The Relative Success of Some Methods for Measuring and Describing the Shape of Complex Objects

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Abstract.—We examined and compared several morphometric methods for describing complex shapes. We chose the leaves of maples (*Acer*) and other tree species because they can all be visually discriminated from each other solely by leaf shape. We digitized the leaf outlines with a video camera and then examined the outlines with several morphometric methods to determine the extent to which margin details could be quantified and compared. Elliptic Fourier analysis provides complete and accurate descriptions of complex outlines and can be used to reconstruct images accurately. We compared several metrics that summarize overall shape complexity. A new measure of margin roughness is useful for quantifying and comparing margin detail independently of overall shape. Fractal dimension is highly correlated with the ratio of perimeter to area (dissection index) and reveals little additional information about shape. In combination, the summaries of shape complexity provide good discrimination of groups. We used canonical discriminant analysis to compare methods for outlines to traditional morphometric analysis of measurements taken between landmark points. Groups were discriminated from each other more clearly with outline methods than with landmark-based analyses. [*Acer*, Fourier analysis, fractal dimension, landmark analysis, leaf shape, margin roughness, *Liquidambar*, morphometrics, *Quercus*.]

Morphology remains one of the richest and most reliable sources of information about systematic, evolutionary, and ecological relationships. Several types of methods have been devised for the quantitative analysis of shape (Rohlf, 1990b; Rohlf and Bookstein, 1990). The majority of effort has been directed toward techniques using information about the locations of several points in an image, or landmarks (Blackith and Reyment, 1971; Cheverud and Richtsmeier, 1986; Bookstein, 1989; Marcus, 1990; Rohlf, 1990b; Rohlf and Bookstein, 1990; Rohlf and Slice, 1990; Marcus et al., 1996). Landmark methods have been extended to three dimensions (Cheverud et al., 1983; Bookstein, 1990), and allow landmark points to be internal as well as on the edge of a structure. Although many aspects of shape can be characterized by a small number of landmark points, there may be additional information about form between the landmarks that is not incorporated into landmark analyses. Morphometric methods that use the entire outline of objects are newer and

less well developed. Several of the methods for outlines are appropriate only for smooth curves or simple closed curves without concave segments, and are often applied only when there are no homologous landmarks (Rohlf, 1990b). However, the utility of outline methods is not restricted to forms in which homologous landmarks cannot be identified.

Outline methods utilize various methods of curve fitting that are sensitive to curvature (Rohlf, 1990a, 1990b). Fourier analysis can be applied both to simple ovals such as skulls (Lestrel and Roche, 1986), insect wings (Rohlf and Archie, 1984), and mussel shells (Ferson et al., 1985), and to the more complicated and irregular outlines of leaves (Kincaid and Schneider, 1983; McLellan, 1993; Premoli, 1996) and insect genitalia (Liu et al., 1996). Eigenshape analysis (Lohmann, 1983), mathematically related to Fourier analysis (Rohlf, 1986), can be applied to closed contours of simple shapes (Kores et al., 1993). Moment invariants are another method for analysis of curves that has been applied to leaf shapes

(White et al., 1988; Lonn and Prentice, 1990).

Many biological structures, such as sutures between bones and the teeth on leaves, vary in fine detail, but the quantitative characterization of the smallest scales of resolution has not been well developed. Fourier analysis can describe small aspects of shape (Kincaid and Schneider, 1983), but has not been used extensively. Chain code descriptors, which assess the change in direction in an outline at the level of individual pixels (Pavlidis, 1978), have been tested with leaf outlines and found to be poor at distinguishing taxa (White et al., 1988).

Leaf shape presents a more complex problem in morphometrics than skulls, jaws, and insect wings, as leaf shapes come in a wide variety of shapes, which vary in overall proportions of length and breadth, the extent of cutting between lobes, and in the size and shape of teeth along the margins. Few landmarks can be designated on leaves that are not lobed. Pseudolandmarks, or points on an outline that are designated geometrically, make the use of landmark-based methods feasible on images without homologous points (Dickinson et al., 1987; Kores et al., 1993). Hybrid methods using both landmarks to define parts of curves and analysis of specified parts of outlines have been developed as appropriate for application to some leaves (Ray, 1992), thereby maximizing the available information.

Many morphometric methods have been developed and characterized one at a time (e.g., Kincaid and Schneider, 1983; Dickinson et al., 1987; Bookstein, 1989; Rohlf and Slice, 1990; Slice, 1993); there have been few studies that made empirical comparisons of alternative approaches (White et al., 1988; McAlarney, 1995). There is now a wide choice of methods available, but it can be difficult to determine the relative merits of the various approaches for a specific application. Moreover, arguments in the literature have been contentious (e.g., Bookstein et al., 1982; Read and Lestrel, 1986; Bookstein, 1987; Lele and Richtsmeier, 1990; Crowe, 1994). Here we demonstrate empirically that many morphometric methods yield similar results in distinguishing the shapes of leaves from some well-known taxa.

MATERIALS AND METHODS

Collections and Image Acquisition

Ten leaves were collected from each of 10 trees in 12 species, consisting of 9 maple species, *Acer* (one with two populations), 2 oaks, *Quercus*, and sweetgum, *Liquidambar styraciflua* (Table 1). These species were chosen for comparison of methods for shape analysis because they can be distinguished from

TABLE 1. Species and sources of leaf collections.

Species	Locality	Source	Light
Acer ginnala Maxim.	Univ. of Toronto campus	cultivated	part sun
Acer palmatum Thunberg ex. Murray	Mount Pleasant Cemetery, Toronto	cultivated	part sun
Acer pensylvanicum L.	Brevard, Transylvania Co, NC	wild	closed forest
Acer platanoides L.	Univ. of Toronto campus	cultivated	full sun
Acer pseudoplatanus L.	Mount Pleasant Cemetery, Toronto	cultivated	part sun
Acer rubrum L. (Coastal)	Trenton, Jones Co., NC	wild	sparse forest
Acer rubrum L. (Montane)	Brevard, Transylvania Co., NC	wild	closed forest
Acer saccharum var. *floridanum* (Chapman) Desmarais	Chapel Hill, NC	wild	forest edge
Acer saccharinum L.	College Park, MD	wild	forest edge
Acer spicatum Lamarck	Randolph, NH	wild	closed forest
Liquidambar styraciflua L.	Trenton, Jones Co., NC	wild	sparse forest
Quercus alba L.	Chapel Hill, NC	wild	forest edge
Quercus palustris Muench.	College Park, MD	cultivated	forest edge

each other on the basis of leaf shape alone (Fig. 1). Leaves of these species provide a wide range of variation in several aspects of shape, with differences in the numbers of lobes, in the degree of lobing, in the shape of sinuses and apices, and in the size and number of teeth on the margins (Fig. 1).

Fully expanded leaves were collected from mature trees from within 2 m of the ground, with an effort to collect leaves within each population that had the same exposure to sunlight. Leaves were then pressed and dried before digitization.

Outlines were recorded using a video camera and the program MorphoSys (Meacham and Duncan, 1991) in the Botany Department of the Royal Ontario Museum, Canada. The height of the camera was adjusted for each group of leaves so that the frame was nearly filled by the extreme points on the leaf. The resolution of the video image was 512 × 480 pixels. MorphoSys records outlines as four-step chain codes, and allows manual designation of landmarks and the designation of measurements to be taken between points.

We used three categories of morphometric methods: Fourier analysis, several single-parameter metrics of shape (including fractal dimension, dissection index, margin roughness, perimeter/length ratio, and the number of points in the outline), and traditional morphometric multivariate analysis of linear measurements. The resolution of the various methods was compared as the ability to discriminate groups with canonical discriminant analysis. Except for the commercially available Fractal-D, the computer programs for calculating the descriptors were written in Turbo Pascal version 3.0, and are available from the authors upon request. Statistical analyses were performed with SAS (SAS

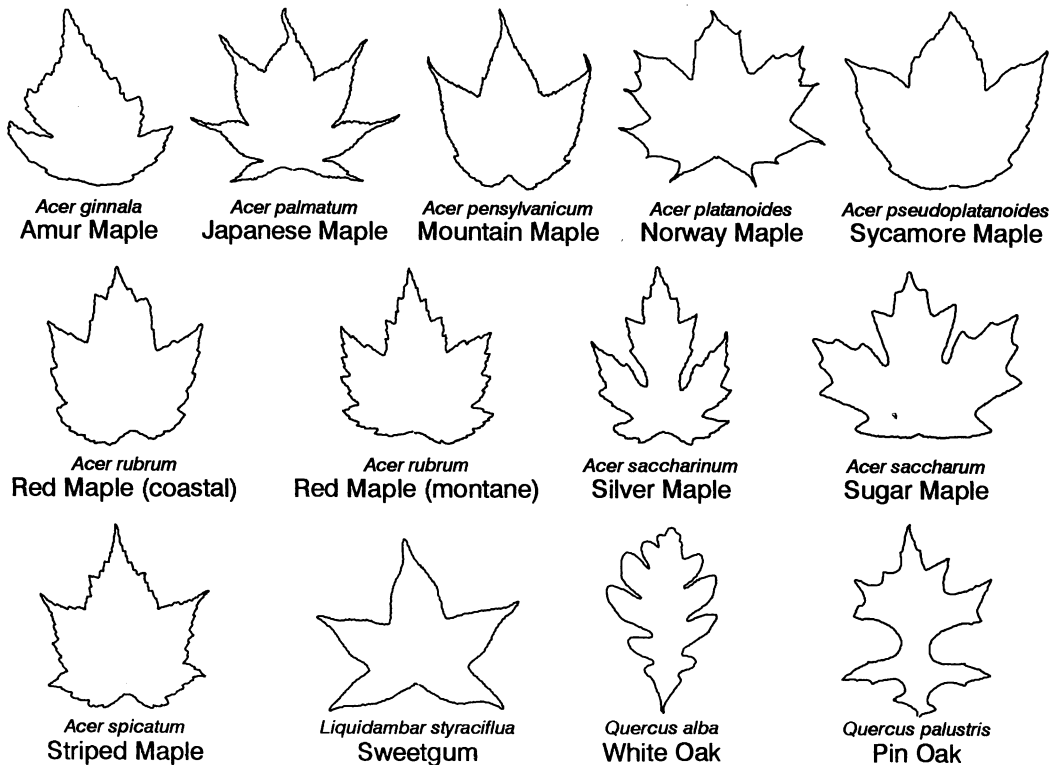|				
Acer ginnala **Amur Maple**	*Acer palmatum* **Japanese Maple**	*Acer pensylvanicum* **Mountain Maple**	*Acer platanoides* **Norway Maple**	*Acer pseudoplatanoides* **Sycamore Maple**
Acer rubrum **Red Maple (coastal)**	*Acer rubrum* **Red Maple (montane)**	*Acer saccharinum* **Silver Maple**	*Acer saccharum* **Sugar Maple**	
Acer spicatum **Striped Maple**	*Liquidambar styraciflua* **Sweetgum**	*Quercus alba* **White Oak**	*Quercus palustris* **Pin Oak**	

FIGURE 1. Outlines of representative leaves of the species examined in this study.

Institute, 1985) and NTSYS-PC (Rohlf, 1993).

Outline Methods: Fourier Analysis

Fourier analysis is a mathematical way of reducing complex curves into their component spatial frequencies. It provides a precise and accurate description of shape over a range of size scales (Kuhl and Giardina, 1982; Kincaid and Schneider, 1983). Fourier analysis therefore seems appropriate for the analysis of outlines such as leaf shapes in which there is a periodic repetition of parts, as well as variation in shape over a range of size scales. The coefficients of the lower order Fourier harmonics correspond to the overall shape, and the higher order harmonics correspond to smaller details of the outline (Kuhl and Giardina, 1982; Kincaid and Schneider, 1983).

We chose elliptic Fourier analysis (Kuhl and Giardina, 1982) because a previous comparison of Fourier methods (Rohlf and Archie, 1984) showed that it produced reasonable results with biological shapes, and, unlike some other Fourier methods for closed contours, it can cope with shapes that include many concave segments. We performed elliptic Fourier analysis on the digitized leaf outlines with a program written to utilize xy coordinates, rather than chain codes, starting at the first point in each image at the juncture of the petiole and lamina. First, chain code files were translated into xy coordinates. We used every fourth point in the outlines, due to computer memory limitations. Four coefficients are produced for each harmonic. We normalized the coefficients to the first harmonic to remove differences in size and orientation according to a method given by Kuhl and Giardina (1982). We calculated the power series of the normalized Fourier coefficients by squaring each coefficient, summing the two components for each x and each y, and then taking the square root of each sum, resulting in two coefficients for each harmonic, rather than four.

The asymmetry of images and other information about shape is reduced with the power series of the Fourier coefficients. However, some of that information may not be informative in making comparisons of shape, particularly in this data set. Simple images with clearly bilateral symmetry may be aligned to have the same major axis (Rohlf and Archie, 1984), but alignment is not so straightforward with leaves because the several lobes may occur at different angles from each other.

The elliptical Fourier coefficients for 64 harmonics were calculated for each leaf, and we used the means of each coefficient for 10 leaves from each of 130 trees in multivariate statistical analyses. During the analysis, the number of harmonics employed was varied in order to evaluate the number of harmonics necessary for discrimination of taxa, and was in some instances limited by the capacities of the programs. We used canonical discriminant analysis to compare the discrimination of groups based on elliptic Fourier coefficients that had been normalized only with the power series of the normalized coefficients. Power series of coefficients from 4, 8, 16, and 32 harmonics were also compared to evaluate the importance of the number of harmonics in discrimination of groups. Previous studies with Fourier analysis of shapes have shown that larger numbers of harmonics can provide greater resolution of groups (Kincaid and Schneider, 1983; Mou and Stoermer, 1992).

Principal component analysis is frequently used to evaluate Fourier coefficients when groups are not known a priori (Rohlf and Archie, 1984; McLellan, 1993; Liu et al., 1996). We employed principal component analysis (SAS Institute, 1985) based on correlation matrices of the coefficients to examine the extent to which groups were defined by this method, and subsequently used the values of the first two principal components as variables in comparisons with other metrics of leaf shape.

We reconstructed the leaf outlines

from raw Fourier coefficients using the same number of points as in the original image, to assess the aspects of shape that different numbers of Fourier harmonics describe. We estimated the goodness of fit between the original image and the reconstruction (referred to as the Fourier error, e_f) by summing the distances between each point of the original image and the closest point of an image reconstructed from Fourier coefficients for 1 to 64 harmonics, according to

$$e_f = \frac{1}{k} \sum_{i=1}^{k} \sqrt{(x_i - r_i)^2 + (y_i - s_i)^2}$$

where x_i and y_i are the ith x and y coordinates in the original image, r_i and s_i are the ith coordinates in the reconstructed image, and k is the number of points in the outline. The points in the reconstructed image (r_i, s_i) are those that have the shortest distance to the matching (x_i, y_i) points. The (r_i, s_i) points are found by measuring the Euclidean distance between each original outline point (x_i, y_i) and all points on the reconstructed outline (r_j, s_j) from $j = (I - 20)$ to $j = (I + 20)$, and using the point (j) with the least distance to the given (x_i, y_i). The smaller the e_f, the better the fit to the data, and the units of e_f are millimeters. We log-transformed e_f for parametric statistical tests. We analyzed the results of Fourier errors for 4, 8, 16, 32, and 64 harmonics in detail and examined correlations with other measures of outline complexity (see later discussion).

Single-Parameter Shape Descriptors

Several single-parameter descriptors were calculated for outline data for 100 leaves within each species. Each of them summarizes some aspect of shape into a single metric, making statistical comparisons straightforward. The disadvantage of one-parameter measures is that they do not provide unique descriptions of shape; they are not information preserving (Pavlidis, 1980). However, if each measure is sensitive to a different aspect of shape, then their use in combination

might facilitate discrimination of groups. To examine the ability of these metrics to discriminate among the species, discriminant analysis and canonical discriminant analysis were used on all of the single-number descriptors, as well as principal components 1 and 2 from the analysis of the power series of Fourier coefficients for 16 harmonics. The single-parameter measures we used were fractal dimension, dissection index, margin roughness, perimeter/length ratio, and the number of points in the outline.

The *fractal dimension* characterizes complex lines with values between one and two (Mandelbrot, 1982; Vlcek and Cheung, 1986; Slice, 1993) and can serve as a measure of complexity or scale of shape. It is based on the use of fractal geometry, which is characterized by self-similarity of structure or complexity of an image at all scales (Mandelbrot, 1982; Barnsley, 1988; Peitgen et al., 1992). It seems appropriate for leaves to apply fractal geometry, because the structure of fine scale details can resemble the larger scale aspects of leaf shape (Lindenmayer, 1977; McLellan and Dengler, 1995), thus showing self-similarity. Fractal dimension is not itself a measure of self-similarity, but should correspond to the degree of complexity of an image, based on the assumption of self-similarity.

We calculated fractal dimension using the program Fractal-D (Slice, 1993), provided by Dennis Slice. The program requires the designation of the sizes of measurements taken from outlines, and we used the file leaf2.stp provided with the program. We tested the options for standardization of the images and randomization of the starting point. Standardization of the size of the images resulted in slightly lower values for non-standardized images than for images standardized to the same size. Randomization of the starting point of the images made no difference to the values of fractal dimension, compared to using the same starting point for all images. Values reported here are for calculations based on standardized images, in which the

outline always started at the same point, at the juncture of the petiole and leaf blade, and measuring clockwise.

The *dissection index* is the ratio of an outline's perimeter to the square root of its area, standardized so that a circle has a value of 1.0 (Kincaid and Schneider, 1983; McLellan, 1993). This dimensionless number gives a simple measure of the degree to which an outline is more complex than a circle.

We devised a new measure called *margin roughness* to measure small changes in direction of an outline. Margin roughness is similar to chain code descriptors that examine the frequency of angles between adjacent points as an indication of fine-scale fluctuation of an outline (Pavlidis, 1978). The absolute values of angles between lines defined by sequential pairs of points, *P* (Fig. 2), are determined, summed, and averaged over all pairs of points, then corrected for the average that would have been found for a circle with the same number of points, according to

$$MR = \frac{1}{k} \sum_{i=1}^{i=k-2} \left| \arctan\left(\frac{\sqrt{1-A_i^2}}{A_i}\right) - 180 \right|$$
$$- \frac{360}{k}$$

where

$$A_i = \frac{(\overline{P_iP_{i+1}})^2 + (\overline{P_{i+1}P_{i+2}})^2 - (\overline{P_iP_{i+2}})^2}{2(\overline{P_iP_{i+1}})(\overline{P_{i+1}P_{i+2}})}$$

Video-digitized images consist of points in pixels that are adjacent to each other in an array of squares, and therefore they can be rougher than the actual outline. In addition, the roughness of a digitized line will depend on its direction
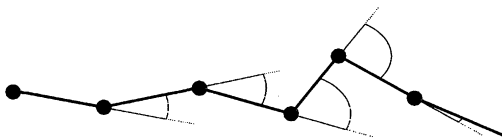

FIGURE 2. Measurement of margin roughness, determined by the sum of angles between lines defined by adjacent points.

relative to the rectangular digitizing grid. A vertical or horizontal line has no changes in angles, while a straight, diagonal line consists of 90° steps. As a compromise between the roughness of a video-digitized image and the resolution of margin detail, lines were defined by pairs of points separated by four intervening points on the outline.

We selected the distance between points at the tip of the major lobe and the largest sinus on one side of each leaf as a measure of length of part of the leaf (Fig. 3). The length of the perimeter of the outline between these two points was divided by the linear length to yield the *perimeter/length ratio*, and the values were log-transformed for statistical analysis. This is a measure of the shape complexity that incorporates information from part of the outline; low values correspond to smooth curves, and higher values should correspond to more complicated margins. An additional single parameter was the total number of points in each image. If each image fills the

FIGURE 3. Measurements taken for measure of ratio of perimeter to length for one part of the outline. The straight line is the linear distance between points at the tip of the major lobe and at the major sinus. The perimeter of the outline between these points was measured, and the ratio of the two was used.

video frame, then the number of points should correspond to the complexity of the shape.

Traditional Landmark Methods

We took linear measurements between 10 landmark points on each leaf at the tips of lobes, bases of sinuses, and the juncture of the petiole and lamina. Four sets of measurements were compared with each other. One included all 20 measurements, and three sets consisted of 9 measurements each: one in the direction of radial growth, one mostly orthogonal to the direction of growth, and a third in a pattern of a truss, where measurements were taken between adjacent points on the outline, and pairs separated by one other point (Strauss and Bookstein, 1982) (Fig. 4). Means of the two values of measurements taken from both sides of a leaf were used. Each of the four data sets was subjected to Burnaby's transformation (Rohlf and Bookstein, 1987) using

NTSYS-PC (Rohlf, 1993), in which principal component 1 is used to make an orthogonal projection of the data matrix to control for differences in size, so that comparisons of shape can be made independently of differences in size.

Comparisons of Methods

The single-number metrics were compared with analysis of variance between trees within species and between species. The three types of morphometric methods were compared using canonical discriminant analysis (CDA) and discriminant function analysis (DFA), following comparisons made by White et al. (1988). CDA is one type of ordination technique for characterizing predetermined groups of individuals. The between-group variance is maximized relative to that within groups, thus emphasizing those descriptors that best discriminate among samples. A plot of the sample positions on the first two canonical axes
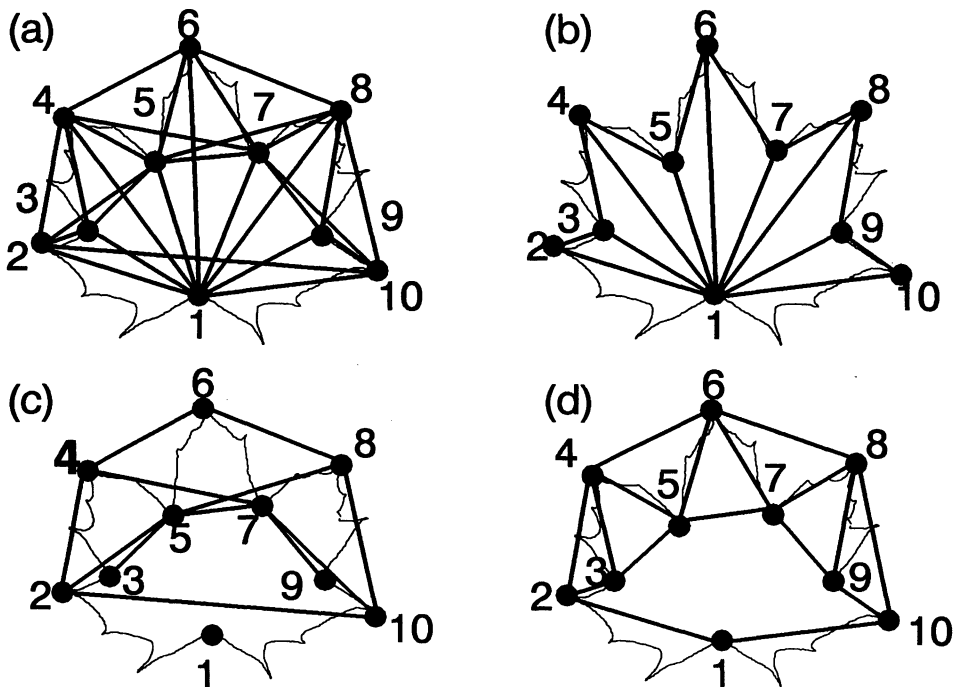

FIGURE 4. Linear measurements. (a) Full set of 20 measurements, (b) radial measurements, (c) measurements that are orthogonal to the radial direction of growth, and (d) truss pattern of measurements.

gives the best two-dimensional projection of the differences between groups. Wilks' lambda provides a test for the significance of differences between groups; small values correspond to highly significant differences. The percent misclassification found by discriminant analysis provides another indication of the resolution of the methods (White et al., 1988).

RESULTS

Fourier Analysis

We calculated normalized elliptic Fourier coefficients and the power series of normalized Fourier coefficients for each leaf, and then used mean values of coefficients for the 10 leaves from each tree in subsequent analyses. We compared the coefficients of the first 16 Fourier harmonics using canonical discriminant analysis and principal component analysis. The definition of groups with the first two canonical variates is somewhat different with the power series of normalized coefficients and with normalized coefficients alone (Fig. 5). With the power series, the two species of *Quercus* cluster together, and *Acer platanoides* forms a group distinct from other species, while these groups overlap others with the normalized coefficients. Though all values of Wilks' lambda are very small and highly significant, the values are lower for the power series than for normalized coefficients, and the power series produced greater discrimination among groups (Table 2). Even though the number of variables, or descriptors, is halved by taking the

power series, the discrimination of groups is improved. An increase in the number of harmonics ought to provide additional information for shape comparisons, because additional harmonics describe the shape more completely (Kuhl and Giardina, 1982; Kincaid and Schneider, 1983). Canonical discriminant analysis based on 4, 8, 16, and 32 harmonics of the power series of Fourier coefficients showed greater discrimination of groups with more harmonics (Table 2). Thirty-two harmonics resulted in 100% correct classification, and 16 harmonics provided 99% correct classification by discriminant analysis.

The most common application of Fourier analysis for finding groups of shapes involves subjecting the coefficients to principal component analysis of the coefficients (Rohlf and Archie, 1984). A few of the known groups, *A. palmatum*, *A. saccharinum*, and *L. styraciflua*, are distinct from each other in the plot of the first two principal components (Fig. 6) based on the power series of Fourier coefficients for 16 harmonics. Although principal component 1 (PC1) accounts for 40.8% of the total variation, it appears to contribute little to the discrimination among groups.

The eigenvectors and the reconstructions of images from Fourier coefficients together provide a way of interpreting the aspects of shape to which multivariate analysis of Fourier coefficients is sensitive. The eigenvectors of the first two principal components for both the x and y components of the Fourier coefficients demonstrate the relative contributions of the harmonics to these principal

TABLE 2. Comparison of Fourier descriptors of shape.

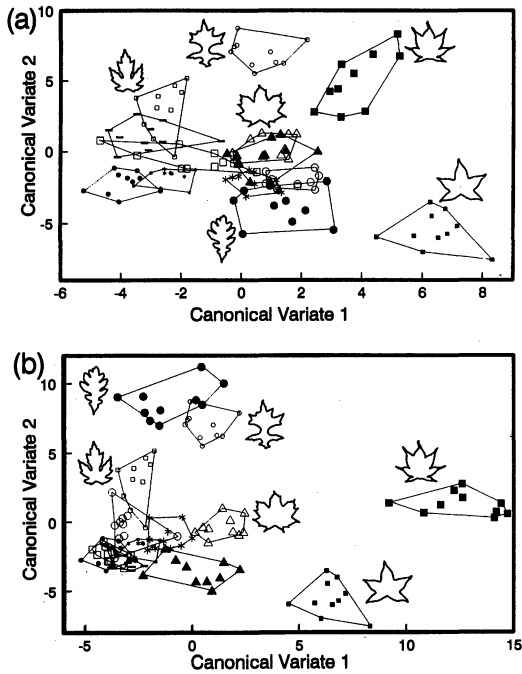|	Fourier coefficients				
	Normalized		Power series of normalized		
Number of harmonics	16	4	8	16	32
Number of descriptors	60	6	14	30	62
Number of significant axes	6	4	5	5	5
Wilks' lambda	0.00000020	0.00067079	0.00000154	0.00000001	0.00000000
Correct assignment (%)	96	83	95	99	100

FIGURE 5. (a) Canonical variates. Large open circles = *Acer ginnala*; large solid squares = *Acer palmatum*; dashes = *Acer pensylvanicum*; open triangles = *Acer platanoides*; solid triangles = *Acer pseudoplatanus*; small solid circles = *Acer rubrum* (montane); large open squares = *Acer rubrum* (coastal); small open squares = *Acer saccharinum*; large stars = *Acer saccharum* var. *floridanum*; small stars = *Acer spicatum*; small solid squares = *Liquidambar styraciflua*; large solid circles = *Quercus alba*; small open circles = *Quercus palustris*. (a) Normalized elliptic Fourier coefficients. Canonical variate 1 accounts for 30% of the variation, and canonical variate 2 accounts for 23%. (b) Power series of normalized elliptic Fourier coefficients. Canonical variate 1 accounts for 35% of the variation, and canonical variate 2 accounts for 23%.

components (Fig. 7). PC1 is largely accounted for by harmonics 5 and higher for both the *x* and *y* components, and PC2 has a large contribution from the first and second harmonics, and lesser contributions from harmonics 8, 9, and 10 for the *x* component and 12, 13, and 14 for the *y* component.

Reconstructions of images from different numbers of harmonics show that a small number of harmonics correspond to the major lobes of the leaves (Fig. 8). *Acer saccharum* and *A. saccharinum* both
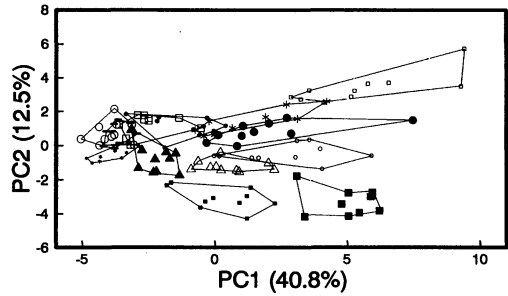

FIGURE 6. Plot of the first two principal components (PC) based on the power series of normalized elliptic Fourier coefficients. Means of the Fourier coefficients of 10 leaves in each tree were used in the principal component analysis. For symbol definitions, see Fig. 5.

have leaves with five lobes. In *A. saccharum*, there are a median lobe and two secondary lobes of nearly the same size, while in *A. saccharinum* the median lobe is larger than the flanking secondary lobes. In both species, the smallest pair of
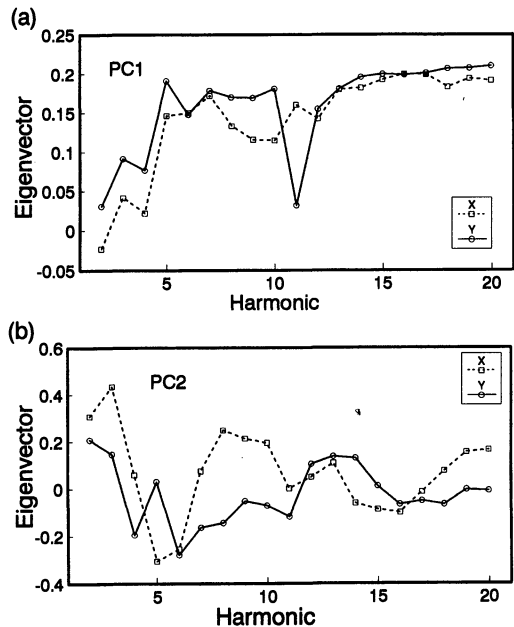

FIGURE 7. Eigenvectors of the first 20 Fourier harmonics based on the power series of normalized elliptic Fourier coefficients. X and Y are the *x* and *y* coefficients, respectively, of each Fourier harmonic. (a) Principal component 1. (b) Principal component 2.

4   8   16   32   64   Original
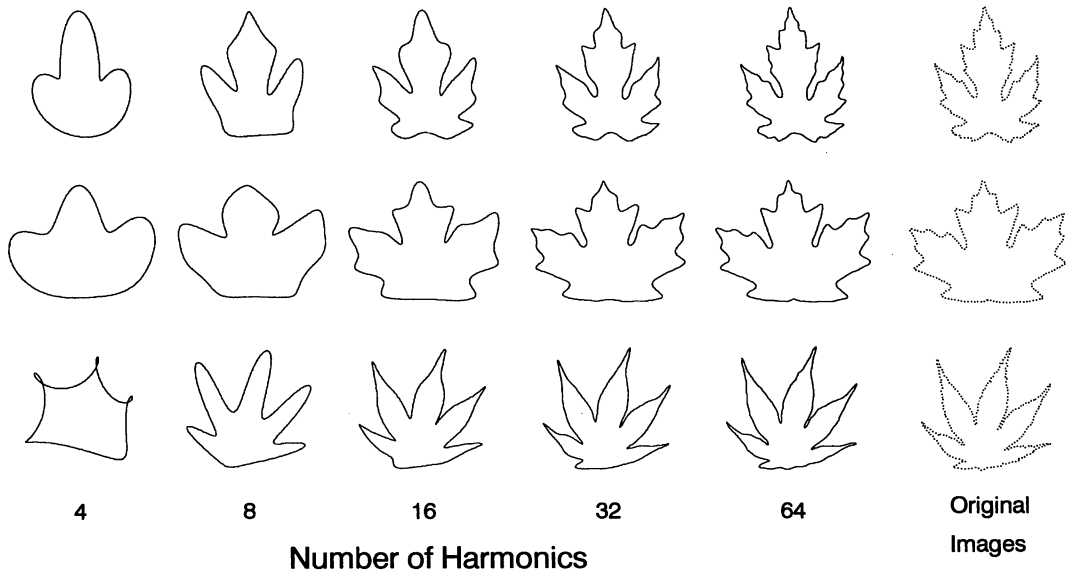Images

Number of Harmonics

FIGURE 8. Reconstructions of images from different numbers of Fourier harmonics. (Top) *Acer saccharinum*, (middle) *Acer saccharum*, and (bottom) *Acer palmatum*.

lobes occurs near the base of the leaf. Reconstructions of the images based on four harmonics for these two species show the difference between them in the relative sizes of the median and secondary lobes. Leaves of *A. palmatum* usually have seven lobes, including a median lobe and a pair of secondary lobes of nearly the same size that flank it. Another two (paired) smaller lobes are located distal to the secondary lobes, and there is a much smaller pair at the base of the leaf. Reconstructions based on four harmonics of the image of a leaf of *A. palmatum* do not resemble the leaf closely; there are three looped points and two square corners. However, this shape is distinctly different from those of *A. saccharum* and *A. saccharinum*. With eight harmonics, each of the reconstructions of these species resembles the major pattern of lobes. With additional harmonics the reconstructions are closer to the original images. However, sharp angles at sinuses of *A. saccharinum* are not reconstructed with complete accuracy even with 64 harmonics.

We estimated the error of fit (e_f) between original images and reconstruc-

tions from Fourier coefficients based on harmonics 1 to 64. To make reconstructions that fit the original images equally well, more complicated shapes should require a greater number of harmonics than simpler shapes. Therefore, a measure of the error of fit between the two might provide an indication of the general complexity of the shape. The plots of e_f for each species are similar in shape, with a rapid decline from one up to about 10 or 15 harmonics, and a slow decrease after about 20 harmonics (Fig. 9). Those species with high values of e_f for a small number of harmonics also have high values for many harmonics. There is little additional improvement to the fit between reconstructions and original images with more than about 20 harmonics, as can be seen in the reconstructions based on 32 and 64 harmonics (Fig. 8).

Single-Parameter Shape Descriptors

The single-parameter shape descriptors are similar to each other in their distributions (Fig. 10). There are many cases in which mean values differ significantly
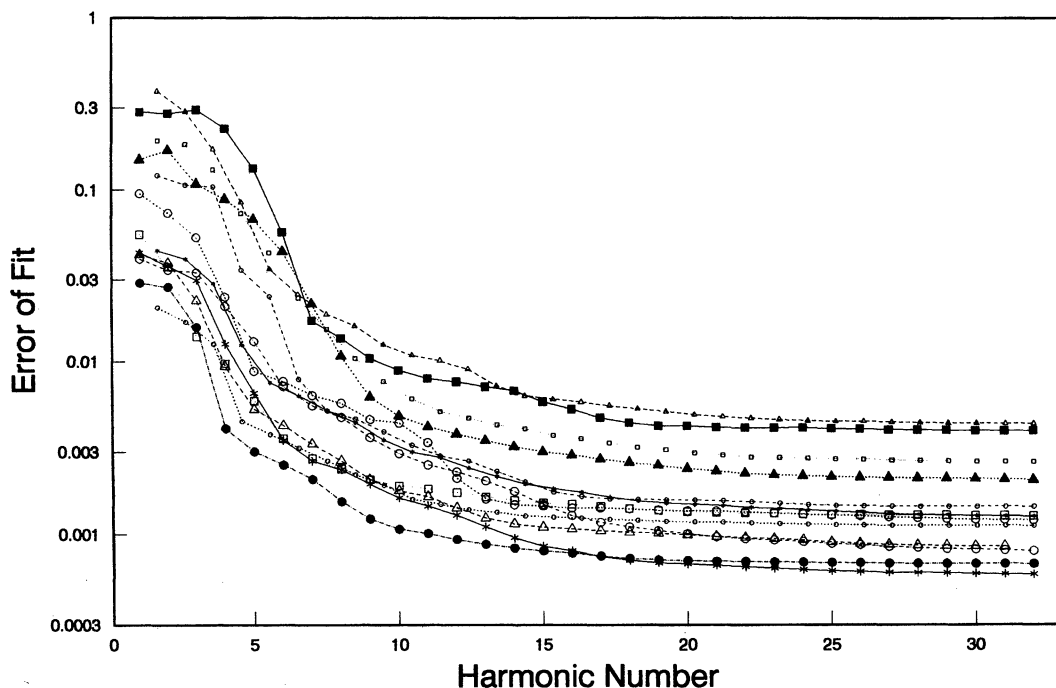
FIGURE 9. Error of fit between original digitized image and reconstruction from Fourier coefficients for 1 to 32 harmonics. Points represent means of the Fourier fit for each of 10 trees in a species. For symbol definitions, see Fig. 5.

among species, but the entire ranges of values in the samples of 100 leaves for each species overlap among several species. In defining characters in systematics, the range of variation within each taxon is important (Stevens, 1991), just as significant differences among means are important.

Values of fractal dimension ranged from 1.066 to 1.401. The lowest values were found in *A. spicatum*, *A. pensylvanicum*, and *A. pseudoplatanus*, which are the least incised and have the fewest lobes of the leaves considered here. Highest values were found in *A. palmatum*, with a large number of lobes, and *A. saccharinum*, which is deeply incised. The values of fractal dimension produced some nonoverlapping groups, but the broad range within each species, particularly the two *Quercus* and *Liquidambar*, precludes clear discrimination of species or groups of species using the entire range of values (Fig. 10).

The values of the dissection index ranged from 4.28 to 11.99 (Fig. 10). The lowest values were found in *A. pseudoplatanus* and the coastal population of *A. rubrum*, while the highest values were found in *A. palmatum* and *A. saccharinum*, as with fractal dimension.

Margin roughness ranged from 12.12 to 39.62 degrees (Fig. 10). The lowest values were found for the two *Quercus* species, *A. saccharum*, and *A. platanoides*, all of which have smooth margins. The highest values were found for the montane population of *A. rubrum*, *A. spicatum*, and *A. pensylvanicum*, all of which have teeth of moderate size (Fig. 1). The latter two, which can be visually distinguished from each other by the size and number of teeth, are not separable by values of margin roughness. Although *L. styraciflua* has numerous small teeth on the margins, margin roughness is fairly low with a mean of 19.81 degrees. These small teeth are below the resolution of
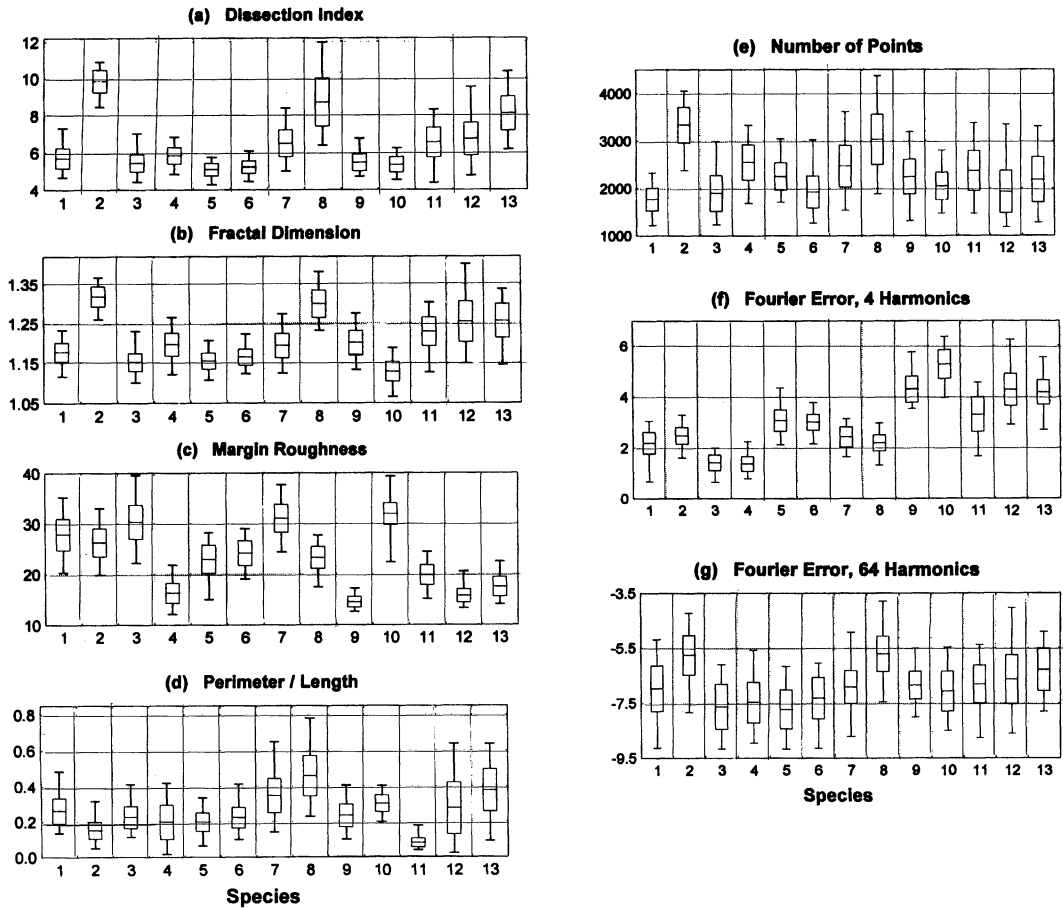
FIGURE 10. Box plots showing mean, standard deviation, and minimum and maximum of measures of shape complexity for 100 leaves of each species. 1, *Acer ginnala*, 2, *A. palmatum*, 3, *A. pensylvanicum*, 4, *A. platanoides*, 5, *A. pseudoplatanoides*, 6, *A. rubrum* (coastal), 7, *A. rubrum* (montane), 8, *A. saccharinum*, 9, *A. saccharum* var. *floridanum*, 10, *A. spicatum*, 11, *Liquidambar styraciflua*, 12, *Quercus alba*, and 13, *Q. palustris*. (a) Dissection index, (b) fractal dimension, (c) margin roughness, (d) perimeter/length, (e) number of points, (f) Fourier error of fit with four harmonics, and (g) Fourier error of fit with 64 harmonics.

the digitized image and the calculations based on lines defined by every fifth point.

The perimeter/length ratio ranged in value from 0.018 to 0.781 (Fig. 10). This measure should be close to 1.0, and therefore have log-transformed values approaching zero, when there is a straight margin between the two points, and have higher values when the margin is more complicated. The highest mean values were found in *A. saccharinum* and the two *Quercus*, while the lowest values occurred in leaves from *L. styraciflua*.

The number of points in' the outline ranged from 1,188 to 4,364, and was greatest in *A. palmatum* and *A. saccharinum*, and lowest in *A. rubrum* (coastal), *A. ginnala*, and *A. spicatum*.

The error of fit for four Fourier harmonics should correspond with overall large-scale shape, since that is what is apparent in images reconstructed with the first four harmonics (Fig. 7). Leaves that are more deeply incised, *A. palmatum* and *A. saccharinum*, have the highest mean values of e_f (Fig. 10). Intermediate mean values and wide overall range were

found for the two *Quercus* and *L. styraciflua*, while the remainder of the maples had lower values. This pattern is similar to that found for fractal dimension and dissection index.

The Fourier error for 64 harmonics could correspond to the smaller scale aspects of shape, since many harmonics are necessary to fully describe the details of margins. *Acer palmatum* and *A. saccharinum* had the highest mean values, as they did for dissection index, fractal dimension, and Fourier error with four harmonics. Each species has a broad range of values relative to the range of all species (Fig. 10). This pattern might have resulted from sensitivity to small differences specific to each image, rather than to aspects of shape that are found in all 100 leaves from a species.

Each of the single-parameter descriptors showed highly significant differences among species and among trees within species (Table 3). Higher values of *F* correspond to greater ability to discriminate among species. Dissection index, margin roughness, and Fourier error for four harmonics have the highest *F* values for the comparisons both among species and among trees within species. Perimeter/length ratio and Fourier error for 64 harmonics have the lowest values for comparisons of trees within species, and perimeter/length ratio, the number of points in the outline, and the second principal component of the PCA of Fourier coefficients have the

lowest values for comparisons between species. Therefore, these descriptors might provide less information to discriminate among groups.

There are many statistically significant and positive correlations between the shape measures, with the exception of margin roughness and PC2 of Fourier coefficients (Table 4, Fig. 11). Margin roughness varies independently of the other metrics, except for fractal dimension, Fourier error for four harmonics, and PC1, with which it is negatively correlated. Plotted against each other, fractal dimension and margin roughness together show three groupings of species (Fig. 11). *Acer palmatum* and *A. saccharinum* have moderate values of margin roughness and high values of fractal dimension. *Quercus alba*, *Q. palustris*, *L. styraciflua*, *A. saccharum* and *A. platanoides* have low values of margin roughness and a range of values of fractal dimension, while the others have relatively low values of fractal dimension and high margin roughness.

Fractal dimension and dissection index are highly positively correlated with each other in the shapes included in this study (Fig. 11), and both are highly correlated with the Fourier errors for 4 and 64 harmonics and the number of points. The estimation of fractal dimension involves measuring perimeter using ever-decreasing steps, and thus it is not surprising that its estimate is highly correlated with a measure of the ratio of

TABLE 3. Analysis of variance of single-number descriptors.

Effect	Species			Trees within species		
	df	F	P<	df	F	P<
Dissection index	12	1222.45	0.0001	116	18.92	0.0001
Fractal dimension	12	525.64	0.0001	116	7.11	0.0001
Margin roughness	12	1310.51	0.0001	117	12.41	0.0001
Perimeter/length	12	215.92	0.0001	116	7.77	0.0001
Number of points	12	195.53	0.0001	117	5.84	0.0001
Fourier error 4	12	1356.37	0.0001	116	12.21	0.0001
Fourier error 64	12	964.60	0.0001	116	3.95	0.0001
PC1[a]	12	602.65	0.0001	117	9.69	0.0001
PC2[a]	12	203.69	0.0001	117	3.17	0.0001

[a] PC1 and PC2 are the first and second principal components from the PCA of Fourier coefficients.

TABLE 4. Correlations among measures of complexity. Means of values for 10 leaves in each of 130 trees were used.

	Dissection index	Margin roughness	Perimeter/ length	Number of points	Error 4	Error 64	PC1[b]	PC2[b]
Fractal dimension	0.916 ****	−0.317 ***	0.215 *	0.718 ****	0.946 ****	0.820 ****	0.936 ****	−0.059 n.s.
Dissection index		−0.054 n.s.	0.276 ***	0.766 ****	0.861 ****	0.849 ****	0.871 ****	−0.117 n.s.
Margin roughness			−0.193 *	−0.034 n.s.	0.467 ****	−0.119 n.s.	−0.426 ****	−0.003 n.s.
Perimeter/length				0.168 n.s.	0.099 n.s.	0.316 ***	0.252 **	0.604 ****
Number of points					0.649 ****	0.538 ***	0.768 ****	−0.136 n.s.
Error 4						0.767 ****	0.927 ****	−0.165 n.s.
Error 64							0.767 ****	0.074 n.s.
PC1								0.005 n.s.

[a] Associated probabilities: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$, n.s., not significant.
[b] PC1 and PC2 are the first two principal components of analysis of the power series of 16 elliptic Fourier harmonics.

perimeter to area. The estimation of fractal dimension depends on the use of appropriate step files (Slice, 1993) and can vary greatly with the resolution of the image. Our attempts at utilizing this program with hand-digitized images, consisting of 200–300 points rather than the several thousand points in video digitized images, resulted in negative values for fractal dimension, which cannot be correct. The ratio of area to perimeter can also vary with image resolution, but does not depend on as many assumptions as fractal dimension, thus making it a less error-prone measure. It seems likely that the high correlations between fractal dimension, dissection index, Fourier error for four harmonics, and PC1 are
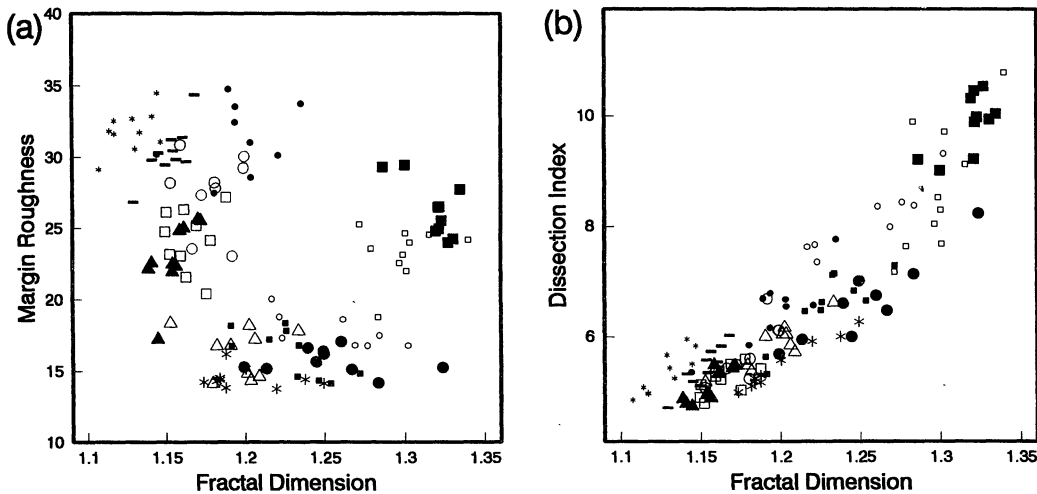

FIGURE 11. Fractal dimension plots. (a) Margin roughness. (b) Dissection index. Points represent mean values for 10 leaves from each of 130 trees. For symbol definitions, see Fig. 5.

due to measurement of similar aspects of shape. If this is the case, then dissection index would be the most efficient to use, because its calculation is the simplest of these metrics.

Traditional Landmark Methods

Four sets of linear measurements were transformed according to the method of Burnaby (Rohlf and Bookstein, 1987). The transformed data sets were then subjected to canonical discriminant analysis. The radial and orthogonal data sets have similar discriminating power, while the data set of 20 measurements and the truss data set were better at discriminating the species (Table 5). However, none of the sets of measurements is as good at discriminating groups as either Fourier coefficients or the single number metrics.

Discussion

Our analyses demonstrate greater discrimination among groups with morphometric methods that utilize outline information than with methods utilizing measurements between a few points. However, the difference in discrimination is not large, and none of the methods showed great superiority over the others. In the sample of leaf shape considered here, there is variation in the shape and complexity of outlines between landmark points, so it is not surprising that the inclusion of outlines improves discrimination among groups.

Most of the single-number descriptors are highly correlated with each other and appear to correspond to large-scale differences in shape. Some, such as margin roughness, vary independently of most of the others, and therefore must be sensitive to other aspects of shape. Together, these descriptors provide as much information about discrimination of groups as large numbers of Fourier harmonics. Fractal dimension does not reveal information in a novel way when compared to other measures of shape complexity, and its usefulness in comparing leaf shapes has been questioned previously (Vlcek and Cheung, 1986). Fractal analysis of leaf shapes might be developed in a useful way if a measure could be developed that would estimate the the degree of self-similarity in a biological image. Many fractal models of plants mimic development, and thus may provide a way of comparing shapes that corresponds to the ways they develop (Barnsley, 1988; Prusinkiewicz and Lindenmayer, 1990; Gould et al., 1992).

Resolution of images by digitization may pose a limitation for some applications. Video digitizing is rapid, but the resolution of an ordinary video camera is lower than hand digitizing. Some of the margin details, especially in sweetgum, were not apparent in the data analysis. Recording the outlines with higher resolution, or magnifying part of the

TABLE 5. Comparisons of descriptors of shape.

	Outlines		Landmarks (linear measures)			
	Single-parameter measures	Single parameter measures with PC1 and PC2[a]	20	Radial	Orthogonal	Trusses
Number of descriptors	7	9	20	9	9	9
Number of significant axes	4	4	4	4	4	4
Wilks's lambda	0.00005174	0.00000502	0.00000014	0.00002494	0.00007070	0.00003545
Correct assignment (%)	93	100	95	86	86	94

[a] PC1 and PC2 are the first and second principal components from the PCA of Fourier coefficients.

outline for digitization, could save these details. There are prospects for greater resolution with automated digitizing using scanners (Molvray et al., 1993).

We have shown that the power series of Fourier coefficients provided better discrimination of groups in this sample of leaves than coefficients that were normalized only. Information about shape is lost when the power series is used, and images cannot be reconstructed. However, at least some of that information is not valuable in making comparisons of shapes in this sample. Simple images may be aligned to have the same major axis, and the amplitude of Fourier coefficients, even without normalization, may be highly informative. For instance, Liu et al. (1996) used raw Fourier coefficients on images of standard size and alignment of the posterior lobe of the male genital arch of *Drosophila*. PC1 accounted for 80% of the variation in this sample. However, complex leaf shapes present a more complicated problem of alignment of numerous lobes, and the power series of Fourier coefficients can improve discrimination of groups.

Shape analysis methods may be information preserving or not (Pavlidis, 1980); it may or may not be possible to reconstruct the image from some part of the analysis. There is an attraction to information-preserving methods, in that it is clear that they have captured all aspects of shape in the images. There is also a necessity for information-preserving methods when the application is visual recognition of images, as in the detection of tanks and airplanes by robots (Kuhl and Giardina, 1982) or identification of weeds (Franz et al., 1991; Woebbecke et al., 1995). However, sensitivity to every aspect of shape, especially when shape is complicated, may be neither feasible nor desirable.

The individual leaf shape traits that can be used to discriminate among species did not appear in any of the methods in our analyses, perhaps because many other aspects of leaf shape are included in addition to the diagnostic ones. Leaves that are easily identified visually were not always easily discriminated by many methods. Traditional taxonomic descriptions of leaves involve separate terms for overall shape, the shapes of the base and the tip, the extent of incision, and the size and shape of teeth on the margin (Stearn, 1983; Radford et al., 1985), and species are often distinguished on the basis of some, but not all, of these aspects of shape. The U- and V-shaped sinuses of *A. saccharum* and *A. rubrum*, respectively, are good traits to discriminate between these species, but form a small part of overall shapes that are similar to each other. Many of the analyses shown here do not separate these species from each other because all parts of the outlines are equally weighted, whereas human pattern recognition can concentrate on specific components of an object. Much as aspects of shape are considered independently of each other in the verbal descriptions of leaf shape (Radford et al., 1985; Stearn, 1983), the use of several measures of shape may be practical for finding traits that distinguish species.

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