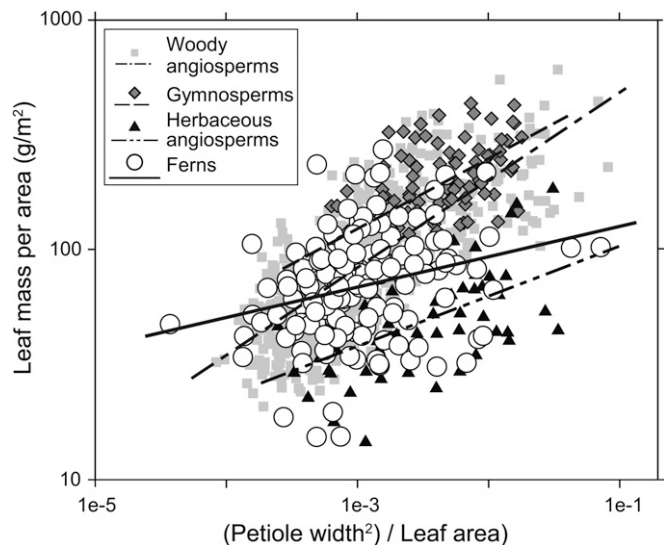

Fig. 4. Scaling relationships between petiole width normalized for leaf area (PW^2/A) and leaf dry mass per area (M_A). PW and A units in meters, M_A units in g/m^2. Fern primary and secondary pinnae: log M_A = 0.134·log (PW^2/A) + 2.237; n = 144 species–site pairs, r^2 = 0.07, $F_{1,143}$ = 11.33, P = 0.01. Woody dicot angiosperms compilation from Royer et al. (2007): log M_A = 0.3820·log (PW^2/A) + 3.070; n = 3.070; n = 667 species–site pairs, r^2 = 0.55, $F_{1,666}$ = 11.33 P < 0.0001. Herbaceous angiosperm and broad-leaved gymnosperm compilation from Royer et al. (2010). Herbaceous angiosperms: log M_A = 0.2204·log (PW^2/A) + 2.245; n = 58 species–site pairs, r^2 = 0.32, $F_{1,57}$ = 11.33 P < 0.0001. Gymnosperms: log M_A= −0.3076·log (PW^2/A) + 3.015; n = 93 species–site pairs, r^2 = 0.44, $F_{1,92}$ = 11.33 P < 0.0001.

being equal, the beam structure that supports both organ types should be co-optimized for similar features—both biomechanical and hydraulic. Furthermore, some previous studies indicated a consistent scaling relationship between fern petiole diameters and frond and pinnae length and width (Arcand et al., 2008; Creese et al., 2011). However, somewhat unexpectedly, our results suggest that there may be different functional explanations for the PW–M_A relationships in fronds and pinnae, perhaps related to balancing the demands of support and hydraulic conductivity of the frond vs. fern pinna (e.g., Brodribb et al., 2005;

Table 6. Correlation coefficients (r) for pairwise comparison between leaf physiognomic variables and mean annual temperature. Physiognomic variables from Peppe et al. (2011) and Royer et al. (2005).

Physiognomic variables (unit)	Simple ferns	Compound ferns	Simple and compound
Leaf area (cm^2)	0.04	0.05	0.04
ln (Leaf area) (cm^2)	0.05	–0.10	0.05
Compactness	0.00	0.27	0.00
Feret's diameter	–0.15	–0.14	–0.15
Perimeter / leaf area	–0.03	0.35	–0.03
Perimeter ratio	0.11	0.28	0.11
No. of teeth	0.00	0.16	0.00
No. of teeth / perimeter (cm^{-1})	0.03	0.08	0.03
No. of teeth / internal perimeter (cm^{-1})	0.04	0.10	0.04
No. of teeth / blade area (cm^{-2})	–0.02	0.18	–0.02
Tooth area (cm^2)	–0.06	–0.06	–0.06
Average tooth area (cm^2)	–0.03	–0.55	–0.03
Tooth area / blade area	–0.20	–0.22	–0.20
Tooth area / perimeter (cm)	–0.03	–0.67*	–0.03
Tooth area / internal perimeter (cm)	–0.02	–0.68*	–0.02

Notes: * $P < 0.05$.

untapered cantilever beam (structure A) or a tapered cantilever beam (structure B) is indistinguishable (df = 1, $P = 0.102$) (Fig. 3A) indicating model D explains the scaling relationship of all fern frond types equally well.

The area-normalized PW and M_A relationship in fronds differed significantly between terrestrial and ephiphytic ferns (Fig. 3B). It may be that the differing relationships reflect the petiole (stipe) of the frond serving different functional roles in ferns with different growth habits. That is, in terrestrial ferns, the petiole must hold laminar tissue erect for photosynthesis, remain anchored to the ground by rhizomes, and remain flexible. Epiphytic, epipetric, and pendant leaves do not always raise themselves vertically, thus requiring less structural support from their petiole. Additionally, the hydraulic conductivity of the petiole may also play a role in influencing the scaling relationship because there are differences in leaf hydraulic conductivity potential and cavitation resistance in terrestrial and epiphytic ferns (Watkins et al., 2010). Given this evidence, it seems plausible that petiole width in epiphytic ferns is more a reflection of hydraulic supply than of mechanical support requirements, potentially explaining the different scaling relationship in terrestrial and epiphytic ferns.

With relatively small sample sizes in several fern clades, we only had limited power to quantify clade-specific scaling relationships. Although the PW–M_A scaling was statistically significant in Polypodiales only, there was no significant evidence of any *difference* in scaling slopes among groups. Thus, the cantilever beam model explains the scaling relationship between PW and M_A reasonably well for all fern groups.

Comparison of ferns and seed plants—The biomechanical model that best explains the scaling relationship in frond and primary and secondary pinnae combined (model D) differs from the model that best explains the relationship in woody dicots, herbs, and gymnosperms (model B) (Fig. 4). The scaling relationship in ferns is best explained when fronds are modeled as a cantilever beam, whereas in seed plants the scaling relationship is best explained when leaves are modeled as a vertical pole supporting a singular laminar mass. Model D does an especially poor job explaining the scaling relationship in herbs, suggesting that canopy position and lack of wood do not drive the difference between ferns and the other vascular plant groups. Together our results indicate a distinctly different functional relationship between PW and M_A in the seed plants and ferns. This difference is somewhat surprising given that other studies have suggested some convergence in the mechanical design of petioles in ferns and angiosperms (e.g., Niklas, 1991, 1993). These differences between ferns and seed plants may be related to the significant differences in the variability in foliage architecture and/or vascular systems between ferns and seed plants or to their differing evolutionary histories (e.g., Roth-Nebelsick et al., 2001; Schneider et al., 2004; Boyce, 2005).

Additionally, in agreement with Karst and Lechowicz (2007), we observed that ferns have generally lower M_A values compared with woody seed plants, even though the ranges broadly overlap (Fig. 4).
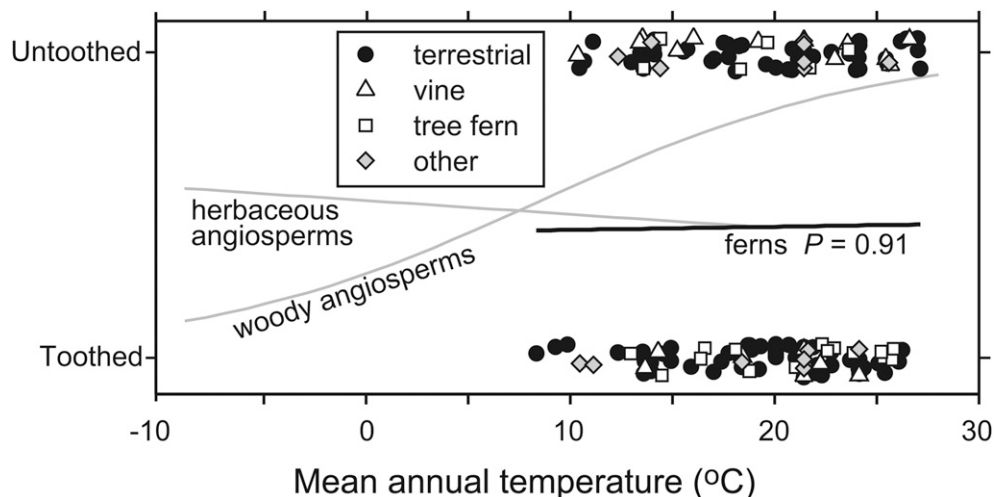

Fig. 5. Relationship between leaf-margin state and mean annual temperature for ferns. The slope of the line is not significantly different than zero (df = 1, $P = 0.91$). For comparison, gray lines show logistic regression for woody dicot angiosperms (df = 1, $P < 0.001$) and herbaceous angiosperms (df = 1, $P = 0.41$) from Royer et al. (2012). All points have been slightly jittered to better visualize data for all fern growth habits.

Fossil applications of biomechanical models—Model D models the biomechanical scaling relationships between area-normalized PW and M_A in both fronds and primary and secondary pinnae reasonably well. There are no differences in the scaling relationships in different fern lineages, including those that likely evolved in the Paleozoic (e.g., Marattiales; Taylor et al., 2009). These results suggest that the models for both fronds and primary/secondary pinnae presented here (Tables 4, 5) can be applied to fossil ferns. The interrelationship of M_A with other leaf economic variables in seed plants (e.g., Wright et al., 2004) has been used to estimate leaf life span in fossils (e.g., Royer et al., 2007, 2010). Although they did not measure leaf life span, Karst and Lechowicz (2007) found that some leaf economic variables in ferns (M_A, nitrogen, and net photosynthetic rate) were interrelated in ways that were consistent with seed plants. Additionally, Karst and Lechowicz (2007) found that fronds that overwintered had significantly higher M_A values than those that did not, which is the expected relationship based on the leaf economic spectrum. Given the similarities in the interrelationships of leaf economic variables in ferns and seed plants and the limited evidence suggesting a positive relationship between M_A and leaf life span (Karst and Lechowicz, 2007), a similar relationship seems likely between M_A and leaf life span in ferns and seed plants. Thus, estimates of M_A in ferns could be used as an approximation of leaf life span providing important information about the ecological strategies of ancient ferns. However, given the uncertainties of these models, we suggest that they should only be applied to fossil ferns in conjunction with other paleoecological methods.

Leaf physiognomy—Fern leaf physiognomy, including leaf margin state, was insensitive to MAT (Table 6, Fig. 5). For compound fronds, tooth area/perimeter and tooth area/internal perimeter show a significant relationship with MAT (Table 6); however, in both cases, the relationships were driven by a single outlying data point (both relationships become nonsignificant when the data point is removed).

This lack of sensitivity to climate is similar to that found in herbs (Fig. 5; Royer et al., 2012). This may be because both ferns and herbs are generally understory plants and the subcanopy microclimate may be distinctly different from regional climate (e.g., Bailey and Sinnott, 1916). In the herb *Chloranthus japonicus*, leaf teeth may serve to release excess positive root pressure (Feild et al., 2005). Given that many ferns share a similar low-statured growth habit to herbs, it is possible that ferns may also be sensitive to damage from increased root pressure, providing another possible explanation for the poor relationship between leaf physiognomy and MAT. Alternatively, the lack of climatic sensitivity in ferns may be related to the differing evolutionary histories of leaf morphology in ferns and angiosperms (e.g., Roth-Nebelsick et al., 2001; Schneider et al., 2004; Boyce, 2005; Benca et al., 2013).

Summary—Our observations indicate that allometric scaling relationships between area-normalized PW and M_A are different among all hierarchical levels of fern fronds and that the scaling relationship in ferns is most accurately described as behaving like an end-loaded cantilever beam (model D). This modeled relationship differs somewhat according to growth habit, but not by mechanical leaf structure (i.e., tapered vs. untapered cantilever beam) or phylogeny. Given the lack of phylogenetic influence, the model can be applied to fossil ferns in conjunction with other paleoecological proxies (e.g., leaf

venation). Fern leaf physiognomy appears insensitive to climate and should not be used to reconstruct paleoclimate.

We found evidence that biomechanical and leaf–climate relationships in ferns were fundamentally different from those in seed plants. These differences between seed plants and ferns may be related to differences in their usual growth habits and hydraulic conductivity and/or to the distinctly different evolutionary histories of their leaf morphology and vein architecture.

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