APPENDIX 4

STATISTICS AND MORPHOMETRICS
IN PLANT SYSTEMATICS

Statistics is a branch of mathematics dealing with collecting, organizing, analyzing, and interpreting numerical data. Morphometrics is the study of shape or form, and generally uses statistical methods. Statistical and morphometric analyses often go hand in hand and are used in plant systematics to quantify structural features. The quantified features can be used to derive a new character or to refine or define two or more character states of an existing character. These quantitative characters may be used in two major ways: 1) to discriminate between two or more taxonomic entities; e.g., to assess the distinctiveness of taxa, such as a species, infraspecies, or hybrids; and 2) to serve as data in phylogenetic analyses.

The following is a very brief overview of these statistic and morphometric techniques and how they might be used to address taxonomic and systematic problems. See References for Further Study for detailed information.

QUALITATIVE AND QUANTITATIVE CHARACTERS
Characters may be divided into two general types: qualitative and quantitative. Qualitative (or categorical) characters are those in which the states are not directly measured but are based on defined classes of attributes. Examples of qualitative characters are: 1) petal color, with states red and yellow; 2) ovary position, with states superior and inferior; and 3) leaf shape, with states ovate and elliptic. Qualitative characters are assessed by observation and comparison with self-evident or predefined terms. Nothing is measured; features are observed and compared to the definitions of our terminology.

Quantitative characters are those in which the states are measured and based on numbers. There are two general types of quantitative characters: continuous and discrete. Continuous quantitative characters are those in which individual measurements are not necessarily integers and may potentially form a continuum. Examples would include: 1) inflorescence length, with states ranging from 6.2–10.4 cm versus 14.6–28.1 cm; or 2) leaf area, with states 4–8 cm² and 11–22 cm². Discrete quantitative characters are those in which measurements are always integers, including 1) number of parts (also called meristic characters), such as stamen number (e.g., 5, 10, or 15 per flower); and 2) presence/absence data (e.g., presence/absence of a corolla).

The difference between qualitative and quantitative characters is arbitrary. Any qualitative character can be quantified (e.g., “petal color” can be quantified in terms of ranges of visible light wavelength) and quantitative characters can be made qualitative by defining and naming classes of attributes (e.g., a leaf area of 4–8 cm² can be arbitrarily termed “small,” and one of 11–22 cm² can be termed “large.” Qualitative characters are in reality a product of our terminology and may actually represent continuous variation or be arbitrarily divided into discrete states made to conform to these preexisting terms. When variation is carefully examined, clear breaks in the character may not be present. In these cases, it may be necessary to standardize or more precisely define qualitative characters and character states (see Stevens 1991). Perhaps the most important criterion in defining either type of character is whether (and by what criteria) the states are nonoverlapping versus overlapping. Statistical methods are used to evaluate this.

STATISTICS IN TAXONOMY
One use of statistical methods in taxonomy is ascertaining if and how two or more taxa are different from one another, e.g., to evaluate if two or more infraspecies or morphologically similar species are the same or different with respect to the features that have been used to distinguish them. The past differentiation of these taxa may have been based on observations of specimens, but with no supporting data presented. A careful statistical study can corroborate or refute these past classifications, or provide evidence for new groupings.

For example, suppose two very similar plant species have been distinguished solely by fruit length: species Q with fruits 0.4–1 mm long, species R with fruits 1.2–1.8 mm long.
Are these species really distinct from one another? Or do the features used to differentiate them form a continuum with no clear breaks? The following summarizes some steps in acquiring data, calculating simple statistics, graphing the data, and evaluating differences by statistical tests.

**Data**

One consideration in statistical methods is the sample, the subset of the total that is actually measured (given that the entire “population” cannot be feasibly measured). Ideally, the sample should be large enough in size (represented by n), and random enough in distribution to be representative of the statistical population. However, taxonomic studies are often restricted to herbarium specimens, which, unfortunately, may be limited in number and not necessarily representative of the range of variation of the taxon. The ideal situation, in a rigorous study, is to make extensive, new collections of the taxa of interest, over their entire geographic and habitat range.

A second consideration in such a study is comparability, an assessment that the features measured are homologous and heritable, in order that a study can assess evolutionary change. Plants and plant parts can show environmental plasticity. Things like temperature and sunlight exposure, soil moisture and mineral content, and interactions with other organisms can cause considerable variation in the size, number, and shape of plant parts. In addition, one should always measure features that are comparable sexually (e.g., males compared with males and females compared with females in a dioecious species), positionally (e.g., leaves, flowers, or fruits comparable in position on the plant, e.g., the periphery versus center of the inflorescence, or base versus apex of the plant), and developmentally (having comparable stages of maturation).

A third consideration is precision. Features to be measured should, of course, be defined carefully, so there is no ambiguity as to what is actually quantified. In addition, the device used to make the measurements must be precise. Use of a digital camera (mounted, as needed, on a microscope), scale, and computer-interfaced software (see References for Further Study) may give the best and most consistent type of data, one that has a permanent, easily accessed record.

**Simple Statistics**

Recall the taxonomic problem cited above: Is species Q, traditionally defined as having fruits 0.4–1 mm long, different in this feature from species R, defined with fruits 1.2–1.8 mm long? One method of tackling this question is simply to measure the fruits of numerous individuals of the two putative species, and determine if they: 1) sort into two groups, one with shorter fruits and one with longer, corresponding to the two groups; 2) do not sort into groups at all; or 3) sort into groups that are novel with respect to the previous taxonomy.

Because this problem is to assess if two previously defined and name species are indeed different, the appropriate approach is to examine features of separate individuals of each of the two species, typically from plant herbarium specimens (each of which is assumed to represent a separate individual plant). The factors mentioned earlier, a large and unbiased sample size, comparability, and precision of definition and measurements should be taken into account.

Given that numerous fruits from each sample (individual plant specimen) are measured, several standard statistics are typically calculated: 1) an average of the values, typically the mean (the numeric average; Table A4.1, illustrated in Figure A4.1); 2) the range of values, from the minimum to maximum measurements (e.g., as in Figure A4.4); 3) quartiles, the three data points (1st, 2nd, and 3rd quartiles) that divide the data into four equal parts (example in Figure A4.4); the second quartile is the median.

Also often calculated are simple statistical parameters that measure how much the data vary relative to the mean. For example, of two samples of n = 5, one with fruit lengths of 0.5, 0.6, 0.7, 0.8, and 0.9, and another with fruit lengths of 0.65, 0.675, 0.7, 0.725, and 0.75, both have the same mean (0.7 mm), but the former has a greater variability relative to the mean. The standard deviation of a sample is a measure of the differences of individual values from the mean of all values, calculated as the square root of the “sum of squares” (SSx) divided by the sample size minus one (Table A4.1). With a normal distribution of the data, ±1 standard deviation from the mean encompasses approximately 68% of the data; ±2 standard deviations of the mean account for approximately 95% of the data. Standard error is a measure of how close the sample mean is to the mean of the entire population. Standard error is calculated as the standard deviation divided by the square root of the sample size (Table A4.1). A range of ±2 standard errors will include about 95% of all sample means. As with standard deviation, calculations of standard error are accurate only if the data have a normal distribution (conforming to parametric statistics).

**TABLE A4.1** Some simple statistics.

| Sample size (total number of observations): | n |
| Mean: \( \bar{x} = \frac{\sum x}{n} \) |
| Standard deviation: \( s_x = \sqrt{\frac{SS_x}{n-1}} = \sqrt{\frac{\Sigma (x - \bar{x})^2}{n-1}} \) |
| Standard error: \( s_{\bar{x}} = \frac{s_x}{\sqrt{n}} \) |
Figure A4.1  Univariate graphs and associated statistics of fruit length (mm), plotted by increasing mean for individual specimens of species \( Q \) (fruits defined as 0.4–1.0 mm long) and species \( R \) (fruits defined as 1.2–1.8 mm long). Mean is indicated by dots, ranges by vertical line, and ±1 standard deviation by bars on each side of the mean. P values indicate results of t-test, comparing species \( Q \) and \( R \). A. Plot showing nonoverlapping fruit lengths between species, although the boundaries of the features are different than originally defined: \( Q \) now defined by fruits 0.5–1.2 mm long, \( R \) by fruits 1.4–2.0 mm long. Note small p value. B. Plot showing mostly clear break in fruit length between species (by original definitions), but with some samples overlapping in range. Note larger p value. C. Plot showing continuous grade of fruit length, with no clear breaks. D. Plot showing generally smaller and larger-fruited groups, but with some individuals intermediate in fruit size.

Univariate and Bivariate Plots

In the example cited above (assessing whether fruit length differentiates between species \( Q \) and \( R \)), a graph or plot of this feature can be informative (Figure A4.1). Note in this example that individual specimens are sorted on the X-axis by increasing sample means, with range and ±1 standard deviation of values shown. Because only a single feature (fruit length in this case) is being graphed, this is called a univariate plot.

This simple plot may reveal any number of trends. One scenario is that the two species show a clear break in fruit length (Figure A4.1A), corroborating their distinctiveness and recognition as separate entities; however, note that the ranges of fruit length characterizing the two taxa is different than originally proposed. A second scenario is similar to the first, only in this case the ranges of the two groups overlap for some specimens (Figure A4.1B). Can we be sure that the two species are distinct? A third scenario is that the samples show a continuously overlapping grade in fruit length (Figure A4.1C). In this case, one might conclude that the fruit length character originally used to distinguish the two species was arbitrarily determined and does not reflect real taxonomic entities. Thus, the separation of these two species comes into question; perhaps they are best merged into one species. Yet another scenario is that two entities are generally recognizable in the original definition of fruit length differences, but with some intermediate individuals (Figure A4.1D). The two species in this case could be genetically different but intergrading, e.g., among samples intermediate to their geographic ranges. This might be interpreted as evidence that the two “species” have incompletely diverged and are best treated as infraspecies of one species. Or, the intermediates could represent occasional hybrids between the two parent species, where they come into contact. (Other types of data, e.g., detailed molecular studies or correlation with biogeography or habitat, would be needed to tease apart these possibilities.) Tests of statistically significant differences (see later discussion) can be done to assess the delimitation of taxa in the various scenarios of Figure A4.1.

Another graph sometimes used to assess character differences among taxonomic groups is a bivariate plot, in which the relationship between two variables are graphed...
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Figure A4.2: Bivariate plot of two morphological features, in this case seed papillation and seed diameter, for species A–E. Note clustering of points into five species groups, with some overlap between species C and D.

In this example, species A–E are assumed to be discrete entities, and the mean values for each individual within the taxa are plotted. The advantage of a bivariate plot is that boundaries between taxa can often be better established by using two characters. Note that in this example, seed diameter alone would show considerable overlap among taxa, but a combination of seed diameter and seed papillation separates the taxa into more differentiated groups (Figure A4.2).

One type of bivariate plot traces the development (or ontogeny) of a feature. Developmental data require plotting one variable, Y, against time, either as real time or as time estimated by some other criterion (e.g., the size of a particular organ, which increases with time). Such a plot of a variable as a function of real or relative time for a given taxon yields a so-called ontogenetic trajectory (Figure A4.3). A comparison of the ontogenetic trajectories between different taxa may be used both to refine character definition and to assess the evolutionary change of a given feature.

Tests of Significance
Graphing measurements from a study can give insight as to the differentiation of taxa. However, statistical tests are often needed to determine if the groups are really different, given the variation in the samples. In these tests, the “null hypothesis” is that there are no significant differences between the groups being compared. If any differences between populations are due to chance or random variation, then the differences are nonsignificant and the null hypothesis is not rejected. However, if the differences are likely not due to chance, then differences between groups are significant and the null hypothesis is rejected. Typically, the null hypothesis is rejected if the probability of differences between groups being due to chance alone is less than 5% (p < 0.05), though sometimes values of less than 1% (p < 0.01) are considered.

The commonly used tests for significance are valid only with data that have a normal (Gaussian) distribution, and in the purview of parametric statistics (as mentioned earlier with standard deviation and standard error calculations). Most morphological data used in taxonomic studies will apply to parametric statistics, if the sample size is large enough and there are no biases in selecting the individual plant or character. [Note that there are statistical tests to determine if the data are normally distributed. If they are not, the data can be transformed, e.g., by converting all values to their logarithms; these transformed data may often have a normal distribution and be subject to parametric statistics.]

Linear regression analysis is a statistical procedure for fitting a straight line onto a bivariate plot of two variables, X and Y, and testing the significance of relationship between them. Linear regression analysis assumes that the average relationship of Y to X can be represented as a straight line. This is a big assumption, and nonlinear methods may need to be considered. Linear regression is not often used in systematic or taxonomic work, but might possibly be used to evaluate slopes of curves in tracing a developmental pathway (Figure A4.3) or a bivariate plot of two morphological features. The character states in these cases could be the slopes of the curves.
One test of significance between sample means is the \textit{t-test} (or \textit{student’s t-test}). One form of this, the “unpaired t-test,” may be used to statistically compare two samples in order to evaluate the probability of their being the same (the null hypothesis). The relationship of this statistic to probability is a function of the number of \textit{degrees of freedom}, which is related to sample size. For example, in the scenarios of Figure A4.1, a t-test of the two groups corresponding to species \textit{Q} and \textit{R} of Figure A4.1A, in which all samples of the two groups are combined, yields a probability of < 0.01, meaning that the two species are statistically different for fruit length. Similarly, a t-test for the combined species samples of Figure A4.1B, showing a slight gap in the data, yields a higher probability but one < 0.05, generally the cut-off that the two groups are significantly different.

\textbf{Analysis of variables} (ANOVA) is used to evaluate differences among more than two groups in a single test of significance. ANOVA is somewhat complex (see references listed), but basically compares variability between samples with variability within samples. A statistic, known as the F-value (= between-sample variance/within-sample variance), is calculated, along with the p significance value. Additional tests, known as \textbf{post hoc tests}, are used to determine which samples are different from others. For example, Figure A4.4 represents a graph of a feature (adaxial sepal length) derived for six taxa. An ANOVA, followed by a \textit{Tukey post hoc test} showed that taxon \textit{T} and taxon \textit{X} are significantly different from all other taxa in the analysis at the p < 0.01 level, but no other combinations show pair-wise differences.

\textbf{Multivariate Statistics}

Multivariate statistical methods are able to evaluate the analysis of two or more (often numerous) variables simultaneously. These separate variables might include morphological characters such as leaf length, leaf width, calyx length, corolla tube length, corolla limb width, stamen length, ovary width, etc.

\textbf{Principal components analysis (PCA)} is a multivariate method that transforms the data variables into other variables called principal components. The first principal component projects the greatest amount of variance in the data, the second principal component the second greatest amount of the remaining variance, and so forth. One use of these new variables is a “scatter plot,” which permits visualization of the relationships of the totality of data variables as clusters, which might otherwise not be apparent. For example, Figure A4.5A shows the plot of the first and second “factors” of a PCA, calculated from 12 different morphological variables. Note that the five species are (to various degrees) clustered and separable from the others, although the outgroup taxon overlaps (Figure A4.5A).

\textbf{Discriminate function analysis} is a similar multivariate technique that determines the variables that discriminate among (in this case) taxonomic groups.

\textbf{MAPPING OF MORPHOLOGICAL DATA}

Data obtained from statistical taxonomic studies may be mapped to illustrate correlations between morphological features and geographic distribution. Various graphics and symbols are often used to represent one or more characters, permitting visualization of correlations (Figure A4.5B).
MORPHOMETRICS

Morphometrics is the study of shape and form and may utilize the statistical methods mentioned earlier. Morphometric techniques can be used in systematics and taxonomy to characterize differences in morphology between two or more taxa, typically for a complicated structure that may be broken down into numerous, separate features. It can be used to assess if two or more taxa are different, derive new characters and character states, or to trace and compare the development of a given structure through time.

Geometric morphometrics is a class of techniques that utilizes landmarks, points on the object that generally correspond to homologous features (Bookstein 1991, Zelditch et al. 2004). For example, a shape comparison of three species differing in corolla and androecium position could be conducted by taking measurements from longitudinally-sectioned corollas, spread flat; from these structures, homologous points are defined, such as the corolla base, corolla lobe apices and sinuses, point of stamen insertion, filament apex, stamen apex, etc. (Figure A4.6A–C). (In addition, sometimes “pseudolandmarks” may be defined, these being relative and not directly attributed to a homologous point but formed, e.g., by the intersection of or arbitrary distances from true landmark points or lines between them.)

One morphometric technique involves connecting every homologous point with every other homologous point and measuring the real distance between them (Figure A4.6A–C), this pattern of lines sometimes called a “truss” network (Strauss and Bookstein 1982). These data are typically log transformed. The log-transformed measures are then used as parameters in a principal components analysis. The resulting pattern of principal components can be used to assess differences between complicated structures among taxa, e.g., those distinguished by fine differences of morphology (Figure A4.6D). (See Dickinson et al. 1987 and Shipunov and Bateman 2005 for examples.)

However, there are many other morphometric techniques (some quite mathematically sophisticated), including elliptic Fourier functions, which analyze shape outlines (Kuhl and Giradina 1982, Premoli 1996, McLellan and Endler 1998, Olsson et al. 2000, Jensen et al. 2002), and relative warp scores (Bookstein 1989, Rohlf 1993, Jensen et al. 2002). An explanation is beyond the scope of this introduction, but see references cited for more information.

QUANTITATIVE CHARACTERS IN PHYLOGENETIC ANALYSES

The statistical methods reviewed earlier can be used to more precisely define a morphological character and its character states, often used to help differentiate between two or more taxa, e.g., in defining the circumscription of infraspecies, close species, or hybrids. Another use of statistical methods is to clearly derive characters and character states that may be either used directly in phylogenetic analyses or traced (optimized) on a tree to evaluate evolutionary changes in these features.

Although some feel that character states used in phylogenetic analyses should always be discrete and nonoverlapping, there are many cases of characters that show no absolute breaks between taxa, and yet obviously have some information reflecting evolutionary history. For example, Figure A4.7A
shows a plot of a feature (sepal width) in ten taxa. Although no taxa are significantly different from all other taxa (as would be assessed, e.g., with ANOVA), there is obviously information that might be valuable in elucidating relationships or at least trends in this feature.

One method of dealing with continuous data like this is to refine the morphological analysis to smaller clades. Morphological data of the entire study group (ingroups and outgroups) may overlap, but within a smaller subset (e.g., the outgroup alone or a well-supported clade within the ingroup) a character may show discrete character states.

Another method of dealing with this type of continuous data is dividing more or less continuous variation into discrete states, a process known as gap-coding, gap-weighting, or homogeneous-subset coding (Mickey and Johnson 1976, Almeida and Bisby 1984, Archie 1985, Baum 1988, Goldman 1988, Chappill 1989). In these methodologies, character states that overlap are placed into different subsets by some criterion. The pooled within-group standard deviation ($s_p$, calculated from the total data of all taxa studied) multiplied by some constant ($c$, which is often =1) is often used as a measure to obtain discrete states from a continuum. One method of deriving these subsets...
is to compare the difference of means between adjacent taxa (Figure A4.7B). If this difference is less than the $c_{\text{SP}}$ value, they are included within the same subset; if adjacent means are greater than $c_{\text{SP}}$, they are placed in different subsets, which may overlap (Figure A4.7C). Finally, these subsets form the basis for character coding. For example, in homogeneous-subset coding (Archie 1985), taxa in the lowest subset receive a character state of 0. For successive taxa, the state value is increased by 1 if the taxon is part of a different subset and increased by another 1 if it is no longer part of the previous subset (Figure A4.7C). In this fashion, a continuously overlapping character (as in Figure A4.7C) can be subdivided into character states (typically treated as “ordered”; see Chapter 2) that reflect differences between subgroups (Figure A4.7C). Other methods for dealing with character coding have been proposed (e.g., Strait et al. 1996).

Another method for coding overlapping characters uses gap-weighting. In one gap-weighing technique (Thiele 1993), character states are placed into subsets according to the formula: $x_{\text{SP}} = n (x - \text{min}) / (\text{max} - \text{min})$, where $x$ is the mean value of the original character state, $x_{\text{SP}}$ is the gap-weighted character state, “max” and “min” are the maximum and minimum state values for all taxa, and $n$ is the maximum allowable number of character states (dependent on the computer algorithm). From the same example of overlapping character states cited earlier (Figure A4.7A), the mean of each taxon is placed into a subset, often called a “frequency bin” (Figure A4.7D), based on its linear distance from the minimum to maximum values. The character states obtained are, again, typically treated as an ordered character, which may be proportionally scaled relative to other characters. Thus, in this example, a greater distance, in terms of state changes, occurs between adjacent taxa $I$ and $J$ (5 steps) than between adjacent taxa $A$ and $B$ (zero steps). (Frequency bins can also be used to code polymorphic characters; see Wiens 1995.)

FIGURE A4.7  A. Graph of 10 taxa, showing continuous grade of feature, with no clear breaks into character states. B,C. Example of homogeneous-subset coding (see text). D,E. Frequency coding (after Weins, 1995). Note that the 24 frequency “bins” correspond to ranges of the data, each assigned to a character state between 0 and 23. Character weight is scaled to 1/23 (= min. no. state transformations) = 0.0434.
REVIEW QUESTIONS

1. What is the difference between a qualitative and quantitative character? Give two examples of each.
2. What features of data and data acquisition need to be assessed?
3. Define mean, median, range, quartiles.
4. What does standard deviation measure? Standard error?
5. What are univariate plots and how might these be used in systematics and taxonomy?
6. What is a bivariate plot and what two general types are used in systematics? What is its advantage over a univariate plot?
7. What is linear regression analysis and how might it be used?
8. Explain what is meant by a test of significance in terms of the null hypothesis and p values.
9. What is the basic function of a t-test?
10. When and how are analysis of variables (ANOVA) used? What is a post-hoc test?
11. What are the two major types of multivariate statistics and how are they used in systematics?
12. How might mapping of statistical data be done, and how is it useful?
13. What is morphometrics?
14. Explain one way morphometric techniques might be used in systematic studies.
15. How might statistics be used in coding quantitative characters?
16. Name one method of coding overlapping features into character states that may be used in phylogenetic analyses.

EXERCISES

1. Select about 10 herbarium specimens (or samples of live plants) of 2–3 morphologically similar species or infraspecies. For each specimen, measure two features (e.g., leaf length and width), from a minimum of 10 organs. Carefully define the features to be measured, taking into account homology of structures, variation due to position, and environmental plasticity. For each sample calculate (e.g., in a spreadsheet) mean, range, and standard deviation. Prepare a bivariate plot of the two features and assess the discreteness of taxa from the results. If statistical software is available, run a t-test (for two taxa) or ANOVA (for three taxa) and assess if the differences between taxa are statistically different.
2. For 10 herbarium specimens each of two taxa, measure up to 10 features. Pool these features by taxon and conduct a principal components analysis. Graph the first two components and evaluate if the clusters of points are discrete.
3. Select three or more closely related taxa and identify a complex organ or plant part (e.g., one used primarily to distinguish between the taxa) to subject to a morphometric analysis. Identify homologous points on the structure in question. If possible, quantify at least 10 objects per taxon for these points and conduct any number of analyses, e.g., a principal components analysis from a “truss” network. How might the data be valuable in systematic studies?

REFERENCES FOR FURTHER STUDY

RESEARCH ARTICLES AND CHAPTERS:

SOME STATISTICS AND MORPHOMETRIC BOOKS:

COMPUTER SOFTWARE
http://rsb.info.nih.gov/ij
Free, multiplatform software for morphometric analyses.
http://life.bio.sunysb.edu/morph
Extensive software downloads and information on hardware, date, courses, and investigators in morphometrics.
http://www3.canisius.edu/~sheets/morphsoft.html
SYSTAT software. 2009 onwards.
http://www.systat.com
Very widely used commercial software in the biological sciences.