

Digital Future for Paleoclimate Estimation from Fossil Leaves? Preliminary Results

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Leaf size and shape are selected by climate and are strongly correlated with climatic variables. Accordingly, fossil dicotyledonous leaves are considered to be among the most reliable indicators of terrestrial paleoclimates. However, the methods used have much potential for improvement. All currently rely on discrete, usually binary characters, but leaf morphology is better evaluated and more reproducibly measured using continuous variables. Digital leaf measurement offers the possibility of continuous, reproducible variables, with the potential to improve paleoclimate estimates as well as the ecological and evolutionary understanding of leaf form. Results are reported for five variables measured on 238 leaves representing the woody species from three living test floras—two temperate and one humid tropical. The variables are: shape factor (an area:perimeter ratio standardized to a circle); the ratio of feret diameter (diameter if leaf's area is contained in a circle) to major axis length; the ratio of tooth area to leaf area; tooth count; and tooth count standardized to leaf perimeter. It is well known that increased mean annual temperature is correlated with a high percentage of untoothed species. By extension, increasing temperature is expected to be associated with relatively undissected, more circular leaves (i.e., with high shape factor, low tooth-area:leaf-area ratio, and low tooth count). These trends were observed in the test floras, both between the tropical and the temperate sites and between the two temperate sites. Shape factor was the variable that resolved the three sites most significantly. The five variables show promise for testing on an expanded set of floras sufficient to develop statistical models for paleoclimatic studies.

INTRODUCTION

Paleoclimate estimation from fossil plants is one of the most important contributions of paleobotany to the earth sciences, with impacts on diverse research areas such as climate history and modeling, paleoenvironmental reconstruction, comparisons of past climates with greenhouse gas concentrations, and the climatic context of biotic turnover and

evolution (see Parrish, 1998). Among the most productive approaches for estimating paleoclimate from fossil floras is the use of correlations observed in living forests between climatic and leaf size and shape variables (Bailey and Sinnott, 1915; Wolfe, 1979, 1993; Greenwood and Wing, 1995; Herman and Spicer, 1996; Wing et al., 2000; Gregory-Wodzicki, 2000; Jacobs, 2002; Spicer et al., 2002; Wilf et al., 2003). Of these methods, by far the most widely and easily used, known as leaf-margin analysis, is based on the correlation between mean annual temperature (MAT) and the percentage of woody dicot species in a sample that has untoothed leaf margins (Bailey and Sinnott, 1915; Wolfe, 1979; Wilf, 1997). The statistical precision of leaf-margin analysis is about $\pm 2^\circ\text{C}$ before consideration of sampling and taphonomic factors (Wilf, 1997; Burnham et al., 2001). The physiological basis of the correlation remains poorly understood. However, work to date suggests that leaf teeth are convergent adaptations that, as edge features, help to optimize photosynthetic capability by affecting water balance and acquisition of CO_2 during short, cool growing seasons (Canny, 1990; Wolfe, 1993; Baker-Brosh and Peet, 1997; Wilf, 1997). The correlation often decreases or changes in extremely cold, dry, saline, or other physiologically stressful environments (e.g., Bailey and Sinnott, 1915; Wolfe, 1993).

The potential for greater precision, and for the estimation of climatic variables besides MAT, has been demonstrated through the multivariate analysis of up to 31 size and shape (physiognomic) variables, also scored on a percentage basis as part of a collection and analysis procedure known as CLAMP (climate-leaf-analysis multivariate program; Wolfe, 1993; Wolfe, 1995). For variables other than mean annual temperature, the accuracy of CLAMP analyses and derivative methods have been questioned and debated, as discussed elsewhere (Jordan, 1996; Burnham, 1997; Wilf, 1997; Wilf et al., 1998, 1999; Wolfe and Uemura, 1999; Gregory-Wodzicki, 2000; Jacobs, 2002). In the case of mean annual temperature, analytical methods based on CLAMP, which theoretically should produce significantly improved estimates, often do not yield more accurate results than does leaf-margin analysis when tested on living floras (Wilf, 1997; Wiemann et al., 1998; Gregory-Wodzicki, 2000; Kowalski and Dilcher, 2001). This outcome may be related, in part, to the reproducibility of the leaf-scoring procedures for CLAMP that were presented by Wolfe (1993). For the same leaves, different investigators appear to score the presence or absence of leaf teeth with near uniformity, but the same is not necessarily true, for example, for apex and base states (Wilf, 1997). The noise from variance in scoring can exceed the additional temperature signal from extra characters (Wilf, 1997; Wiemann et al., 1998).

The general lack of improved estimates from the CLAMP method poses a challenge to paleobotanists, many of whom continue to use leaf-margin analysis. What should be done next to improve leaf-climate science, besides gathering new data from living forests for continued testing of equations that represent variations on the existing methods (e.g., Gregory-Wodzicki, 2000; Kowalski and Dilcher, 2001; Jacobs, 2002)? One possible solution would be to address the scoring problem directly through revision and rigorous testing of the scoring definitions for the multivariate characters, followed by rescaling of the calibration data.

Alternatively, a fully digital approach to leaf scoring, explored here, has the potential to produce either replace-

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ments for leaf-margin and CLAMP analyses or, at the least, complementary and informative techniques. Digital image processing has improved exponentially along with computing power (e.g., Starck et al., 1998), and digital techniques have been used to investigate leaf outlines (Kincaid and Schneider, 1983; Sagasti et al., 1993; Moraczewski, 1998; McLellan and Endler, 1998; Jacobs, 1999). With the recent advent of inexpensive digital photography, fast personal computers, and capable imaging software, the potential now exists to use digital leaf physiognomy in almost any laboratory and at relatively low cost.

Digital leaf physiognomy potentially offers several major advantages over leaf-margin analysis and CLAMP scoring. First, the reproducibility of scoring is greatly increased over that of many of the CLAMP characters because computational algorithms for shape measurement are unambiguous by definition. Second, binary presence-absence data for individual variables, represented by percentages, are replaced or supplemented by continuous measurements, which should lead to significant improvement in the overall precision of calibration data and to better understanding of the variation of climatically informative leaf traits. For example, with continuous data, a more powerful set of statistical tests and analyses is available for analyzing the means and distributions of species measurements within a floral sample. Third, continuous measurements allow the correlations of previously unquantified variables, such as tooth size and area:perimeter ratios, with leaf traits that are well-known ecologically, such as lifespan, nitrogen content, thickness, herbivore resistance, and others (Coley et al., 1985; Ackerly and Reich, 1999; Reich et al., 1999), potentially leading to better understanding of the ecology of leaf form and thus of the underlying selective forces that control leaf-climate correlations. Fourth, unlike voucher specimens that are dispersed among museums and personal collections, digital leaf images can be shared easily via the Internet.

Here, preliminary tests of a suite of digital physiognomic variables are presented, and their potential is considered for paleoclimatic reconstruction and ecological studies. The variables were chosen for ease of use with commercially available software and include absolute measurements and various ratios of leaf area, leaf perimeter, tooth size, and tooth number, measured on 238 leaves from three study areas. The goal of this contribution is to make the first baseline measurements, tests, and comparisons of the digital variables for nearly perfect leaves. Application to fossils, which often are incomplete, must await work on a larger set of test floras, development of the most promising variables, and tailoring of the methods.

STUDY AREAS AND SPECIMENS

Two of the three test floras are located in temperate forests and one in a tropical moist forest. The temperate study areas are Allegheny National Forest (ANF), northern Pennsylvania, USA (MAT = 7.2°), and York County (York), southern Pennsylvania (MAT = 11.8°C); the tropical site is on Barro Colorado Island (BCI), Republic of Panama (MAT = 27°C). The collections investigated here are the same ones described as subsamples by Wilf (1997) and include 47, 56, and 137 species of woody dicots, respectively. Detailed climatic and collection data for the three sites

are provided elsewhere (Wilf, 1997, and references therein). Leaf-margin analyses of the collections indicate respective MATs of 8.6°C, 9.6°C, and 25.8°C (Wilf, 1997). The Pennsylvania collections were made from multiple transects by P.W., from both live foliage and leaf litter, and they are permanently housed at the Morris Arboretum, University of Pennsylvania. The Barro Colorado Island collection was made by R. Burnham and S. Wing from a single hectare of forest, and it is housed in the Division of Paleobotany of the National Museum of Natural History, Smithsonian Institution, and in the University of Michigan Museum of Paleontology.

The tropical-temperate comparison provides a first-order test of the sensitivity of the digital variables to fundamentally different climates: for example, does the BCI site have species with smaller teeth, as suggested by anecdotal observation of tropical vegetation, and more circular leaf shape than the Pennsylvania sites? The latter, with a difference in MAT of only ~5°C, pose a more difficult, second-order challenge within the cold end of the spectrum, especially because they have similar species composition, and leaf-margin analysis differentiates only 1°C of their actual temperature difference.

The three study collections, like those in most herbaria, were not made with attention to acquiring perfectly intact margins, and leaf overlaps in mounts were common. Accordingly, photographically distinct leaves with intact margins were rare, and it became clear that in most cases, more than one leaf per species could not be analyzed without making new collections. It was assumed that the main effect of using one leaf per species rather than a suite of replicates was noise in the analyses, biasing against the observation of climatic trends. Also, preliminary tests showed low variance of measurements within species, in comparison to variance among species (Appendix 1). Leaf specimens were selected for photography using the following protocol: for each species in each of the three collections, the first leaf specimen found, or leaflet if a compound leaf, was selected for analysis that had a nearly intact and photographically distinct margin, was not mounted under, over, or immediately next to other specimens or tape, and did not appear to inhabit the extremes of a species' morphological range. If no specimens meeting these criteria existed in the collections for a particular species, a specimen from the University of Michigan Herbarium or the National Herbarium was substituted, chosen for the closest possible proximity of its original collection site to the respective study area (specimen data for substitutions are available from the SEPM Data Archive, <http://www.ngdc.noaa.gov/mgg/sepm/archive>). Two species from the Barro Colorado Island collection were missing, and no substitutions were available, so 135 species were analyzed for the BCI sample. For facultatively toothed species, a leaf with the most abundant margin type in the respective collection for that species was selected. Future collections for leaf-climate studies should prioritize leaf specimens with perfect margins, enabling the rapid analysis of replicates, and should not be mounted with glue or other restrictive materials.

VARIABLES AND EXPECTED RESULTS

Variables explored here, and defined in Table 1, are shape factor; the ratio of feret diameter to major axis length (here-

TABLE 1—Definitions of leaf-shape variables.

Variable	Definition
Shape factor	4π (leaf area/leaf perimeter ²)
Feret-diameter ratio	feret diameter/major axis length
Tooth-area ratio	area of all teeth/leaf area
Tooth count	total number of teeth
Standardized tooth count	tooth count/leaf perimeter

Notes: Feret diameter is the theoretical diameter of an object (here, a leaf blade) if it were circular in shape. Major axis length is the longest measurable dimension, which correlates strongly with the formally defined length of a leaf (Ash et al., 1999). All primary measurements were made in cm or cm².

after “feret-diameter ratio” for convenience); the ratio of tooth area to leaf area (hereafter “tooth-area ratio”); tooth count; and tooth count standardized to leaf perimeter. These variables are derived directly from computerized measurements of length, area, and perimeter, or from simple counting of teeth (details of measurement techniques are described in the next section). Other, less-promising variables are mentioned briefly. Fourier analysis represents an alternative but labor-intensive means for describing leaf shape (e.g., Kincaid and Schneider, 1983; Sagasti et al., 1993). However, this approach is not well suited for the accurate reproduction of fine features such as small teeth (Moraczewski, 1998; McLellan and Endler, 1998). Descriptive leaf terminology below follows Ash et al. (1999).

Two variables, shape factor and feret-diameter ratio, express the proportional length of leaf margin that is available for interaction with the atmosphere. Shape factor is an area:perimeter ratio that is standardized to a circle; a circle has a shape factor of one, whereas a straight line has a shape factor of zero. Thus, a toothy, dissected leaf will have a relatively low shape factor. Feret-diameter ratio also is a comparison of a shape to a circle. Feret diameter is the theoretical diameter of an object if its area were contained in a circle, and the major axis of an object is the longest straight line that connects any two points. For example, the feret-diameter ratio of a circle is one, whereas the measurement approaches zero for objects approaching a straight line. Like shape factor, feret-diameter ratio can be computed automatically, without any decisions needed concerning, for example, which leaf features are teeth or lobes. Major axis length correlates well with the traditional measurement of leaf length (Ash et al., 1999), which must be measured manually ($r = 0.99$ for both ANF and York), but for rare leaves with greater width than length, the major axis is a dimension most similar to conventional width, not length. Colder climates should be associated with a greater proportion of margin exposed to the atmosphere and thus low values for shape factor. The same may hold for feret-diameter ratio, but low values also can result for this variable when leaves have a high ratio of length to width. Because length:width ratio is positively correlated with temperature (Wolfe, 1993), feret-diameter ratio may be affected both positively and negatively by temperature.

The remaining variables involve measurement of teeth. Wolfe (1993) introduced five discrete characters to describe teeth, in addition to presence or absence. Although some of

these are related to the density of teeth on the blade, none involves tooth size. To the extent that leaf teeth are adaptive edge features associated with water loss, gas exchange, and carbon fixation, at least for young leaves (e.g., Canny, 1990; Baker-Brosh and Peet, 1997), greater tooth size should enhance these effects by creating stronger edge gradients. Therefore, colder climates generally should be associated with larger teeth. One variable, tooth-area ratio, refers to the proportion of a leaf's area that is occupied by teeth and requires manual selection of teeth, as discussed below (the ratio of tooth area to leaf perimeter yielded unpromising results and is not shown). Tooth count is simply the total number of teeth on a leaf blade and is counted manually. Leaves with many teeth present relatively more leaf edge to the atmosphere, therefore, colder climates should correlate with higher tooth counts.

Tooth count was standardized by dividing it by leaf perimeter (standardized tooth count). Because teeth contribute perimeter to the blade, the numerator and denominator of the standardized tooth counts covary. The effect of the covariance is to reduce the sensitivity of this variable to toothiness, and thus potentially to climate, with the principal effect being on leaves with many large teeth. Alternative standardizations were not attractive. Standardization to area yielded skewed results (not shown). Software dilation filters were capable of smoothing the margin and preserving overall shape, but they inflated leaf size unpredictably in the process. Therefore, the standardization to perimeter was retained for ease of use and repeatability.

IMAGE PROCESSING AND LEAF MEASUREMENT

Specimens and centimeter scales were photographed on white backgrounds using a Nikon Coolpix 990 digital camera, 2048 × 1536 pixels resolution. Wavy or curled leaves were pressed flat under glass or small weights to minimize image distortion. Images were prepared in Adobe® Photoshop® 6.0 (Adobe Systems Inc., San Jose, CA). The petiole was removed from the image, using a straight line cut defined by the two points of intersection of the petiole and leaf lamina (Fig. 1). If a minute portion of the leaf margin was damaged, the margin was reconstructed as a straight line. Error produced by this approximation procedure was assumed to be negligible.

Digital size calibrations and measurements were made using Sigma Scan Pro® 5.0 (SPSS Science, Chicago, IL). Images were calibrated using a 2-point rescaling routine native to the software. Sigma Scan Pro® operates by measuring overlays created from images that it detects. To create a first overlay, the application's fill tool was used to select all contiguous pixels within an automatically detected threshold range. An additional overlay filter, “fill holes,” was used, even when there were no apparent holes, because even missed pixels that are not visible to the naked eye at low zoom can have a significant effect on measurements. The output measurements used here were area, perimeter, shape factor, feret diameter, major axis length, and minor axis length. Sigma Scan Pro®'s standard algorithm for estimating original perimeter was used, which reduces the pixelated perimeter into vertical, horizontal, and diagonal components. The accuracy of this procedure increases with increasing image size and is assumed to be adequately high for the images studied.

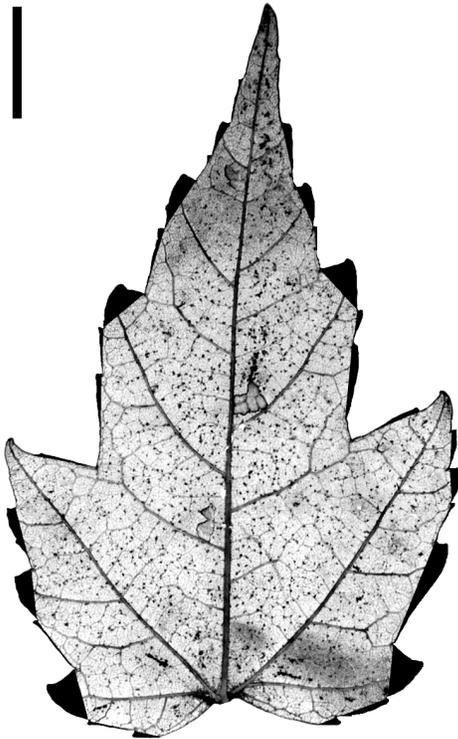


FIGURE 1—Example of image preparation and tooth selection. *Acer rubrum* (red maple) leaf with three primary veins, tooth selection shown in black. The petiole is removed, no selection crosses a primary vein, and the most basal teeth are selected along the same line as the superjacent teeth (see text for details). Scale bar = 1 cm.

In order to measure tooth area, separate image files, each comprised of every tooth on a leaf, were made for each toothed specimen; the procedure for whole leaves outlined above was repeated, but the only applicable measurement was area. Untoothed leaves were simply assigned a tooth area of zero. No existing software can discriminate teeth from leaf blade with sufficient reliability, so tooth selection was manual. This stage was therefore the most labor intensive and the most likely to introduce human error. In addition, the precise definition of a tooth, critical for reproducibility, occasionally is ambiguous for certain features of leaf margins. Accordingly, great effort was directed towards the reproducible and accurate selection of teeth.

Within Adobe® Photoshop®, teeth were selected along a straight line from sinus to sinus (Fig. 1). For unlobed and palmately lobed leaves, a tooth was considered to be any vascularized extension of the margin (Wilf, 1997) such that the straight-line segment between the sinuses of the extension did not cross a primary vein as defined by Ash et al. (1999). This definition allowed for an objective and repeatable distinction, applicable to the great majority of specimens, between an extension that is a tooth and one that is a lobe. Experience justified two technical modifications to the rule above, and more modifications may be necessary in the future. First, the lobes of bilobed and pinnately lobed leaves (Ash et al., 1999) were not selected, even if they did not contain a primary vein. Second, some pinnate leaves have both lobes and teeth following a similar pattern along the margin (e.g., many *Quercus* species).

In these cases, a majority-rule decision was made as to whether the majority of the similar extensions were teeth or lobes, according to whether their sinuses reached less or more, respectively, than one quarter of the distance to the midvein (Ash et al., 1999). If teeth were the majority, then every extension following the same pattern as these teeth was considered a tooth, and likewise for lobes. An additional technical note is that teeth with no basal sinus, such as the most basal teeth on a blade or lobe, were selected from the apical sinus along the same line as that used to select the superjacent tooth (Fig. 1).

Errors in measurement are estimated as less than 1 mm². Summary data are shown in Appendix 2. Measurement and collection data for individual specimens are available at the SEPM Data Archive (<http://www.ngdc.noaa.gov/mgg/sepm/archive/>). All raw and processed images are available, either on hard media directly from P.W., or from servers linked from his home page, <http://www.geosc.psu.edu/~pwilf> (as accessed 2–10–03).

RESULTS

Shape factor and feret-diameter ratio displayed normal results without transformation (Fig. 2A, B). However, in order to better approximate normality for tooth-area ratio, tooth count, and standardized tooth count, untoothed species were removed. For tooth-area ratio, a natural logarithmic transformation also was applied (Fig. 2C). The ratio of the logs was tried as an alternative transformation (not shown), but this produced a less significant result. For tooth count and standardized tooth count, a marginal increase in normality was obtained through logarithmic transformation, but results are plotted without transformation for the sake of a more intuitive presentation (Fig. 2D, E). Both of the tooth-count variables exhibited significant outliers, which are retained here for illustrative purposes.

Unidirectional trends in the means and medians were found that matched expectations for four variables (Fig. 2); feret-diameter ratio showed no trend (Fig. 2B). Shape factor increased with increasing MAT, whereas tooth-area ratio, tooth count, and standardized tooth count decreased.

Analysis of variance (ANOVA) tests for all pairs of sites are shown in Table 2. Statistically significant ANOVA results are not required for a variable to be useful for paleoclimatic estimation. However, the variables with significant ANOVA results are currently the most promising because they show the potential to discriminate sites.

Shape factor had significantly different means between all pairs of sites and was the only variable that discriminated the ANF and York samples, passing the second-order test (Table 2). Of the other variables, tooth-area ratio had the most significant ANOVA results because it discriminated both temperate sites from BCI. Thus, shape factor discriminated both large and small differences in temperature, but no tooth variable could discriminate the Pennsylvania sites. However, the statistical power of the three tooth variables is limited, in comparison to shape factor, by the removal of untoothed species and consequent loss of degrees of freedom (Table 2).

Feret-diameter ratio discriminated York from BCI (Table 2), and BCI showed a lower mean feret-diameter ratio than both ANF and York. The low mean of feret-diameter ratio for BCI, in contrast to the high mean of shape factor,

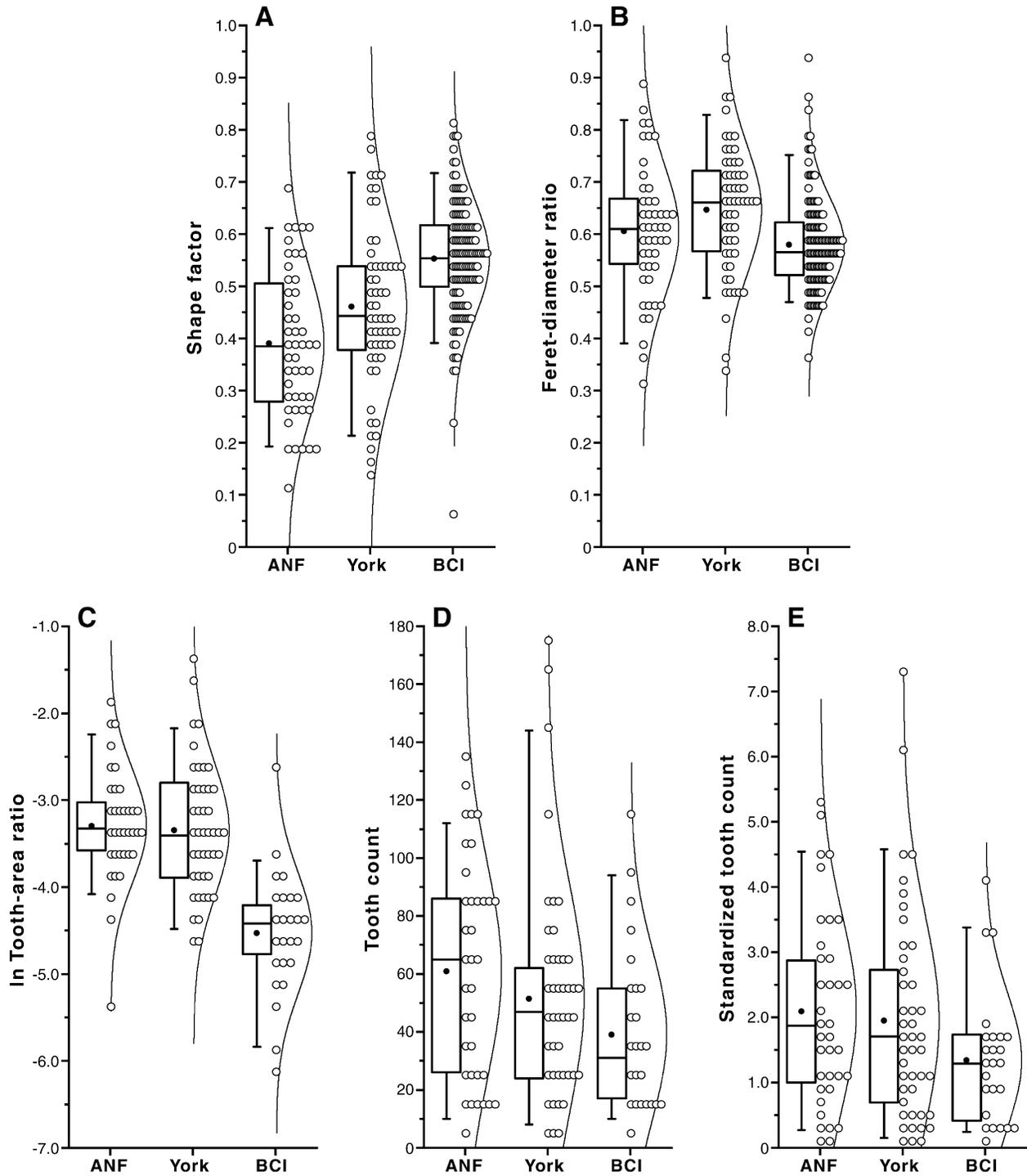


FIGURE 2—Box plots showing the distributions of leaf-shape variables (Table 1) among the three test floras (see text), which are arranged left-to-right within each plot in order of increasing MAT. Each species in each sample is represented by one datapoint, which shows the measurements of a single leaf. Boxes show the 25th to 75th percentile values with median bars and means as large dots. Error bars show the 5th and 95th percentile values. Individual data points are shown in binned form with accompanying normal curves for comparison. All primary measurements were made in cm or cm². Supporting data are available in Appendix 2 and from the SEPM Data Archive (www.ngdc.noaa.gov/mgg/sepm/archive). (A–B) Results for variables measured for all species in a sample. (C–E) Results for variables measured only for the toothed species in a sample.

TABLE 2—ANOVA results for each pair of sites.

Variable	Species	ANF vs. BCI	York vs. BCI	ANF vs. York
Shape factor	all	$F_{1,180} = 65.1, p < 10^{-10}$	$F_{1,189} = 21.5, p < 10^{-5}$	$F_{1,101} = 5.94, p = 0.017$
Feret-diameter ratio	all	$F_{1,180} = 2.32, p = 0.130$ ns	$F_{1,189} = 17.9, p < 10^{-4}$	$F_{1,101} = 2.86, p = 0.094$ ns
ln (Tooth-area ratio)	toothed	$F_{1,59} = 48.9, p < 10^{-8}$	$F_{1,67} = 41.2, p < 10^{-4}$	$F_{1,78} = 0.086, p = 0.770$ ns
Tooth count	toothed	$F_{1,59} = 6.08, p = 0.017$	$F_{1,67} = 1.96, p = 0.167$ ns	$F_{1,78} = 1.24, p = 0.270$ ns
ln (Tooth count)	toothed	$F_{1,59} = 4.42, p = 0.040$	$F_{1,67} = 1.71, p = 0.196$ ns	$F_{1,78} = 0.970, p = 0.328$ ns
Standardized tooth count	toothed	$F_{1,59} = 4.86, p = 0.031$	$F_{1,67} = 2.76, p = 0.101$ ns	$F_{1,78} = 0.174, p = 0.678$ ns
ln (Standardized tooth count)	toothed	$F_{1,59} = 3.00, p = 0.088$ ns	$F_{1,67} = 1.18, p = 0.281$ ns	$F_{1,78} = 0.465, p = 0.497$ ns

Notes: See Table 1 and text for variable definitions. ns = not significant ($p \geq 0.05$).

shows that length:width ratio was more important than leaf circularity in affecting feret-diameter ratio. Mirroring the pattern for feret-diameter ratio (Fig. 2B), the average length:width ratios for the three samples are highest for BCI, intermediate for ANF, which includes five narrow-leaved *Salix* species, and lowest for York, with two *Salix* species (Wilf, 1997). For all species in this study, feret-diameter ratio showed a strong negative correlation with the ratio of major to minor axis length ($r = -0.88$), which is an approximation of length:width ratio, but a low correlation with shape factor ($r = 0.38$). The ratio of major to minor axis length resulted in trends that were nearly the same among sites as feret-diameter ratio (but less significant; raw data in SEPM Data Archive, <http://www.ngdc.noaa.gov/mgg/sepm/archive>).

Although further testing is needed, the results suggest that warmer climates may be associated with species bearing more circular leaves and with smaller and fewer teeth than those found in temperate climates. Feret-diameter ratio should be tested further, given the ease with which it is measured and the normality of the results (Fig. 2).

DISCUSSION

Given the promising preliminary results, the potential of digital variables for paleoclimate estimation is considered here. The variables might be most useful in multivariate combination with each other and/or with traditional but reproducible variables such as leaf-margin percentage. In this way, for example, variables scored for only toothed species (e.g., Fig. 2C–E) could be combined with others measured for all species in a sample.

Shape factor is the variable tested that appears to carry the greatest climatic signal. Adding to the appeal of shape factor is the rapidity and reproducibility of measurement: no identification or selection of teeth is required, and the measurements are independent of scale, eliminating human error in the calibration process. In addition, untoothed species can be included without sacrificing normality, increasing statistical power (Table 2). Unfortunately for application to fossils, the leaf margin must be nearly intact, or it must be possible to reconstruct the leaf outline with reasonable precision, in order to make accurate measurements of shape factor. Experimentation with randomly cut leaf images showed that the discriminatory power of shape factor decreases sharply as outlines are altered. Even in fossil floras with excellent preservation, for which leaf shapes have been reconstructed wherever possible (Johnson and

Ellis, 2002), a significant proportion of species cannot be fully restored. However, further analyses may reveal shape factor to be climatically useful when applied to the best-preserved fraction of fossil floras, so long as shape does not bias which are the best-preserved species. Standard techniques for good collecting, such as intensive quarrying and careful tracking and gluing of leaf pieces, can improve greatly the proportion of species represented by complete specimens. These methods have proven their value repeatedly in other paleobotanical contexts (e.g., Johnson, 2002), but they are not always practiced.

Tooth-area ratio and tooth count variables also appear robust and should be applied more easily to fossils than variables that require a complete leaf outline. It is likely that tooth-area ratio can be approximated in a number of ways. One possible technique would measure the area of the preserved teeth compared to the area of the corresponding portion of the blade between the teeth and the primary vein (this area presumably would be circumscribed parallel to the secondary veins, not perpendicular to the midvein). Although absolute tooth count may be difficult to derive from some incomplete specimens, standardized tooth count can be derived easily from the preserved portion of the leaf margin.

Digital leaf physiognomy may have applications to ecological studies of living plants. For example, toothiness, thought to be associated with deciduousness and short leaf lifespan, can be measured continuously using most of the variables presented here (i.e., a toothy leaf has low shape factor, high tooth-area ratio, and high tooth count). A testable hypothesis is that toothiness correlates negatively with leaf lifespan, thickness, density, and herbivore resistance and positively with nitrogen content, based on the established correlations of leaf lifespan with the other variables (Coley et al., 1985; Ackerly and Reich, 1999; Reich et al., 1999). Correlations of toothiness with herbivory resistance can be examined with fossils using existing methods for quantifying insect herbivory on fossil leaves (Wilf et al., 2001), potentially improving the paleoecological characterization of ancient plant species.

CONCLUSIONS

The results presented here show the potential of continuous variables that measure leaf physiognomy both for paleoclimatic reconstruction and for ecological studies of living and fossil plants. Warmer climates may be associated with fewer and smaller teeth per toothed species than

colder climates and leaf shapes that are relatively circular, in addition to having fewer toothed species as previously known. The preliminary data justify closer examination of digital leaf physiognomy at a number of sites sufficient to allow the development and testing of univariate and multivariate statistical models for estimation of mean annual temperature and other climatic variables. The models also can use conventional variables, such as the percentage of untoothed species, so long as these are reproducible. Another phase of development and testing, using manipulated modern leaves, will be required to assess applications to incomplete fossil leaves. Whenever possible, species in both modern and fossil collections that are to be used for climate analysis should be represented by specimens with intact leaf outlines.

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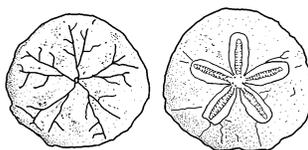
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APPENDIX 1

Within-species variance.

In order to obtain a preliminary evaluation of the variation within (versus among) species for some of the variables measured, twenty leaves (or leaflets) from single trees of each of two species found in the Pennsylvania samples were collected on the University of Michigan campus. These were chosen to represent nearly circular shape (*Robinia pseudo-acacia*) and highly dissected shape (*Quercus rubra*). For *R. pseudo-acacia*, the standard deviations of shape factor and feret-diameter ratio were 0.048 and 0.044, respectively, and corresponding values were even lower for *Q. rubra* (0.039 and 0.029). These results were considerably less than the standard deviations among species for these variables (Appendix 2). Although more testing is in order, these comparisons suggest that low numbers of replicates, or perhaps no replicates, might be adequate for paleoclimate estimation from digital leaf physiognomy. If so, this is good news for paleobotanists, who commonly work with species represented by a single specimen.

APPENDIX 2

Summary data.

Variable	ANF		York		BCI	
	Toothed	All	Toothed	All	Toothed	All
<i>N</i>	36	47	44	56	25	135
Shape factor						
mean	0.356	0.390	0.415	0.461	0.554	0.553
median	0.345	0.385	0.414	0.440	0.565	0.554
range	0.424	0.577	0.534	0.635	0.423	0.770
σ	0.115	0.142	0.122	0.153	0.101	0.110
Feret-diameter ratio						
mean	0.618	0.605	0.647	0.646	0.586	0.579
median	0.628	0.610	0.657	0.657	0.569	0.565
range	0.555	0.555	0.525	0.607	0.284	0.575
σ	0.136	0.126	0.119	0.121	0.076	0.089
ln (Tooth-area ratio)						
mean	-3.298	n.a.	-3.345	n.a.	-4.530	n.a.
median	-3.334	n.a.	-3.425	n.a.	-4.416	n.a.
range	3.489	n.a.	3.226	n.a.	3.486	n.a.
σ	0.656	n.a.	0.755	n.a.	0.706	n.a.
Tooth count						
mean	60.972	46.702	51.500	40.464	39.120	7.244
median	62.5	39.0	46.5	33.0	31.0	0.0
range	128	132	166	170	107	113
σ	37.195	41.636	38.506	40.173	28.828	19.532
Standardized tooth count						
mean	2.095	1.604	1.948	1.530	1.344	0.249
median	1.801	1.137	1.611	1.053	1.290	0.000
range	5.283	5.374	7.084	7.200	3.962	4.109
σ	1.471	1.565	1.640	1.659	1.025	0.680

Notes: All primary measurements in cm or cm²; n.a. = not applicable because the measurement is not defined for all species. Numbers of toothed species listed above differ from Wilf (1997) because in that publication, facultatively toothed species were scored as half-toothed, following Wolfe (1993). Here, a single leaf was chosen to represent the most common margin state of the species in the sample. All raw data are available from the SEPM Data Archive (<http://www.ngdc.noaa.gov/mgg/sepm/archive>). Definitions of variables (see Table 1 and text): shape factor = 4π (leaf area/leaf perimeter²); feret-diameter ratio = feret diameter/major axis length; tooth-area ratio = area of all teeth/leaf area; tooth count = total number of teeth; standardized tooth count = tooth count/leaf perimeter.