

RESEARCH ARTICLE

Experimental evidence for species-dependent responses in leaf shape to temperature: Implications for paleoclimate inference

Melissa L. McKee¹, Dana L. Royer^{1*}, Helen M. Poulos²

1 Department of Earth and Environmental Sciences, Wesleyan University, Middletown, Connecticut, United States of America, **2** College of the Environment, Wesleyan University, Middletown, Connecticut, United States of America

* droyer@wesleyan.edu

Abstract

In many woody dicot plant species, colder temperatures correlate with a greater degree of leaf dissection and with larger and more abundant leaf teeth (the serrated edges along margins). The measurement of site-mean characteristics of leaf size and shape (physiognomy), including leaf dissection and tooth morphology, has been an important paleoclimate tool for over a century. These physiognomic-based climate proxies require that all woody dicot plants at a site, regardless of species, change their leaf shape rapidly and predictably in response to temperature. Here we experimentally test these assumptions by growing five woody species in growth cabinets under two temperatures (17 and 25°C). In keeping with global site-based patterns, plants tend to develop more dissected leaves with more abundant and larger leaf teeth in the cool treatment. Overall, this upholds the assumption that leaf shape responds in a particular direction to temperature change. The assumption that leaf shape variables respond to temperature in the same way regardless of species did not hold because the responses varied by species. Leaf physiognomic models for inferring paleoclimate should take into account these species-specific responses.

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Introduction

In most regions of the world, the proportion of toothed woody dicot species, as well as species-mean leaf tooth size, tooth number, and leaf dissection, inversely correlate with mean annual temperature (MAT) [1–10]. In short, leaves in cold climates are more likely to have toothed leaf margins with larger and more numerous teeth. Many leaf-functional traits, including size and shape (physiognomy), can be measured in leaf fossils. As such, the paleobotanical community has developed both univariate and multivariate approaches, or leaf-physiognomy climate-models, to quantitatively reconstruct aspects of paleoclimate from leaf fossils [3–10].

Royer and Wilf [11] measured photosynthesis and transpiration in toothed and untoothed leaf margins to investigate the biological underpinnings between leaf teeth and MAT. They found that toothed species often had higher gas-exchange rates in their teeth than in their leaf interior during the first few weeks of the growing season. The corresponding increase in sap

flow in young, expanding leaves should increase the delivery of nutrients to the entire lamina. This function of leaf teeth could be increasingly adaptive in colder climates (with corresponding shorter growing seasons) because leaf teeth could help plants ramp up to maximum carbon production rates sooner relative to an equivalent leaf without teeth. In warmer climates with longer growing seasons, the water cost associated with teeth could outweigh any benefits for maximizing the growing season length [11]. Alternative functional explanations for leaf teeth include the release of excess root pressure [12], the mechanical support associated with leaf thickness [13], and the pattern and duration in which leaf primordia are packed into resting buds [14].

All current leaf-physiognomy climate-models are site-based [3–10]: that is, climate is estimated from the across-species mean of leaf physiognomy. A tacit assumption with these models is that site-mean leaf shape responds rapidly (tens-to-hundreds-of-years) and predictably to climate change. By growing identical seed lines of red maple (*Acer rubrum* L.) in growth cabinets, Royer [15] found that leaves that developed under cooler temperatures were more dissected and had more teeth. These experiments provide strong evidence for the direct and very rapid effect of growth temperature on the morphology of leaf teeth. These experiments also show a response in leaf shape within a single species—and within a single generation—that is consistent with global site-based patterns. Chitwood et al. [16] found similar patterns in field-grown *Vitis* within the span of just two growing seasons, where one year was colder and dryer than the other.

How applicable are plastic responses in a single species to site-based methods, especially considering that: 1) fossil deposits rarely resolve timescales relevant for plastic responses; 2) in present-day compilations of natural populations across the globe, the primary reason why site-mean leaf physiognomy covaries with climate is because species composition differs across sites [1–10], and in cases where physiognomy in natural populations covaries with climate within a single species (e.g., *A. rubrum* [17]), common-garden experiments suggest that differences in genotype are driving at least some of the differences in physiognomic expression [18]; and 3) leaf physiognomy within species does not always respond to climate, either plastically or through changes in genotype [17], [19].

We see at least two reasons for why documenting plastic responses can be relevant for leaf-physiognomy climate-models. First, growth-cabinet experiments provide strong evidence for a causal link between leaf physiognomy and climate because—in contrast to observations in natural populations—other environmental factors can be controlled for. Establishing a causal link boosts confidence that these models can be applied generally, including to fossil settings. Second, if leaf physiognomy can respond to changes in climate within the lifetime of an individual, this increases confidence that fossil assemblages (site means) can faithfully record climate, even during periods of rapid climate change, and not a preceding climate state.

With these motivating principles in mind, how general are the plastic responses exhibited by *A. rubrum*? Here we test how the leaf physiognomy in five dicot tree species responds when grown in growth cabinets of contrasting temperature. If the plasticity observed in *A. rubrum* is common in other taxa, then this would increase the likelihood that reconstructions of climate from the site-mean of fossil leaf physiognomy are robust, even during times of rapid climate change.

We evaluated three key questions: 1) Are leaves grown in cooler temperatures “toothier” and more dissected? This is arguably the most important question for paleobotanists aiming to reconstruct climate from fossil leaf physiognomy. 2) How quickly can leaf physiognomy respond to temperature change? We grew four species from seed and three from saplings (two of the species were grown from both seed and saplings). Because the saplings had previously experienced a different environment, any physiognomic response to temperature in the

growth cabinets would underscore the speed at which these changes happen, even within a single growing season. 3) Do all species respond in the same way? Because current leaf-physiology climate-models are site-based, they require an assumption, on some level, that leaf physiognomy responds to climate in the same way, regardless of the species [5], [20–22]; or, more directly, that the across-species mean of leaf physiognomy always responds to climate in the same way. Our multi-species study design allows us to partly address this question.

Materials and methods

We grew four species from seed (*Acer negundo* L., *Betula lenta* L., *Carpinus caroliniana* Walter, and *Quercus rubra* L.) and three species from transplanted saplings (*Acer negundo*, *Carpinus caroliniana*, and *Ostrya virginiana* (Mill.) K.Koch) (see [S1 Text](#) for details). We selected these species because each has natural populations along the east coast of the United States with leaf shapes that tend to covary with climate in a manner that mirrors site-mean patterns [19].

Seeds and saplings were randomly divided into two temperature treatments. Each group was grown in an independently controlled growth cabinet (Conviron E7/2; Winnipeg, Manitoba, Canada). Both cabinets were set to a 17-hour photoperiod with a 30-minute simulated dawn and dusk, and a 500 ppm CO₂ concentration (actual = 503 ± 10.4 ppm [1σ]). The warm treatment had a target day/night temperature of 28/19°C (actual = 27.9 ± 0.5°C and 19.2 ± 0.7°C), with a time-weighted mean temperature of 25.0°C. The cool treatment had a target day/night temperature of 20/11°C (actual = 20.0 ± 0.3°C and 11.3 ± 1.1°C), with a time-weighted mean temperature of 17.1°C. The cabinets differed somewhat in relative humidity (76.5 ± 1.8% and 90.0 ± 3.6%); because of this, the warm and cool treatments were alternated between cabinets every two weeks to minimize cabinet effects.

Leaves were harvested after three months. From each plant, we selected five young, fully expanded leaves that developed from buds that formed while the plant was in the growth cabinet. In most cases, seven plants per species × temperature were analyzed (exceptions: ten for *B. lenta* and five for *O. virginiana*). Leaves were photographed immediately (Nikon D5300 camera, Nikon, Melville, New York, USA). The procedure for measuring leaf physiognomy is discussed fully by Royer et al. [19] and Huff et al. [23]. Briefly, in Photoshop (Adobe Systems, San Jose, California, USA), minor defects along the leaf margin were corrected using the line tool and petioles were separated from the leaf blade. Teeth were then digitally separated from the leaf blade; most teeth are bounded by two sinuses, but see Royer et al. [19] and Huff et al. [23] for exceptions. Leaf physiognomy was then quantified with ImageJ software (<http://rsbweb.nih.gov/ij/>). In addition, we measured the fractal dimension of leaves (see [S1 Text](#) for details). All measured variables are related to tooth abundance, tooth size, or degree of leaf dissection; see [Table 1](#) for a complete list, along with definitions. All data are presented in [S1 Dataset](#). Tooth size in *Q. rubra* was highly variable, including many instances of lobes (following Royer et al. [19]), leading to difficult-to-interpret patterns; because of this, we exclude tooth-size variables for *Q. rubra*.

The unit of replication for all statistical tests was the number of plants per species × temperature treatment. For most analyses, we first divided the data into plants grown from seed and saplings. We did this because the physiognomy for the two species grown from both seed and saplings was often quite different (*A. negundo* and *C. caroliniana*; e.g., [Figs 1D, 2E](#) and [3E](#); see also [S5 Table](#)). We tested for differences in each physiognomic variable between warm and cool treatments with linear models using the `lm` function in R [24]. First, we evaluated species-level differences in leaf physiognomy with temperature via estimated marginal means in the `emmeans` package in R [25], with a Dunn-Šidák correction to account for multiple comparisons. Second, we compared leaf physiognomy across species, with species, growth

Table 1. Physiognomic variables and definitions.

Variable	Definition
Tooth abundance	
Number of teeth	Total number of primary and secondary ^a teeth
Number of teeth / internal perimeter	Total number of teeth / internal perimeter (perimeter of leaf after leaf teeth are removed) (cm ⁻¹)
Number of teeth / blade area	Total number of teeth / blade area (cm ⁻²)
Tooth size	
Tooth area	Total area of teeth (cm ²)
Average tooth area	Tooth area / number of primary teeth (cm ²)
Tooth area / internal perimeter	Tooth area / internal perimeter (cm)
Tooth area / blade area	Tooth area / blade area (dimensionless)
Leaf dissection	
Circularity	$4\pi \times [\text{leaf area} / (\text{leaf perimeter})^2]$ (dimensionless)
Perimeter ratio	Leaf perimeter / leaf internal perimeter (dimensionless)
Feret diameter ratio	Feret diameter (diameter of a circle with the same area as the leaf) / major length (longest measured line across the leaf blade) (dimensionless)
Fractal dimension	Degree of an object's boundary fragmentation over multiple scales (dimensionless)

Global patterns [6] predict a negative correlation with mean annual temperature for all variables except circularity.

^a Secondary teeth are teeth that are located on larger, primary teeth [19].

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temperature, and the species × temperature interaction as the fixed effects. To test the significance of temperature across species, we computed an ANOVA within the linear model using the `anova` function in R. An advantage of pooling species is that the statistical power is improved for discerning small but pervasive differences that may not be statistically significant within individual species. Third, to test if the physiognomic responses to temperature were dependent on life stage, we constructed a linear model based on the two species grown from both seed and saplings, with life stage, temperature, and the life-stage × temperature interaction as the fixed effects. For all tests, $P < 0.05$ was considered statistically significant.

Results

The directionality of leaf physiognomic responses to temperature are generally consistent with global site-based patterns: leaves from the cool treatment tend to have more numerous teeth, larger teeth, and be more dissected (lower circularity and higher perimeter ratio) (Figs 1–3). Two leaf dissection variables—feret diameter ratio and fractal dimension—show a more mixed response. At the level of individual species, though, very few of these temperature comparisons are significant (asterisks in Figs 1–3; see also S1 & S2 Table).

When we pool species, we find that the differences in leaf physiognomy between temperature treatments are often significantly different. For species grown from seed, all three tooth abundance variables (number of teeth [$P = 0.002$], ratio of number of teeth to internal perimeter [$P < 0.001$], ratio of number of teeth to blade area [$P = 0.002$]), two of the tooth size variables (ratio of tooth area to internal perimeter [$P = 0.003$] and ratio of tooth area to blade area [$P < 0.001$]), and two of the leaf dissection variables (circularity [$P < 0.001$] and perimeter ratio [$P < 0.001$]) are all significantly greater in the cool treatment, which is in keeping with global site-based patterns (S3 Table). Six out of the eleven variables have significant interaction

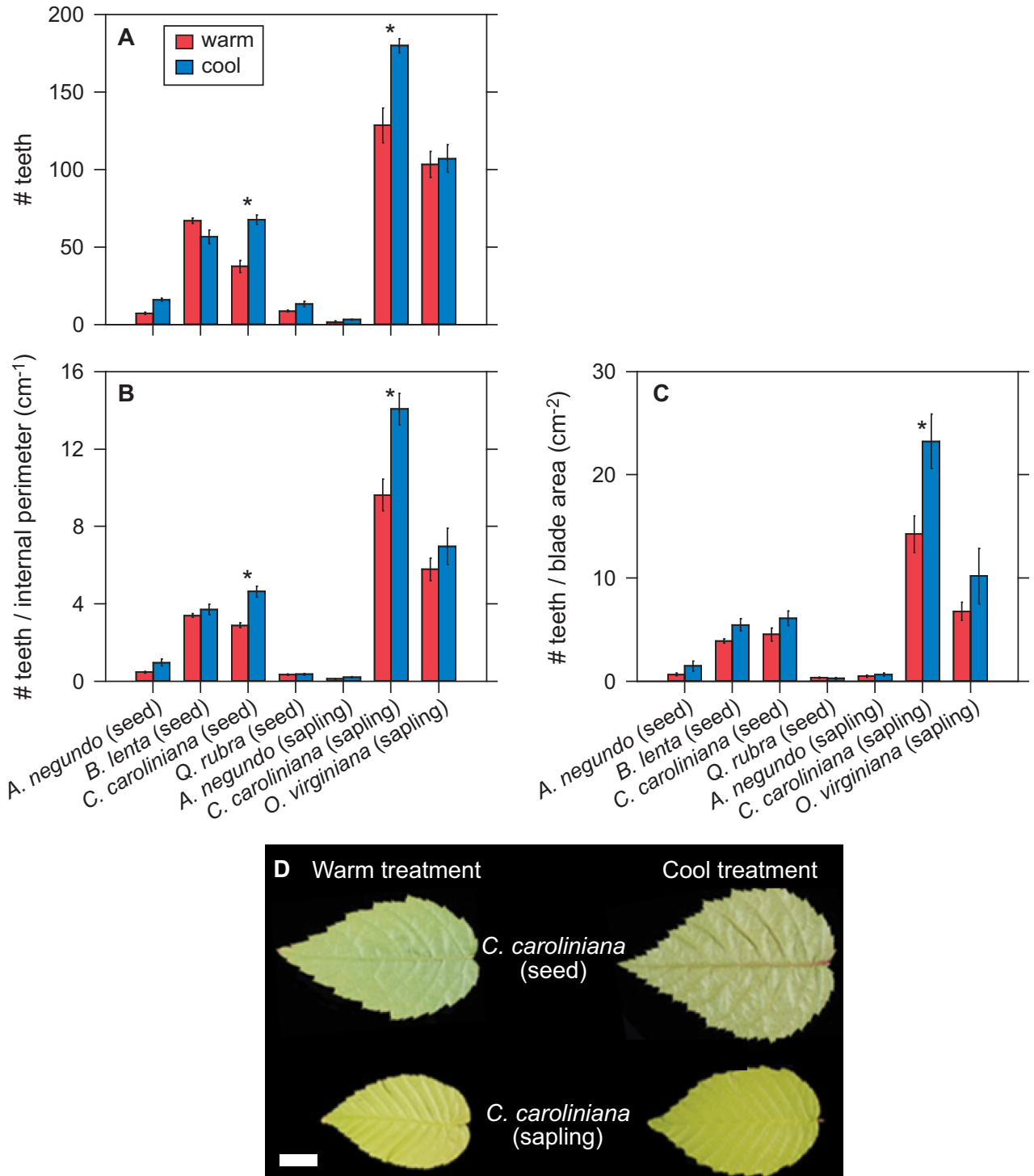


Fig 1. Sensitivity of tooth abundance variables to temperature. (A-C) Comparisons of the species grown in the growth cabinet experiment for tooth abundance variables. Values are means \pm 1 s.e.m. * indicates a significant difference ($P < 0.05$) based on estimated marginal means (see Materials and Methods). Global site-based patterns [6] would predict higher values in the cool treatment for all variables. (D) Representative leaves from species in this experiment that show significant differences in tooth abundance between the two treatments. Scale bar = 1 cm.

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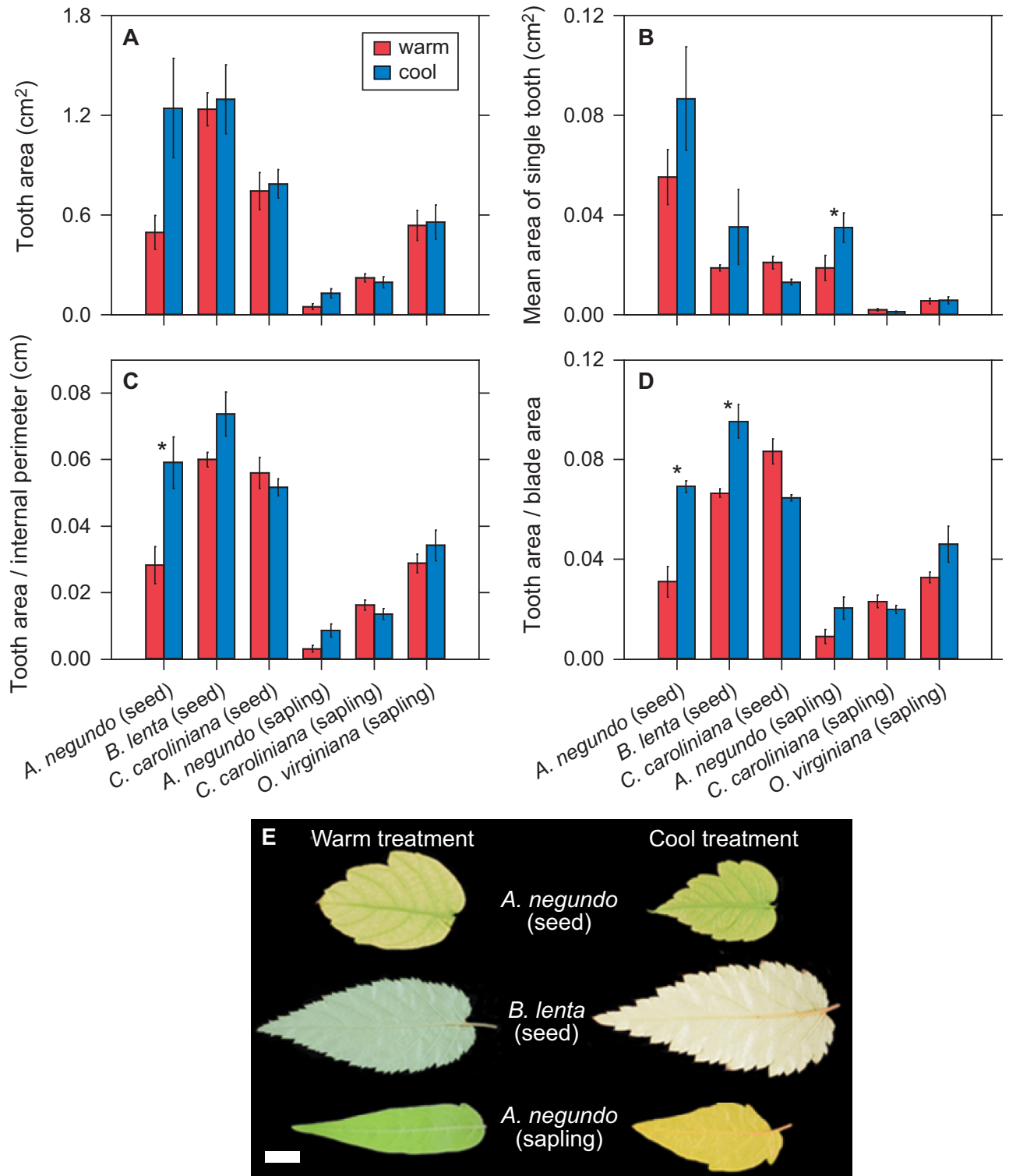


Fig 2. Sensitivity of tooth size variables to temperature. (A-D) Comparisons of the species grown in the growth cabinet experiment for tooth size variables. Values are means ± 1 s.e.m. * indicates a significant difference ($P < 0.05$) based on estimated marginal means (see Materials and Methods). Global site-based patterns [6] would predict higher values in the cool treatment for all variables. (E) Representative leaves from species in this experiment that show significant differences in tooth size between the two treatments. Scale bar = 1 cm.

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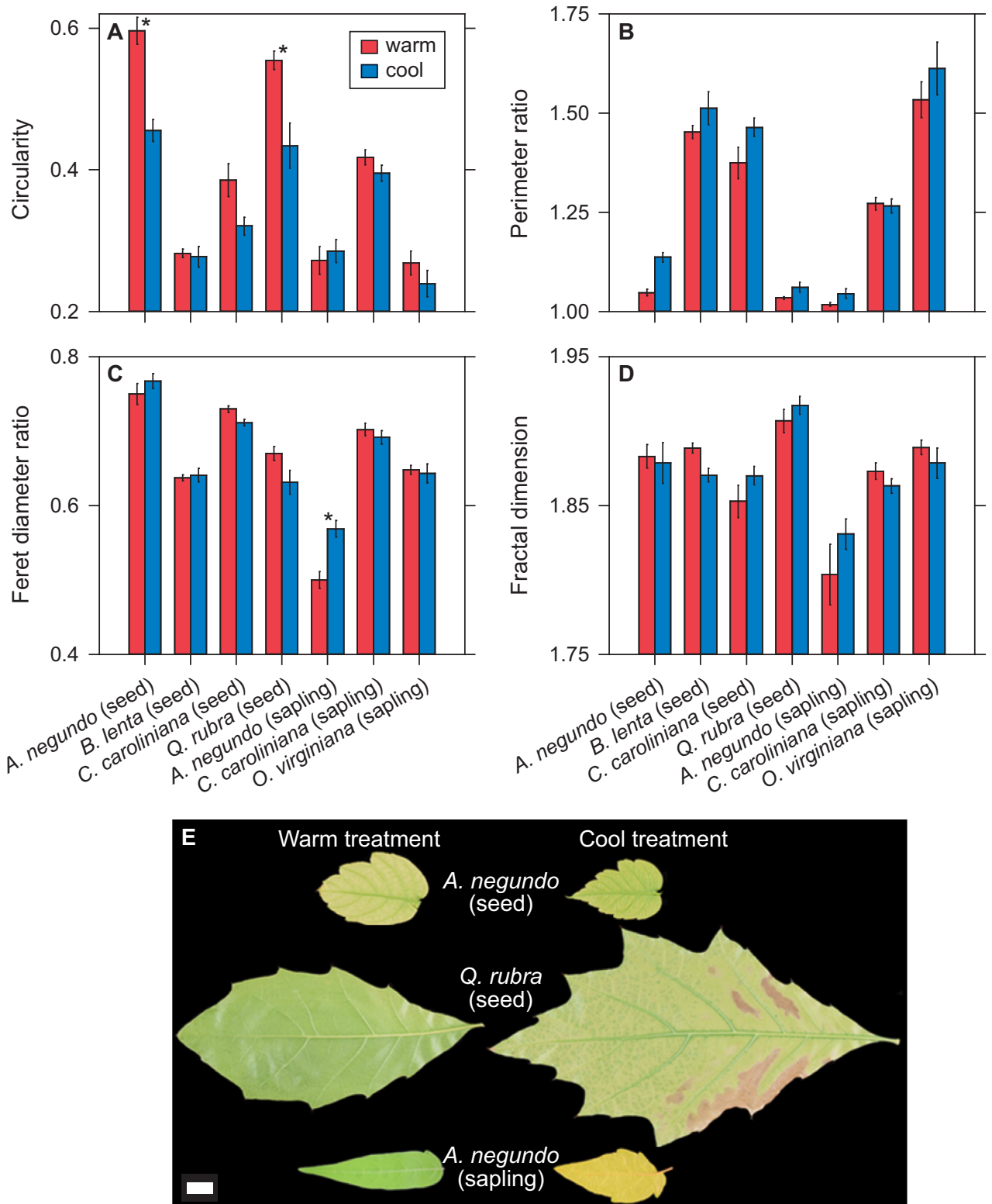


Fig 3. Sensitivity of leaf dissection variables to temperature. (A-D) Comparisons of the species grown in the growth cabinet experiment for leaf dissection variables. Values are means \pm 1 s.e.m. * indicates a significant difference ($P < 0.05$) based on estimated marginal means (see Materials and Methods). Global site-based patterns [6] would predict higher values in the cool treatment for perimeter ratio, feret diameter ratio, and fractal dimension; and lower values for circularity. (E) Representative leaves from species in this experiment that show significant differences in leaf dissection between the two treatments. Scale bar = 1 cm.

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terms in the effect of temperature among species, meaning that the physiognomic responses to temperature are often species-specific (S3 Table).

The saplings show similar but fewer significant patterns: consistent with global site-based patterns, all three tooth abundance variables (number of teeth [$P < 0.001$], ratio of number of teeth to internal perimeter [$P < 0.001$], ratio of number of teeth to blade area [$P = 0.005$]), one tooth size variable (ratio of tooth area to blade area [$P = 0.04$]), and one leaf dissection variable (feret diameter ratio [$P = 0.02$]) are significantly greater in the cool treatment. Five variables have a significant temperature \times species interaction (S4 Table).

For the two species that were grown both from seed and saplings (*A. negundo* and *C. caroliniana*), only two physiognomic variables have a significant temperature \times life-stage interaction: tooth area ($P = 0.05$) and circularity ($P = 0.05$) (S5 Table). This means that for these two variables, temperature does not have the same effect on plants grown from seed vs. saplings.

Discussion

For plants grown from seed, seven out of the eleven measured leaf physiognomic variables respond significantly to temperature. In all cases, physiognomy varies in the same direction predicted from global site-based patterns, with leaves from the cool treatment tending to be more dissected and to have larger and more numerous teeth. These results are consistent with the previous experiments with *A. rubrum* [15], [18]: leaf physiognomy in deciduous, toothed tree species can respond quickly to temperature and in the direction predicted from global site-based patterns. These results are encouraging for those who wish to use leaf physiognomic variables for paleoclimatic reconstructions using leaf fossil data.

For plants grown from saplings, five physiognomic variables respond significantly to temperature. Similar to the plants grown from seed, all three tooth-abundance variables respond, but the sapling responses are comparatively weaker for the tooth area and leaf dissection variables. This may mean that saplings transplanted to a new environment are not as responsive to temperature as plants grown from seed in a single environment. But the difference in our experiment is not large (seven vs. five significant variables); moreover, for the two species grown both from seed and saplings, only two physiognomic variables have a significant temperature \times life-stage interaction. Longer-term experiments with more than two species are needed to test more fully how quickly established plants can alter their leaf physiognomy to changes in temperature. This issue is important for fossil studies because if leaf teeth cannot respond quickly enough to rapid changes in their environment, fossil leaves may reflect the environmental conditions of a time prior to their deposition.

A significant temperature \times species interaction is present for most of the physiognomic variables that respond significantly to temperature. This means that the response of leaf physiognomy to temperature can be species-specific. All current leaf-physiognomy climate-models are site-based [3–10], where different sets of species are compared across sites. If species-specific responses in leaf physiognomy to temperature change are common, then this raises the concern that the site-based physiognomy-climate relationships that we observe today may change as temperature changes. Multiple studies, based on species-rich data sets from North America, South America, and Asia, identify the presence of a species-specific signal in many of the correlations between climate and tooth size, tooth number, and leaf dissection [5], [20–22]. Our results support the more general call to incorporate phylogenetic signal into leaf-physiognomy climate-models [22].

Conclusions

Our experiments support the view that leaf shape generally changes predictably and rapidly in response to temperature change. Critically, the temperature effects on leaf shape are usually

species-specific. Because of this, we recommend that leaf-physiognomy climate-models for reconstructing paleotemperature from fossil leaves take into account these species-specific responses.

Supporting information

S1 Text. Supplementary methods. Methods for cultivating plants in the growth cabinet experiment and for fractal dimension measurements.

(PDF)

S1 Table. Linear model results for seedlings testing for the effect of temperature on leaf shape within species.

(PDF)

S2 Table. Linear model results for saplings testing for the effect of temperature on leaf shape within species.

(PDF)

S3 Table. Analysis of variance of a linear model testing for differences in leaf shape in seedlings across species, temperature (cool or warm), and the species \times temperature interaction.

(PDF)

S4 Table. Analysis of variance of a linear model testing for differences in leaf shape in saplings across species, temperature (cool or warm), and the species \times temperature interaction.

(PDF)

S5 Table. Analysis of variance of a linear model testing for differences in leaf shape in *Acer negundo* and *Carpinus caroliniana* across life stage (seedling or sapling), temperature (cool or warm), and the life stage \times temperature interaction.

(PDF)

S1 Dataset. All leaf physiognomic data.

(XLSX)

Author Contributions

Conceptualization: Melissa L. McKee, Dana L. Royer.

Data curation: Melissa L. McKee, Dana L. Royer.

Formal analysis: Melissa L. McKee, Dana L. Royer, Helen M. Poulos.

Investigation: Melissa L. McKee.

Methodology: Melissa L. McKee, Dana L. Royer.

Supervision: Dana L. Royer.

Validation: Melissa L. McKee, Dana L. Royer, Helen M. Poulos.

Visualization: Melissa L. McKee, Dana L. Royer, Helen M. Poulos.

Writing – original draft: Melissa L. McKee.

Writing – review & editing: Melissa L. McKee, Dana L. Royer, Helen M. Poulos.

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