PBDB Summer Course July 2006

Introduction to Geometric Morphometrics

Mark Webster
Department of the Geophysical Sciences, University of Chicago

Morphometrics

The mathematical quantification of organismal morphology.

- Measures of size (scalars).
- Measures of shape (multidimensional summaries).

Quantification of morphology introduces more rigor into organismal (paleo)biology.

- More rigorous description of morphologies.
- More rigorous comparison among morphologies.

Quantification of morphology:

- Removes the need for analogies.
  - "X is subcircular; Z is more elliptical."
- Removes the speculative aspect of morphological study.
  - "Would you call Y subcircular or elliptical?"

Organisms differ in morphology for many reasons:

- Phylogeny.
- Age (ontogeny).
- Dimorphism/polymorphism (sexual; ecological; functional).
- Environment (ecophenotypy).
- Mutation/variation.
- Disease/injury.

What's The Use?

Rigorous quantification of morphology is therefore important for:

- Species delimitation.
  - Geographic variability (ecophenotypy).
  - Biostratigraphy and correlation.
  - Diversity estimates.
- Species comparison.
  - Disparity estimates.
  - Characters for cladistic analysis.
- Ontogenetic studies.
  - Growth patterns within species (including modularity, etc.).
  - Evolutionary processes (heterochrony, etc.).
- Documentation of temporal trends.
  - Phylogenetic/ecophenotypic morphological change.

Many research fields rely on accurate species recognition and characterization.

- (Paleo)ecology.
- Speciation/extinction patterns and processes.
- Micro- and macro-evolutionary trends.
- Systematics and phylogenetics.
- Biostratigraphy and correlation.

Morphometric data impinge on all branches of the evolutionary sciences!

Types of Morphometrics

Traditional morphometrics:

- Length measurements, angles, etc.
    - Univariate analyses.
    - Bivariate analyses.
    - Multivariate analyses.

Geometric morphometrics:

- Landmark configurations summarizing shape.
    - Multivariate analyses, 2- or 3-dimensional.

Outline-based morphometrics:

- Open or closed curves (perimeters).

All approaches have their uses! [Not all are appropriate for every study!]

What This Course Covers

This course serves as an introduction to common exploratory and confirmatory techniques in geometric morphometrics.

- Two-dimensional data.

Emphasis is put on acquiring a basic understanding of how the techniques work and their practical application to real data.

- This is not a comprehensive mathematics or statistics course!

Topic 1

Data acquisition:

- Imaging specimens.
- Landmarks:
    - Landmark types.
    - Landmark selection.
    - How to digitize landmarks.
- Constructing landmark data files.

Case study #1.

Topic 2

The theory of shape:

- What is shape?
- Comparing shapes.
    - Removing non-shape features.
    - Procrustes distance.
- Shape spaces:
    - Configuration space.
    - Shape space (= oriented pre-shape space).
    - Kendall's shape space.
    - Tangent space.

Topic 3

Difference in shape between configurations:

- Superimposition methods.
- Visualizing difference in shape.
- Variation in shape.
- Statistical testing of difference in shape.

Case study #1 (continued).
Case study #2.

Topic 4

Allometry - The relationship between size and shape:

- The importance of allometry.
- How to identify allometry.
- Describing ontogenetic shape change.
    - Pattern of shape change.
    - Rate of shape change.

Case study #3.

Topic 5

Comparing ontogenetic trajectories:

- Trajectories of shape change.
- Comparing trajectories of shape change:
    - "Angle between ontogenies".
    - Rates of shape change.

Removing the effects of allometry:

- Comparison of shape at standard size.
- Comparison of shape variation at standard size.

Case Study #4: Simple ontogenies, two species.

Case Study #5: Simple ontogenies, unknown number of species.

Topic 6

Answering evolutionary questions with geometric morphometrics.

- Disparity.
- Evolutionary modifications to ontogeny.

Computer Requirements

Imaging software for data collection (free).

- NIH Image (Mac):
    - http://rsb.info.nih.gov/nih-image/Default.html
- SCION Image (PC):
    - http://www.scioncorp.com/

Spreadsheet software for data collection, editing/formatting, and basic analysis.

- Microsoft Excel (Mac or PC).

Morphometric software packages.

- Jim Rohlf's software (PC):
    - http://life.bio.sunysb.edu/morph
- Dave Sheets' software (PC):
    - http://www.canisius.edu/~sheets/morphsoft.html
- Jeff Walker's Morphometrika (Mac):
    - http://www.usm.maine.edu/%7Ewalker/software.html
- Not necessary, but useful for exporting nice TPS graphics.

VirtualPC will allow PC software to run on a Mac.

PBDB Summer Course, Morphometrics July 2006

Topic 1: Data Acquisition

Suggested Reading

- Bookstein (1991): Chapter 3 (pp. 55-87) - landmarks.
- Feldmann et al. (1989): Chapter 4 (pp. 24-29) – preparation; Chapter 37 (pp. 336-341) – photography; Chapter 38 (pp. 342-346) – whitening; Chapter 40 (pp. 351-355) - SEM.
- Zelditch et al. (2004): Chapter 2 (pp. 23-50) - photography, landmarks.

Data Acquisition

1. Imaging specimens.
    - Specimen preparation.
        - Cleaning.
        - Whitening.
    - Specimen photography.
        - Mounting.
        - Lighting.
        - Shooting.
    - Image processing.
2. Extraction of morphometric data from the images.
3. Organizing the data in a spreadsheet.

PHOTOGRAPHY OF SPECIMENS

The quality of image determines the maximal quality of the final data.

- Do not compromise! Do not cut corners!
- The image(s) from which the morphometric data are extracted should be the best possible for that specimen.

Specimen Cleaning

Remove all excess material from the specimen.

- Dirt, rock matrix, "fluff".

Prepare the specimen out of the matrix if necessary.

Whitening

It is often advantageous to whiten specimens prior to photography.

- Brings out features of interest.
- Reduces shininess of specimens.

Use ammonium chloride sublimate (temporary) or magnesium oxide (must be washed off).

- It is sometimes useful to blacken specimens with a coating of dilute black ink first (permanent).

Mounting Specimens

All specimens must be mounted in a standard orientation.

- Specimens must not differ in pitch or roll relative to camera view.

Many taxonomic groups have an established standard orientation.

- E.g., Shaw (1957) for trilobites.

The mounting medium used will depend on the size of the specimen.

- SEM stub (prone to specimen damage during removal).
- Toothpick/steel pin, with gum tragacanth.
- Lead shot (or small fishing weights).
- Sand.
- Modeling clay (but often leaves a residue).
- "Props" (sometimes improvised).

Check with museums before mounting loan material.

Lighting

Play with the lighting for optimal illumination of all features of interest.

- Multiple light sources.
- Adjustable intensity.
- Adjustable angle.

For morphometric purposes, asymmetric lighting is often undesirable (shadows).

- For publication, many journals have a requirement for lighting.
    - E.g., "specimens must be lit from the upper left".

Don't use a camera flash: it is likely to cause too much shadow.

Image brightness can be controlled in several ways:

- Proximity of light source(s) to specimen.
- Illumination intensity of light source(s).
- Shutter speed setting on camera.
    - Longer shutter speed can lead to blurred images and enhanced contrast.
- Aperture setting on camera and/or on lens.
    - Aperture setting also affects sharpness and depth of focus.
- The wonders of Photoshop.

Scale Bar

Always include a scale bar in the photograph.

- Must be in the same plane as the specimen.
    - Scale bar is in focus.
    - Minimal inaccuracy when setting the scale.
- Must not obscure or shadow the specimen.
    - Set the scale bar off to one side.

In some instances it may be preferable to take two images of the specimen: one with and one without a scale bar.

- Don't move the specimen or camera between taking the images!

Photography

Choice of photography equipment will depend on the size and three-dimensionality of the specimen:

- SEM.
- Aristophot (all but extinct…).
- Microscope cameraback attachment.
- Camera:
    - Various lens types (35mm, macrolens, etc.).
    - Extension tubes.

Use mounted camera stands when photographing specimens.

- Greatly reduces camera wobble during exposure.
    - Vertically-mounted stand for plan view.
    - Horizontally-mounted stand or tripod for lateral view.
        - Otherwise, specimen must be rotated 90° if using a vertically-mounted stand.
- Check the camera orientation before every shot.

Remote camera operation (e.g., through a computer) also reduces camera wobble.

Camera Settings

Optimal camera settings will vary according to the study organisms (size, three-dimensionality).

- Trial and error process.
- E.g., for most of my trilobite work:
    - Manual focus.
    - Quick shutter speed (1/750).
        - Reduces blurring due to camera wobble.
    - Automatic aperture setting.

Don't use automatic settings for everything.

Don't use the flash.

Image Processing

Digital images can be processed to improve clarity for data extraction (and publication).

- Mode.
  - Grayscale often best.
- Levels adjustments.
- Brightness.
- Contrast.
- Sharpen/smooth features.

Be careful and be honest!

Extracting Data From Published Images

A quick and easy way to collect data, but has many caveats:

- The figured specimen must be mounted in the standard orientation.
- The lighting must be adequate for data extraction.
  - For publication, many journals have a requirement for lighting.
    - E.g., "specimens must be lit from the upper left".
- The scale must be reliable.

"If in doubt, throw it out."

EXTRACTION OF DATA

Traditional Morphometric Data

Include length measurements and angles.

Selecting Measurements

Standard measurement selection criteria may already exist.

- E.g., trilobite cephalon (Shaw, 1956, 1957).

The nature of the hypothesis to be tested may determine selection of variables.

- E.g., "can these species be distinguished by (relative) length of the ocular lobe?"

Landmarks

Points of correspondence on each object that match within and between populations (Dryden and Mardia, 1998).

Biologically homologous anatomical loci.

- Recognizable on all specimens in the study.

Landmark data are more informative than traditional data:

- Interlandmark distances = traditional lengths.
- Landmark coordinates contain positional information.

Landmark Selection

Landmark configurations should be selected to offer a comprehensive summary of morphology.

- Not just of features where you suspect interesting things are happening.

Landmarks provide the only data: what's going on between the landmarks can only be inferred.

- Localized shape differences cannot be detected in regions between landmarks.

Landmarks should:

- Be homologous anatomical loci.
- Have conserved topological positions relative to other landmarks.
  - No heterotopy!
- Provide an adequate summary of overall shape.
  - (Minimally, be appropriate to address the hypothesis of interest).
- Be reliably digitizable (replicable).
- Lie within the same plane.

Landmark "Types"

Type 1: Discrete juxtapositions of structures.

- "Triple junctions", intersections.
- Points surrounded by tissue.

Type 2: Points of maximal curvature.

Type 3: Extremal points.

- Landmarks identified (or constructed) by reference to other features/landmarks.
    - "Furthest from" another structure/point.
    - Intersection or division of hypothetical lines.
    - Centroid.

Examples of Landmark Selection

Trilobite cephalon (Webster et al., 2001, fig. 4.5).
Piranha body (Zelditch et al., 2004, fig. 2.4).
Foraminifera (Bookstein, 1991, figs. 3.4.5, 3.4.12).
Cotton rat skull (Zelditch et al., 2004, fig. 2.5).
Rat skull (Bookstein, 1991, fig. 3.4.1).
Primate skull (Bookstein, 1991, fig. 3.4.3).
Squirrel scapula (Zelditch et al., 2004, fig. 2.3).

Landmark Selection

Different landmark configurations may be needed for different studies.

- Different hypotheses to be tested.
- Different taxonomic groupings.
- Different ontogenetic stages.

Specimens inappropriate for inclusion in one study may be appropriate for inclusion in another.

- Think ahead: Will *any* data from this specimen *ever* be useful?

Length or Landmark Data?

Length measurements often represent distances between landmarks.

- Collect landmark data, then back-calculate length measures using trigonometry.

Efficiency of Data Summary

Number of landmarks = k
Number of interlandmark distances = d

$$d = (k\ (k - 1))/2$$

For a form summarized by 10 landmarks in two dimensions:

- 20 coordinate variables.
    - Includes information on spatial relationships.
- 45 length measurements in a truss.
    - No information on spatial relationships.
- 990 length ratios.
    - No information on spatial relationships.

Why Use Geometric Morphometrics?

The spatial relationships among landmarks on the organism is important.

- *The configuration is the datum.*

Gives the ability to draw morphological transformations.

- Immediate visualization of data.
- Easier to intuitively understand than a table of numbers.

Allows effective extraction and communication of information regarding the spatial localization of morphological variation.

Limitations of Geometric Morphometrics

Configurations provide data about landmark locations only.

- The space between landmarks is unsampled.
- It is hard to adequately sample some morphological regions.
    - E.g., along the margins of curved structures.

It is hard to graphically represent 3-D data.

Linear projections from shape space into tangent space become unreliable when comparing very different shapes.

Digitizing Replicabilty

Error can result from:

- Inconsistent orientation of specimens relative to plane of digitization.
    - "Tilting" (pitch, roll).
- Non-coplanarity of landmarks.
- Difficulty in pinpointing the landmark locus.
    - Biological "vagueness".
    - Differences in illumination.
    - Differences in focus.
- Pixelation or distortion on screen.

ORGANIZING DATA

Traditional Morphometric Data

Arrange in an Excel spreadsheet as follows:

- Specimens as rows.
- Variables as columns.

Landmark Data

Arrange in an Excel spreadsheet as follows:

- Landmarks as rows.
- Specimens as (paired) columns.

Collecting Data

Variables must be organized in the same order for all specimens.

- Homologous variables form columns (traditional data) or rows (landmark data).

Missing Data

A specimen with missing data for a variable cannot be included in an analysis which involves that variable.

- (Although it may be included in other analyses not involving that variable.)

When collecting data from an image, do not "skip over" a missing variable.

- Give it an easily recognizable "dummy measure" (e.g., tiny length; tiny landmark coordinates).
- Manually replace the "dummy measure" value with a period (".") in the spreadsheet immediately after pasting in the data for that specimen.

Inapplicable Variables

Some morphological features may not be present on all taxa in a given data set.

This leads to problematic values for the affected variables:

- Length measures of zero.
- "Synonymy" of landmarks.

Variables with such problematic values must typically be excluded from analyses.

CASE STUDY # 1
The Trilobite *Olenellus chiefensis*

From the images provided:

- Select a configuration of landmarks.
- Digitize those landmarks.
- Paste the landmark coordinates into Excel.

Landmark Data

Arrange in an Excel spreadsheet as follows:

- Landmarks as rows.
- Specimens as (paired) columns.

Topic 2: The Theory Of Shape

The following topics will be covered:

- What is shape?
- Comparing shapes.
  - Removing non-shape features.
  - Procrustes distance.
- Shape spaces:
  - Configuration space.
  - Shape space (= oriented pre-shape space).
  - Kendall's shape space.
  - Tangent space.

The theory of shape is *the key concept* of geometric morphometrics.
An understanding of the theory of shape allows determination of the validity of particular morphometric methods for particular questions.

WHAT IS SHAPE?

Shape is:

- Inherently multidimensional.
  - More than one variable is required to describe the differences between even the simplest shapes (triangles).
- Dimensionless (no units).

Shape is all the geometric information that remains when location, scale, and rotational effects are filtered out from an object (Kendall, 1977).
Translation, rescaling, and rotation do not affect shape.

- Anything else does!

A landmark *configuration* is the datum!

- An individual landmark is not shape.

Comparing Shapes

To compare shapes it is necessary to remove all non-shape differences between configurations.

- Location differences.
- Size differences.
- Rotational differences.

Removing Location Differences

Configurations of the same number of (homologous) landmarks can be aligned so that they share a common location.
This is called "centering a configuration".

- Translate the configuration along the x- and/or y-axis so that the centroid of the configuration is at [0, 0].

Centroid Location

The centroid is the center of the landmark configuration.
Easy to calculate:

- x-coordinate of centroid = mean x-coordinate of all *k* landmarks.

$$x_{centroid} = \frac{\sum_{1 \text{ to } k} x_k}{k}$$

- y-coordinate of centroid = mean y-coordinate of all *k* landmarks.

$$y_{centroid} = \frac{\sum_{1 \text{ to } k} y_k}{k}$$

Centering the Centroid

Translate all coordinates so that the centroid lies at [0, 0].

$$\sum_{1 \text{ to } k} x_k = 0$$
$$\sum_{1 \text{ to } k} y_k = 0$$

Easy to do:

- Subtract the x-coordinate of the (non-centered) centroid from the x-coordinate of each landmark.
- Subtract the y-coordinate of the (non-centered) centroid from the y-coordinate of each landmark.

Removing Size Differences

Configurations of the same number of (homologous) landmarks can be rescaled so that they share a common size.

- "Unit centroid size".

Centroid Size

The square root of the sum of the squared distances between each landmark and the centroid of the form.

- Equivalently, the square root of the mean of all squared interlandmark distances.

Centroid size of configuration A =

$$\sqrt{\sum_{i=1 \text{ to } k} \sum_{j=1 \text{ to } m} (a_{ij} - c_j)^2}$$

…for *k* landmarks in *m* dimensions, where a_{ij} is the landmark *j*-coordinate of landmark *i* in configuration A, and c_j is the centroid *j*-coordinate.

"Size" Measures

There are many commonly-used proxies for "size":

- Length of an axis.
- Area.
- Volume.
- Weight.
- Score on PC 1.
- Centroid size.

Why Use Centroid Size?

Centroid size has the benefit over all other size measures that it is *mathematically uncorrelated with shape in the absence of allometry*.

- It does not induce a correlation between size and shape in the presence of circular normal landmark errors.
- It is statistically orthogonal to shape.
- See Bookstein (1991, chapters 4, 5).

Centroid size has the drawback that it is hard to intuitively relate to an actual size.

- Specimen has glabella length of 8.5 mm.
- Configuration has centroid size of 15.4.

Unit Centroid Size

Meets the criterion that:

$$\sqrt{\sum_{i=1 \text{ to } k} \sum_{j=1 \text{ to } m} (a_{ij} - c_j)^2} = 1$$

Which simplifies to:

$$\sum_{i=1 \text{ to } k} \sum_{j=1 \text{ to } m} (a_{ij} - c_j)^2 = 1$$

Easy to do:

- Divide the x-coordinate of each landmark by the centroid size.
- Divide the y-coordinate of each landmark by the centroid size.

Removing Rotational Differences

Configurations of the same number of (homologous) landmarks can be rotated so that they show minimal offset in location of homologous landmarks.

- "Least squares" fit.

To find the angle of rotation (θ) to bring the target form T into optimal alignment with the reference form R:

$$\theta = \text{arctangent} \left(\frac{\sum_{j=1 \text{ to } k} Y_{Rj}X_{Tj} - X_{Rj}Y_{Tj}}{\sum_{j=1 \text{ to } k} X_{Rj}X_{Tj} + Y_{Rj}Y_{Tj}} \right)$$

Removing Non-Shape Differences

Location, size, and rotational differences between configurations are removed using these operations:

- Centering the configurations to common centroid coordinates.
- Rescaling to unit centroid size.
- Rotating to optimal (least squares) fit.

This is *Partial Procrustes superimposition.*

- Often extended into Generalized Procrustes Analysis (GPA) by iterative rotations around a recalculated mean form.

Configurations now differ *only* in shape.

Partial Procrustes Distance (D_p)

Represents the amount of difference in shape between two configurations.

Square root of the summed squared distances between homologous landmarks on two configurations following Partial Procrustes superimposition.

Shape Space

Morphospaces

There are many "varieties" of morphospace, differing in the types of operations performed on the raw data:

- Configuration space (raw data).
- Pre-shape space.
- Shape space (= oriented pre-shape space).
- Kendall's shape space (most refined).
- Tangent space.

Configuration Space

Raw landmark coordinates.

- Configurations differ in shape and non-shape.

For landmark configurations, the dimensionality of configuration space = *km*.

- *k* = number of landmarks.
- *m* = number of planes (2 or 3).

The location of any shape in that configuration space is specified by *km* components.

- These are the values in the landmark data matrix.
- Configurations are free to differ by *km* degrees of freedom.

Critical Point

Each landmark configuration is summarized by *km* coordinates, but has only one shape.

- Individual landmarks are *not* treated as separate variables.
- *The configuration is the datum.*
- Each configuration occupies one point in (*km*-dimensional) morphospace.

Shape Space

(Oriented pre-)shape space is the morphospace in which shapes are optimally compared.

- All non-shape differences between configurations have been removed.
  - Configurations have been centered, scaled to unit centroid size, and optimally rotated.
    - i.e., configurations have been placed in Partial Procrustes superimposition.
- Has $km - m - 1 - \{m(m - 1)/2\}$ degrees of freedom.

The nature of shape space is easiest to comprehend when dealing with landmark configurations of triangles.

Shape Space For Triangles

Configuration space for triangles has $km = 6$ dimensions.

Oriented pre-shape space for triangles has $km - m - 1 - \{m(m - 1)/2\} = 2$ dimensions.

- Two dimensions lost by translating to common centroid location.
- One dimension lost by rescaling to unit centroid size.
- One dimension lost by rotating to optimal (Partial Procrustes) fit.

Two degrees of freedom separate any two triangles in oriented pre-shape space.

Therefore oriented pre-shape space for triangles is 2-dimensional (a surface).

Shape space has no "edges", so the 2-dimensional surface can be envisioned as the (2-D) surface of a (3-D) hollow ball (or hemisphere).

- Radius of ball = 1 (unit centroid size).
- Ball centered on [0, 0, 0].

Every possible configuration of three landmarks (optimally superimposed) is represented as a point on the surface of the hemisphere.

- Reference shape (e.g., mean of all forms) lies at the pole.
- "Lines of latitude" on the surface represent shapes of equal distance from the reference shape.
- The location of each shape relative to the reference shape is given by two parameters (degrees of freedom).

Moving Beyond Triangles…

Oriented pre-shape space is a $[km - m - 1 - \{m(m - 1)/2\}]$-dimensional surface of a hypersphere centered on the origin and with a radius of 1.

All shapes (in Partial Procrustes superimposition) are represented by points on that surface.

Dimensionality of Shape Space

The dimensionality of configuration space = km.

The dimensionality of oriented pre-shape space = $km - m - 1 - \{m(m - 1)/2\}$.

- m dimensions lost by translating to common centroid location.
- One dimension lost by rescaling to unit centroid size.
- $[m(m - 1)]/2$ dimensions lost by rotating to optimal (Partial Procrustes) fit.

The location of any shape relative to another in oriented pre-shape space is specified by $km - m - 1 - \{m(m - 1)/2\}$ components.

- Shapes can differ by $km - m - 1 - \{m(m - 1)/2\}$ degrees of freedom.

Distance Between Shapes

The distance over the surface of the oriented pre-shape hypersphere between any two points is the *Procrustes distance* (ρ) between the shapes specified by those points.

- Equal to the angle (in radians) between the radii that connect the center of the hypersphere to the points multiplied by the length of the radius.
  - Since radius = 1 [unit centroid size], Procrustes distance ρ = angle between radii.
  - Values for ρ range from 0 to $\pi/2$.

But the shortest distance between the two points passes through the inside of the hollow hypersphere, not over the surface.

This chord is the *partial Procrustes distance* (D_p).

- We've met this before!
- Square root of the summed squared distances between homologous landmarks on two configurations following Partial Procrustes superimposition.

More On Procrustes Distances

The minimal distance between two forms at unit centroid size in oriented pre-shape space is the partial Procrustes distance (D_p).

But the distance can be further reduced if centroid sizes are not constrained to unity.

- The distance is truly minimized when the centroid size of one form is 1 and that of the other is $\cos(\rho)$.
- This is the full Procrustes distance (D_F).

Procrustes Distances (ρ, D_p, D_F)

The amount of difference in shape between two configurations can be quantified as:

- Procrustes distance (ρ).
- Partial Procrustes distance (D_p).
- Full Procrustes distance (D_F).

Fortunately, the three measures are related:

- D_p = chord of arc $\rho = 2\sin(\rho/2)$
- $D_F = \sin(\rho)$

Kendall's Shape Space

The configurations of all shapes can be rescaled to their minimal distance (D_F) from a given reference form.

- Centroid size of reference form = 1.
- Centroid size of every other form = $\cos(\rho)$, where ρ = Procrustes distance from reference form.

This defines a new hypersphere shape space of radius 0.5, tangent to oriented pre-shape space at the reference form.

- This is Kendall's shape space.

Oriented pre-shape space:

- Centered location, unit centroid size, rotation to minimize partial Procrustes distance (D_p) [and therefore Procrustes distance (ρ)].

Kendall's shape space:

- As above, but with centroid size adjusted to minimize full Procrustes distance (D_F).
- Configurations are in *full Procrustes superimposition* with respect to the reference form.
- Same dimensionality as oriented pre-shape space.

Shape Spaces and Tangent Space

Oriented pre-shape space and Kendall's shape space are curved (non-linear, non-Euclidean).

Most statistical tools assume a linear, Euclidean space.

It is typical to work in a Euclidean space tangent to (Kendall's) shape space in order to apply statistical tests.

Tangent Space

Working in tangent space comes at a cost in distortion of relative location of points away from the tangent point to shape space (reference form).

- The further the distance from the reference form, the more severe the distortion.
- Choice of reference form is important!
    - Typically the "average form" (consensus) of all configurations is used.

Summary

Summary of Shape Spaces

There are several "shape spaces", characterized by the operations performed on the raw landmark data and the number of degrees of freedom.

- Configuration space:
  - Configurations differ in location, size, rotation, and shape.
  - df = km
    - For 2D data, df = $2k$
- Oriented pre-shape space:
  - Configurations have common location (centered on centroid), common size (scaled to unit centroid size), and common orientation (rotated to minimize [partial] Procrustes distance).
  - Any remaining differences are in shape.
  - df = $km - m - 1 - \{m(m - 1)/2\}$
    - For 2D data, df = $2k - 4$
- Kendall's shape space:
  - As above, but with centroid size adjusted to minimize full Procrustes distance.
  - df = $km - m - 1 - \{m(m - 1)/2\}$
    - For 2D data, df = $2k - 4$
- Tangent space:
  - Projections of data from curved shape space into a linear space (tangent to shape space at the reference form).
  - df = $km - m - 1 - \{m(m - 1)/2\}$
    - For 2D data, df = $2k - 4$
  - Distances will be distorted.

Topic 3: Difference In Shape

Difference in shape between configurations:

- Superimposition methods.
- Visualizing difference in shape.
- Variation in shape.
- Statistical testing of difference in shape.

Case study #1 (continued).
Case study #2.

DIFFERENCE IN SHAPE

Difference in shape between configurations can be visualized in several ways, e.g.:

- Scatter plots of landmark coordinates.
- Vectors of landmark displacement.
- Deformation grids.

All visualizations of differences in shape require *superimposition* of landmark configurations as a first step.

SUPERIMPOSITION METHODS

What Is Superimposition?

Superimposition removes irrelevant (non-shape) differences in configuration data attributable to specimen orientation, size, and position.

- Reduces the dimensionality (number of shape variables).
- Reduces the degrees of freedom by which configurations can differ.

The Importance of Superimposition

The method of superimposition of landmark configurations determines:

- The visualization of shape difference between configurations.
- The quantification of shape difference between configurations.
- The range of statistical tests available for determining the significance of shape difference between configurations.

Superimposition Methods

There are several superimposition methods available:

- Baseline Methods:
  - Bookstein Registration (Two-point Registration).
    - Produces Bookstein coordinates (shape coordinates).
  - Sliding Baseline Registration.
    - Produces Sliding Baseline coordinates.
- Procrustes Superimposition.
    - Produces Procrustes coordinates.
    - Places the configurations in shape space.
- Resistant-Fit Methods.

BOOKSTEIN REGISTRATION

Select two landmarks to act as end-points of a baseline, which will be used to standardize all configurations.
Configurations are relocated, rotated, rescaled such that one baseline landmark has the coordinates [0, 0] and the other has the coordinates [1, 0].

- The baseline then defines a coordinate system within which all other landmarks can be considered (Bookstein coordinates, BC).

Translation

New x-coordinate of landmark *i*: $x'_i = x_i - x_A$

New y-coordinate of landmark *i*: $y'_i = y_i - y_A$

Rotation

New x-coordinate of landmark *i*: $x''_i = x'_i \cos\theta + y'_i \sin\theta$

New y-coordinate of landmark *i*: $y''_i = y'_i \cos\theta - x'_i \sin\theta$

… where $[\cos\theta = (x_B - x_A)/d]$ and $[\sin\theta = (y_B - y_A)/d]$

Rescaling

New x-coordinate of landmark *i*: $x'''_i = x''_i/d$

New y-coordinate of landmark *i*: $y'''_i = y''_i/d$

Combining Operations

Bookstein Shape x-coordinate of any landmark Z:

$$bcZ_x = \frac{(B_x - A_x)(Z_x - A_x) + (B_y - A_y)(Z_y - A_y)}{(B_x - A_x)^2 + (B_y - A_y)^2}$$

Bookstein Shape y-coordinate of any landmark Z:

$$bcZ_y = \frac{(B_x - A_x)(Z_y - A_y) - (B_y - A_y)(Z_x - A_x)}{(B_x - A_x)^2 + (B_y - A_y)^2}$$

Difference In Shape

Difference in shape is summarized by offset in the position of the free landmark(s).

Many configurations can be compared simultaneously. This is useful for:

- Checking digitizing error.
- Distribution of each free landmark should be circular.
- Identifying shape groupings.
- Identifying trajectories of landmark movement relative to the baseline.

Advantages of Bookstein Registration

Easy to interpret results (location/movement of landmark relative to baseline).

- Especially if the baseline is an important biological axis.
- Orientation of the baseline is respected.

For 2-dimensional data in Bookstein registration, there are $k - 2$ free landmarks and therefore $2k - 4$ free coordinate variables:

- Equals the dimensionality of shape space the configurations would occupy.
- Equals the number of degrees of freedom.
    - One lost to rescaling.
    - One lost to rotating.
    - Two lost to translation.
- Standard statistical tests (e.g., Hotelling's T^2) can be carried out to determine the significance of shape difference without discarding variables.

Disadvantages of Bookstein Registration (1)

Perception of results depends on choice of baseline:

- There is a transfer of variance of baseline landmarks to other landmarks.
    - Transfer is biased: the variance transferred depends on the distance from the baseline.
    - This can induce correlations among landmarks.
- Vectors of landmark displacement.

Choice Of Baseline

The observed difference in shape between configurations depends upon the choice of baseline.

- E.g., AB, AC, or BC.

Does Baseline Choice Matter to Results?

Scatter differs mainly by translation, rotation, and rescaling.

- See Bookstein (1991, pp. 130-133).

Rough guidelines:

- Don't define a baseline using landmarks which are hard to digitize.
- Results are easier to interpret if the baseline is a meaningful body axis.
    - One which doesn't show rotation.
- Make the baseline as long as possible.
    - Minimize the effect of localized shape variation.

Disadvantages of Bookstein Registration (2)

Configurations are rescaled to unit baseline length, not unit (or even the same) centroid size.

- Size differences between the configurations are not entirely removed.

Configurations are rotated to common baseline orientation.

- Rotational differences between the configurations are not entirely removed.

Shape coordinates do not differ solely in shape.

- Bookstein registration does not place configurations in shape space.
    - Even though it results in the same number of degrees of freedom.

SLIDING BASELINE REGISTRATION

Rescale: Rescale to unit centroid size.
Rotate: Fix baseline landmarks at $[x_1, 0]$ and $[x_2, 0]$.
Translate: Fix centroid at $[0, y]$.

Advantages of SBR

Easy to interpret results (location/movement of landmark relative to baseline).

- Especially if the baseline is an important biological axis.
- Orientation of the baseline is respected.

Baseline landmarks are free in the x-direction:

- Reduces the transfer of variance to other landmarks.

Configurations rescaled to unit centroid size, not unit baseline length.

- Size differences are factored out.

Disadvantages of SBR (1)

Perception of results depends on choice of baseline.

- Baseline landmarks fixed in y-coordinate:
    - There is still some transfer of variance to other landmarks.
        - Biased: the variance transferred depends on distance from baseline.
        - Can induce correlations among landmarks.
- Vectors of landmark displacement.

Configurations are rotated to common baseline orientation.

- Rotational differences between the configurations are not entirely removed.

Configuration centroids are not coincident.

- Translational differences between the configurations are not entirely removed.

SBR coordinates do not differ solely in shape.

- SBR does not place configurations in shape space.

SBR fixes the y-coordinates of two landmarks, resulting in $2k - 2$ free coordinate variables.

- But there are $2k - 4$ degrees of freedom.
    - One lost to rescaling.
    - One lost to rotating.
    - Two lost to translation.
- Must exclude two variables before conducting standard statistical tests to determine significance of difference in shape (e.g., Hotelling's T^2).
    - (Although resampling methods are applicable.)

PROCRUSTES SUPERIMPOSITION

Partial Procrustes superimposition.

- Rescaled to unit centroid size (minimizes partial Procrustes distance [D_p] and Procrustes distance [ρ]).
- Places configurations in shape space.
- Least-squares Theta Rho Analysis (LSTRA).

Full Procrustes superimposition.

- Centroid sizes adjusted to find true minimal distance between configurations (full Procrustes distance, D_F).
- Places configurations in Kendall's shape space.

Resistant-fit "Procrustes" superimposition.

- To account for Pinocchio Effect.

Partial Procrustes Superimposition

Translate: Center centroid at [0, 0].
Rescale: Rescale to unit centroid size.
Rotate: Minimum root summed squared distance between corresponding landmarks on configurations.

- Minimize partial Procrustes distance between configurations.

Advantages of Partial Procrustes Superimposition

No baseline:

- All landmarks free to move in all directions:
  - No transfer of variance.
- Perceptions not baseline-dependent.

Configurations differ only in shape:

- Size factored out (unit centroid size).
- Rotation factored out (minimal partial Procrustes distance).
- Position factored out (centered).
- Configurations lie in true shape space!

Disadvantages of Partial Procrustes Superimposition

No landmarks pinned in location, resulting in $2k$ free variable coordinates.

- But there are $2k - 4$ dimensions in shape space and degrees of freedom.
- Standard statistical tests (e.g., Hotelling's T^2) cannot be applied to determine significance of difference in shape.
  - (But resampling methods are applicable.)
  - ($2k - 4$ warp scores can be generated following TPS analysis.)

The configuration is free to rotate in order to minimize partial Procrustes distance.

- The orientation of biological axes are not respected.
- Harder to interpret results.

RESISTANT-FIT METHODS

Pinocchio Effect

Shape difference/change may be localized to one or a few landmarks.

Resistant-Fit Methods

Conventional Procrustes methods rotate configurations by minimizing the root summed squared distance between landmarks.

- Sensitive to large displacements at few landmarks.
    - Transfer of variance to all landmarks.
    - Looks unreasonable.
    - Can induce covariances.

Resistant-fit methods rotate configurations by more robust optimization criteria.

- Less sensitive to large displacements at few landmarks.

There are many robust optimization criteria (see Press et al., 1988).

The most commonly used is Resistant-Fit Theta Rho Analysis (RFTRA).

- Uses "repeated medians" as the optimization for rescaling and rotation.
    - See Zelditch et al. (2004), pp. 119-122.

Advantages of RFTRA

No baseline:

- All landmarks are free to move in all directions:
    - No transfer of variance.
- Perceptions are not baseline-dependent.

Less sensitive to the Pinocchio Effect.

Disadvantages of RFTRA

Configurations do not differ solely in shape:

- Superimposition doesn't utilize the partial Procrustes distance.
- Configurations don't lie in shape space.

All landmarks are free, resulting in $2k$ free variable coordinates.

- But there are $2k - 4$ dimensions degrees of freedom.
- Standard statistical tests (e.g., Hotelling's T^2) cannot be applied to determine significance of difference in shape.
    - (But resampling methods might be applicable.)

The configuration is free to rotate.

- The orientation of biological axes are not respected.
- Harder to interpret results.

SUMMARY

The various superimposition methods can differ in:

- The observed scatter at landmarks.
    - Is there a transfer of variance?
    - Must a baseline be specified?
- The number of free variable coordinates.
    - Does this match the number of degrees of freedom?
    - Which statistical methods are appropriate?
- Whether or not the configurations lie in shape space.
    - Do non-shape differences remain?
- Whether or not the orientation of biological axes are respected.
    - Are the results easy to interpret?

VISUALIZING DIFFERENCE IN SHAPE

There are several ways to graphically depict difference in shape between configurations:

- Scatter plots of landmark coordinates.
  - Good for comparison of any number of configurations.
- Vectors of landmark displacement.
  - Good for comparison of two (groups of) configurations.
- Deformation grid (thin-plate spline).
  - Good for comparison of two (groups of) configurations.

All visualizations of differences in shape require superimposition of landmark configurations as a first step.

VECTORS OF LANDMARK DISPLACEMENT

For two configurations:

- Superimpose the configurations (using the method of your choice).
- Draw a vector linking the homologous landmarks in each configuration.
- The vector represents the displacement of that landmark between those configurations in that particular superimposition.

For two groups of configurations:

- Calculate the average (consensus) form for each group.
- Superimpose the consensus configurations (using the method of your choice).
- Draw a vector linking the homologous landmarks in each consensus configuration.
- The vector represents the average displacement of that landmark between those groups in that particular superimposition.

DEFORMATION GRIDS

Deformation grids take the information regarding shape difference at discrete points (landmarks) and from those data interpolate shape difference between those points (where no data were collected).

- Useful for visualizing difference in shape.

Based on the classic D'Arcy Thompson transformation grids.

The modern equivalent to the Thompson transformation grid is the *thin-plate spline*.

- Construction of the spline is far more mathematically rigorous.
- The deformation can be mathematically decomposed into "warps" for statistical analysis.
- See Zelditch et al. (2004, chapter 6).

The Metaphor of the Thin-plate Spline

1. One configuration is designated as the "reference form".
   - The other ("target") configuration will be compared to this.
2. The target configuration is placed in generalized Procrustes superimposition with the reference form.
   - All non-shape differences are removed.
   - Configurations lie in shape space.
3. Vectors of displacement are calculated at each landmark, joining the reference to the target form.
4. An orthogonal grid is fit over the reference form, "pinned" to it at the landmarks.
5. At each landmark, the vector of displacement is converted into a vertical displacement.
6. The orthogonal grid is deformed by inequalities of the vertical vectors.
   - Bending of the grid is minimized by a curve-smoothing spline function (see below).
   - The result is that the grid is minimally deformed to fit the data.

What is Being Minimized?

The deformed grid (spline) is a curved surface, and can be mathematically differentiated.

- First derivative: slope of the surface.
- Second derivative: rate of change in slope of the surface.

The second derivative is minimized by the smoothing function.

- "Bending energy" is minimized.
- The spline must pass through each landmark (the data).
- No sharp creases or folds are introduced into the spline unless required to fit the data.

Bending Energy and the Spatial Scale of Deformation

Bending energy is a function of the arrangement of landmarks on the reference form.

- It is harder to move landmarks that are close together in different directions.
    - The slope of the spline must change over a smaller distance.
- The more localized the shape difference, the more bending energy required.
- Minimizing bending energy means minimizing spatially localized deformation.

The Metaphor of the Thin-plate Spline

7. The deformed spline surface is projected back into two dimensions.
    - Changes in slope of the surface are represented as spatially localized deformations.
    - Since bending energy was minimized, localized shape change is not interpolated unless the data demand it.

Caveats Of The Thin-plate Spline

There are several things to be aware of before interpreting thin-plate spline surfaces.

- Landmarks provide the only data:
    - The spline interpolates (with a smoothing function) deformation between landmarks.
- Localized shape change in regions between landmarks cannot be detected.
    - How could they be? There are no data!
- Beware of landmarks at tips of long processes.
    - Shape change will be interpolated in regions with no tissue!
- Beware of the Pinocchio Effect.
    - The spline is inappropriate for change at a single landmark.

VARIATION IN SHAPE

Within-Group Variation

Variation in shape within a group can be quantified as the variance in Procrustes distance away from the mean form for the group.

- See Foote (1993).

$$\text{Variance in shape} = \frac{\sum (x_i - \bar{x})^2}{n - 1}$$

…where $x_i - \bar{x}$ is the Procrustes distance between configuration X_i and the mean form of the group.

CASE STUDY # 1
The Trilobite *Olenellus chiefensis*

Data Collection

From the images provided:

- Select a configuration of landmarks.
- Digitize those landmarks.
- Paste the landmark coordinates into Excel.

Data Formatting

In Excel:

- Select a baseline along the sagittal axis of the cephalon.
- Place each configuration in Bookstein registration using this baseline.
- Reflect landmarks from one side of the cephalon over the baseline onto the other side.
- Average the coordinates of all paired (off-baseline) landmarks.
- Rescale each configuration back to natural size.
- Edit the data.
- Create a TPS format file.

Data Exploration

Open the TPS file in CoordGen and explore the data:

- Try different superimpositions.

Check for digitizing or formatting errors.

- Use PCAGen.

Check for distortion in the tangent plane.

- Use tpsSmall (Rohlf website).

Quantify shape variation within the sample:

- DisparityBox.

STATISTICAL TESTING OF DIFFERENCE IN SHAPE

Following visualization of the landmark data, groups of specimens can often be seen to have (subtle) differences in shape.

But are those groups "different enough" to be considered different species?

- Statistical tests are required!

Statistical tests will give a confidence limit with which we can say that the null hypothesis is rejected.

- H_0: There is no difference in shape between these groups.
    - The groups were drawn from the same underlying population.

The type of statistical test that is valid to conduct depends on:

- The number of free variables in the data set.
- The number of degrees of freedom by which configurations can differ.

The two should match!

How Many Free Variables?

	Variables (2-D data)	Degrees of Freedom (2-D)	Shape Space?
Raw landmark coords.	$2k$	$2k$	No
Bookstein coords.	$2k - 4$	$2k - 4$	No
Sliding baseline coords.	$2k - 2$	$2k - 4$	No
Procrustes coords.	$2k$	$2k - 4$	Yes
Warp scores	$2k - 4$	$2k - 4$	Yes

TESTS BASED ON BOOKSTEIN COORDINATES

Do the groups differ in their means over all variables (analyzed simultaneously)?

- To compare two groups, use Hotelling's T^2 test.
    - A multivariate form of the Student's *t*-test.
- To compare more than two groups, use MANOVA (e.g., Wilk's Λ).

Student's Two-Sample t-test

A parametric test to determine whether the difference in means between two samples differs significantly from zero.

- H_0: No significant difference between sample means.

Assumptions:

- Both samples are normal.
- Both samples have equal variance.
    - (A modified version exists if variance is not equal.)

H_0: No significant difference between the sample means.

- The two samples were drawn from the same underlying population.

To test the null hypothesis:

- Calculate the t-statistic (= [difference between means] divided by [SE_{diff}]).
- Find the critical value at (95%) confidence using a t-table.
    - df = sum of sample sizes - 2
- If the observed t-statistic is larger than the critical value, then H_0 is rejected at (95%) confidence.

Hotelling's T^2 Test

Multivariate equivalent to Student's t-test.

- More than one dependent variable.
- One categorical variable with two groups.

Do the groups differ in their means (when all dependent variables are considered simultaneously)?

- H_0: There is no difference between the group means.

See Zelditch et al. (2004, pp. 218-219, 240).

- There is a mistake in the equation for T^2.

MANOVA

A multivariate equivalent to ANOVA.

- More than one dependent variable.
- One categorical variable (two or more groups).
- Equivalent to Hotelling's T^2 test for more than two groups.

Do the groups differ in their means (when all dependent variables are considered simultaneously)?

- H_0: There is no difference between the group means.

See Zelditch et al. (2004, pp. 218-221).

Tests Based On Bookstein Coordinates

There are several caveats of using Bookstein coordinates:

- The fixed baseline landmarks must be excluded prior to analysis.
    - Data are thrown away!
- Some of the difference between forms is attributable to non-shape.
    - The forms do not lie in shape space.
- The results may depend on choice of baseline.

Sample size must be at least $2k - 4$ for 2-D data.

TESTS BASED ON PROCRUSTES COORDINATES

The number of free variables exceeds the number of degrees of freedom by 4, so standard statistical tests are inappropriate.

- Procrustes coordinates cannot be used as free variables in standard statistical tests.
    - (Resampling methods are appropriate.)

To test for difference in shape between groups, use Goodall's *F*-test.

Goodall's *F*-test

A statistical test analogous to (M)ANOVA, specially adapted for Procrustes coordinates.

- A test of difference in shape between groups performed in shape space rather than tangent space.
- H_0: The groups do not differ in mean shape.

The test is based on partial Procrustes distance.

- Measures deviations from group and grand means as sums of squared Procrustes distances.

Goodall's *F*-statistic is the ratio of explained (between-group) to unexplained (within-group) variation in these distances.

- Between-groups df = V(G – 1)
- Within-groups df = V(N – G)
    - V = dimensionality of shape space (= $2k - 4$ for 2-D data).
    - G = number of groups.
    - N = number of specimens.

Assumes that variance is equal at all landmarks.

- "Isotropic normal distribution" of landmark points about the mean.
- The large degrees of freedom are a result of this assumption.
- The assumption is typically not valid for biological data sets.

TESTS BASED ON WARP SCORES

Warp Scores

Warp scores are calculated through mathematical decomposition of the thin-plate spline.

Warp scores have several useful properties:

- Each describes a mathematically independent component of the shape difference between the reference and target forms.
- There are $2k - 4$ warp scores, which equals the degrees of freedom of the shapes in shape space.
    - Standard statistical analyses can be conducted on the warp scores.

There are two kinds of warp scores:

- Uniform warp scores (= explicit uniform terms).
- Partial warp scores (relating to non-uniform deformation).

Styles of Deformation

The styles of deformation that are possible for a given reference configuration fall into two categories:

- Those requiring no bending energy:
  - Uniform (affine) transformations.
    - Parallel lines on the TPS deformation grid are left parallel.
    - Shape difference is uniform across the configuration.
- Those requiring bending energy:
  - Non-uniform (non-affine) transformations.
    - Parallel lines on the TPS deformation grid are bent.
    - Shape difference is not uniform across the configuration.

Uniform Transformations

For two dimensional data there are six mutually independent ways to cause offset in landmark location between forms without bending the deformation grid lines.

- Four do not alter shape (*implicit* uniform deformations):
  - Translation along the *x*-axis.
  - Translation along the *y*-axis.
  - Rescaling.
  - Rotation.
- Two do alter shape (*explicit* uniform deformations):
  - Compression/dilation.
  - Shear.

Uniform Warps

However, the reference and target forms were placed in partial Procrustes superimposition prior to calculation of their shape differences.

- All non-shape differences (translation in *x*, translation in *y*, size, and rotation) had already been removed.
- The configurations could therefore only differ in the explicit uniform components.
  - Compression/dilation.
  - Shear.

Only two (not six) terms are actually needed to describe the remaining uniform components of shape difference.

- These terms describe the contribution of compression/dilation and of shearing to the total deformation.

Non-Uniform Transformations

Deformations may involve bending (flexing, warping) of the spline surface relative to the reference surface.

- Parallel lines do not remain parallel.
  - "Bending energy" is required, proportional to the amplitude of flexure and the spacing between landmarks.

These are "non-uniform" styles of deformation.

Bending Energy

For smooth, non-uniform deformation, the slope of the surface between landmarks is not uniform.

- The slope changes as a function of proximity to the landmarks.
- The "bending energy" required to achieve a given change in slope is inversely proportional to the spatial scale over which that change in slope must be accomplished.

Partial Warps

A partial warp defines non-uniform deformation of the reference form at a particular spatial scale.

- Deformation at that spatial scale is mathematically independent of deformation at all other spatial scales.
- Partial warps describe mathematically independent modes of non-uniform deformation of the reference form.
    - "Idealized modes of bending".

Partial warps have no magnitude or direction.

- They are a function of the reference form alone.
- A magnitude and a direction for each partial warp is imparted when the reference form is compared to a target form.
    - These are expressed in the *partial warp scores* for that partial warp in that comparison.

Partial Warp Scores

A *partial warp score* represents the actual contribution of a particular partial warp to a particular shape comparison.

- Each partial warp score is expressed as two components:
    - Magnitude of deformation in the *x*-direction along the axis of shape change described by the partial warp.
    - Magnitude of deformation in the *y*-direction along the axis of shape change described by the partial warp.
    - For 2-D data there are $k - 3$ partial warps, and therefore $2k - 6$ partial warp score components.
- Together, the two components of a partial warp score define the magnitude and direction of deformation along that partial warp axis for that particular shape comparison.

Warp Scores

The total difference in shape between the reference form and a target form is therefore described by $2k - 4$ parameters.

- $2k - 6$ non-uniform terms.
    - Partial warp scores in the *x*- and in the *y*-direction for each of the $k - 3$ partial warps.
- 2 uniform terms.
    - Difference in shape resulting from uniform compression/dilation and shear.

The number of free parameters (non-uniform plus uniform terms) now equals the number of degrees of freedom by which the configurations differ in shape space.

- Statistical tests are appropriate!

Tests Based On Warp Scores

The statistical significance of any shape difference between groups using warp scores could be determined by:

- Hotelling's T^2 test.
- Canonical variates analysis (or discriminant function analysis).
    - Can groups be statistically distinguished by their warp scores?
    - Can also be used to test assignment of specimens to groups.

Critical Point # 1

In any analysis of warp scores, all configurations must be compared to the same reference form.

- Suppose configuration A is compared to reference form X, and configuration B is compared to reference form Y.
    - The different reference forms will have different partial warps.
    - The partial warp scores for configurations A and B are not homologous.
        - They don't refer to the same partial warps!

Critical Point # 2

An individual warp is a mathematical construct, not a biological feature.

- Although partial warps and uniform terms of any given shape transformation are mathematically independent, they cannot be treated as biologically independent.
    - The observed displacement at any landmark is described by the combined operation of *all* warps.
- Individual warp scores cannot be treated in isolation.
    - Statistical tests must include all warps together (uniform plus non-uniform).

Critical Point

Geometric morphometrics will assist with description of landmark-based shape and shape change.
There is more to morphology than landmark-based shape!

- Outlines.
- Qualitative features:
    - Ornament.
    - Presence/absence of features.

For many paleobiological questions these other aspects of morphology must be considered, too!

CASE STUDY # 2

Comparing Two Samples: *Olenellus gilberti* versus *Olenellus chiefensis*

Useful Software

For basic data exploration:

- CoordGen.
- PCAGen.
- tpsSmall.

For visualizing shape difference between groups:

- TwoGroup (load group files separately).
- PCAGen (with "Load Group Membership List").

For testing shape difference between groups:

- TwoGroup.
- CVAGen (also checks specimen assignments).

To quantify/compare shape variation within/among groups:

- DisparityBox.

Topic 4: Allometry

Allometry - The relationship between size and shape:

- The importance of allometry.
- How to identify allometry.
- Describing ontogenetic shape change.
    - Pattern of shape change.
    - Rate of shape change.

Case study #3.

Why Do Individuals Differ In Shape?

Organisms differ in shape for many reasons:

- Phylogeny.
- Age (ontogeny).
- Dimorphism/polymorphism (sexual; ecological; functional).
- Environment (ecophenotypy).
- Mutation/variation.
- Disease/injury.

Allometry

Allometry refers to a non-zero relationship between size and shape.

This topic will cover:

- The importance of allometry.
- How to identify allometry.
- Describing ontogenetic shape change.

The next topic will cover:

- Comparing trajectories of shape change.
- Removing the effects of allometry.
- Comparing shapes at standard size.

THE IMPORTANCE OF ALLOMETRY

A relationship between size and shape can be a pleasure:

- What are the ontogenetic growth dynamics of this species?
- Do these two species share the same ontogenetic growth dynamics?
- How have growth dynamics been evolutionarily modified?

A relationship between size and shape can be a pain:

- Do these groups have different mean shapes because they are different species, or because they are different mean ages?
- Failure to take a correlation between shape and size into account will lead to:
    - A higher estimate of within-group disparity (variance).
    - A higher chance of incorrectly failing to reject the null hypothesis of no significant difference in mean shape between groups (type II error).

HOW TO IDENTIFY ALLOMETRY

There are several ways to test whether there is a relationship between shape and size:

- Regress Procrustes distance (away from the smallest form) against size.
- Regress shape variables (warp scores) against size:
    - One at a time (bivariate regression).
    - All at once (multivariate regression).

If the correlation is significant, then allometry exists!

- (And it virtually always does!)

Identifying Allometry

If there *is* allometry in the data:

- Shape does change as a function of size.
- There is ontogenetic shape change.

The next step is to investigate the nature of the pattern of ontogenetic shape change.

Trajectory Of Shape Change

The pattern of shape change followed by an organism during its ontogeny is summarized by the path through multidimensional space traced by the shape variables (regressed against size).

- This is the *ontogenetic trajectory* of shape change.

For 2-dimensional data, shape has $2k - 4$ degrees of freedom.

For statistical comparison, the trajectories of shape change must each be described by $2k - 4$ variables:

- Bookstein coordinates.
- Warp scores (uniform plus non-uniform terms).

Tracing the ontogenetic trajectory of shape change through morphospace means regressing those $2k - 4$ variables against an independent scalar (e.g., log centroid size).

- This is a multivariate regression.

Bivariate Regression

The relationship between the dependent variable (Y) and independent variable (X) is given by:

$$Y = bX + a + \text{error}$$

Multivariate Regression

The relationship between the dependent variable (Y) and univariate independent variable (X) is still given by:

$$Y = bX + a + \text{error}$$

…only now Y is a multivariate shape descriptor $[Y_1, Y_2, \ldots, Y_{2k-4}]$, and b, a, and the error term have $2k - 4$ components.

Predicted multivariate response:

$$[Y_1, Y_2, \ldots, Y_p] = (b_1, b_2, \ldots, b_p)X + (a_1, a_2, \ldots, a_p)$$

Is the Trajectory Linear?

For most statistical purposes, the pattern of shape change through the portion of ontogeny covered by the sample must be linear.

- The (multidimensional) trajectory of ontogenetic shape change must be linear.
    - Examine the residuals of shape versus size (or shape versus shape) regressions.

If the trajectory of shape change is linear over the portion of ontogeny covered by the sample:

- Typical statistical procedures can be applied.
- The description of shape change is much simpler.

If it is not linear, try breaking it into linear sections.

- See Webster et al. (2001).

DESCRIBING ONTOGENETIC SHAPE CHANGE

Any of the standard visualizations of difference in shape between forms can be used to describe difference in shape resulting from ontogenetic shape change.

- E.g., plot vectors from small (young) to large (old) specimens.

Developmental "rate" (of shape change relative to size) can also be calculated.

Developmental Rate

The rate of shape change during ontogeny is the slope of the regression line between the "amount of shape difference achieved*" and developmental time (or size).

- *Summarized by the Procrustes distance away from the smallest (and hopefully developmentally youngest) form.

Critical Point

When regressed against size, the slope is telling us the rate of change in shape *relative to size* (not time).

- The relationship between developmental time and size is often not known.
    - The relationship may not be linear.
    - Size doesn't even always increase with age!

Rate of shape change relative to size and relative to time should not be confused!

Describing Ontogenetic Shape Change

Remember that we are paleobiologists, not mathematicians!

Always frame the description in biological terms, not mathematical jargon.

- Relate shape change to an understandable size measure, not just centroid size.
- Describe deformations in terms of anatomy, not landmarks.

CASE STUDY # 3
The Ontogeny of the Trilobite *Olenellus chiefensis*

Data Analysis

Use the techniques we have discussed to investigate the shape of *Olenellus chiefensis*:

- Is there allometry in the data set?
- If so, is the ontogenetic trajectory reasonably linear?
- If so, describe the rate and pattern of ontogenetic shape change followed by the species.
- Estimate shape variance (removing the effects of allometry if necessary).

Use the terminology sheet for assistance with the anatomy!

Look at:

- Coordinate data:
    - Bookstein coordinates.
    - SBR coordinates.
    - Procrustes coordinates.
- Procrustes distances.
    - Which reference form will you use?
- Warp scores.
    - Which reference form will you use?
- Principal component scores.

Useful Software

To generate a reference form other than the consensus of all specimens:

- Use CoordGen.

To calculate warp scores away from a reference form:

- Use Regress (load in reference form).
- Use PCAGen (reference = consensus of all).

To calculate Procrustes distance away from a reference form:

- Use Regress (load in reference form).

Topic 5: Comparing Ontogenetic Trajectories

Comparing ontogenetic trajectories:

- Trajectories of shape change.
- Comparing trajectories of shape change:
  - "Angle between ontogenies".
  - Rates of shape change.

Removing the effects of allometry:

- Comparison of shape at standard size.
- Comparison of shape variation at standard size.

Case Study #4: Simple ontogenies, two species.
Case Study #5: Simple ontogenies, unknown number of species.

Comparing Patterns of Shape Change

It is often very useful to compare the pattern of shape change in one sample with the pattern of shape change in another sample.

- If the samples differ in mean shape and mean size, comparison of patterns of shape change may help determine whether they differ in mean shape *because* they differ in mean size.
- Comparison of patterns of ontogenetic shape change between species can give insight into evolutionary processes.
  - E.g., Webster & Zelditch (2005).

The degree to which two samples share the same pattern of ontogenetic shape change can be assessed by statistically comparing their ontogenetic trajectories.

TRAJECTORY OF SHAPE CHANGE

The pattern of shape change followed by an organism during its ontogeny is summarized by the path through multidimensional space traced by the shape variables (regressed against size).

- This is the *ontogenetic trajectory* of shape change.

For 2-dimensional data, shape has $2k - 4$ degrees of freedom.
For statistical comparison, the trajectories of shape change must each be described by $2k - 4$ variables:

- Bookstein coordinates.
- Warp scores (uniform plus non-uniform terms).

COMPARING ONTOGENETIC TRAJECTORIES

If two samples share the same pattern of shape change, then their ontogenetic trajectories will be parallel (and maybe even coincident) through morphospace.

- The degree of parallelism between trajectories of shape change can be quantified as an *angle* between them.

THE ANGLE BETWEEN ONTOGENIES

The angle between ontogenies represents the degree of parallelism in their respective patterns of shape change.

- An angle of 0º means the trajectories are parallel (share the same pattern of shape change).
- The larger the angle, the more dissimilar the trajectories are in terms of patterns of shape change.

The angle is calculated as the arccosine of the vector correlation (R_V) between the multivariate trajectories.

- R_V = the dot (inner) product of the normalized vectors of regression coefficients.

Determining the angle involves several steps:

- Calculate the ontogenetic vector for each group.
  - Regression coefficients of warp scores or Bookstein coordinates on lnCS.
- Normalize each vector to unit length.
  - Square root of the summed squared coefficients = 1.
- Calculate the dot (inner) product of the two normalized vectors.
  - This is the vector correlation (R_V).
- The arccosine of this dot product is the angle between the vectors.

CRITICAL POINT

When comparing two trajectories of shape change based on warp scores, the warp scores for both trajectories *must be calculated from the same reference form*.

- The choice of that reference shouldn't matter to results (but you should always check!).
- Typical options are:
  - The consensus of all specimens.
  - The smallest form of the first group.
  - The smallest form of the second group.
  - The consensus of the smallest forms of both groups.
- As always, check for distortion in tangent space!

TESTING THE SIGNIFICANCE OF THE ANGLE

Is the angle between the ontogenies larger than you would expect by chance?

- Could an angle that large have been obtained by chance, by random variation within each group?

This can be determined by a (complicated) bootstrapping procedure.

BOOTSTRAPPED ANGLES

The trajectory of shape change for a given sample has error associated with it.

- The residuals from the regression of shape on size.

Each specimen in the original sample has:

- A size.
- A predicted shape (given its size and the regression for that sample).
- A multidimensional set of residuals (with $2k - 4$ elements), describing how far off the predicted shape it lies (given its size).

HOW THE BOOTSTRAP WORKS

We have two groups (A and B) with sample sizes a and b, respectively (where $a \geq b$).
For the larger of the two groups (A):

- Randomly assign (with replacement) the sets of residuals from all specimens within the group to the expected values of shape (derived from the original regression) at the values of size observed in the real data for that group.
- Calculate the "new shape" of each specimen.
  - Expected shape at that size, plus the (randomly assigned) residual.
- You now have a new trajectory (A', n = a), differing from the original (A) in terms of the distribution of "slop" around the regression.
- Do this again for group A, but this time construct the new trajectory (A'') using only b specimens.
- You now have two new trajectories for the group (A', n = a; A'', n = b), which differ in terms of the distribution of "slop" around the regression (and sample size if $a \neq b$).
- Calculate the angle between the trajectories A' and A''.
- Repeat this a huge number of times for the group.
  - Generates a distribution of within-group angles for group A.
- Repeat steps 1 through 7 for the smaller of the two groups (B), but generating new trajectories (B', B'') each of sample size b.
  - Generates a distribution of within-group angles for group B.
- The statistical significance of the between-group angle of the real data is given by comparing it to the 95th percentile of the range of within-group angles for each group.

- If the between-group angle exceeds the 95th percentile of within-group angles for *both* groups, then it is deemed significant.

THE ANGLE BETWEEN ONTOGENIES

If the angle between the ontogenies of two groups is significant, then difference in shape cannot be attributed to any difference in mean size*.

- Extrapolation of shape along one trajectory will not place configurations along the other trajectory.
- (*Unless they lie along different portions of a non-linear trajectory.)

COMPARING RATES OF SHAPE CHANGE

The rate of shape change (relative to size) can be compared between groups using an ANCOVA.

- The homogeneity of slope test tells you if the rates are equal.
  - Dependent variable = Procrustes distance*
  - Independent variables = lnCS, group, constant
  - Categorical variable = group
  - Interaction term = lnCS with group

*The amount of shape difference for specimens within a particular group can be quantified as the Procrustes distance of those specimens away from the smallest form for that group.

- No need to use the same reference form for both groups.

Removing the Effects of Allometry: Size Standardization

The regression of shape on size allows prediction of shape at any size.
Specimens within a group can be methodologically "slid" along the trajectory of shape change for that group to any desired size, and their shape at that size can be predicted.
This allows the shape of all specimens to be predicted at a common size.

- "Size standardization".
- Removes allometric difference in shape between configurations.

ADVANTAGES OF SIZE STANDARDIZATION

Size standardization removes the effect of difference in shape resulting from difference in size.
Removal of this effect gives:

- A better estimate of the mean shape of the group at that size.
- A better estimate of the "standing" variance in shape within the group at that size.

Non-dynamic shape comparison between groups can be made if those groups are standardized to homologous size.

- The same absolute size (e.g., CS = 4).
- The same point in development (e.g., onset of maturity).
  - This might not occur at the same size in different groups.
  - Different groups can be standardized to different size values.

CRITICAL POINT #1

The trajectory of shape change must be linear over the extrapolation range.

- It is dangerous to extrapolate shape prediction beyond the observed size range.

In multi-group comparisons, this applies to each group.

CRITICAL POINT #2

The residuals stick with the specimens.

- Mismatch between predicted shape and observed shape at actual size = mismatch between predicted shape and "observed" shape at standardized size.
- There is no allowance for change in variance as a function of size.
  - Could lead to overestimation of variance when standardized to a small size, or underestimation of variance when standardized to a large size (or vice versa).

In multi-group comparisons, this applies to each group.

Case Study # 4

Comparing Simple Ontogenies: *Olenellus gilberti* versus *Olenellus chiefensis*

The aim of this case study is to familiarize you with the basic techniques of comparing ontogenies:

- Data exploration.
- Data analysis:
- Patterns of shape change within each species.
- Differences in ontogeny between the species:
    - Patterns of shape change.
    - Rate of shape change.
    - Variance.

USEFUL SOFTWARE

To generate a reference form other than the consensus of all specimens:

- Use CoordGen.

To calculate warp scores away from a reference form:

- Use Regress (load in reference form).
- Use PCAGen (reference = consensus of all).

To calculate Procrustes distance away from a reference form:

- Use Regress (load in reference form).

To calculate the angle between ontogenetic trajectories (including confidence limits):

- Use VecCompare (load in BC or warp scores for each group).

To size-standardize a data set:

- Use Standard.

Case Study # 5

Comparing Simple Ontogenies: Unknown Number of Species

You are now in at the deep end! A new locality with an unknown number of species has been discovered. Your mission is to come up with a report detailing:

- How many species there are.
- The growth patterns of each species.
- How the species differ in shape and variance (static and dynamic comparison).

DATA ANALYSIS

You will need to use the following programs:

- CoordGen
- PCAGen
- CVAGen
- Regress
- VecCompare
- DisparityBox
- Standard
- TwoGroup
- tpsSmall
- Excel
- Statistics package

Topic 6: Disparity

Disparity refers to the "range of morphology" expressed by a (multi-species) sample.

- The range of morphology expressed by a single species is typically referred to as "variation".

Disparity and variation are *relative* measures.

- Single specimens do not have disparity or variation.

See Foote (1997), Ciampaglio et al. (2001), Wills (2001) and Zelditch et al. (2004, chapter 12).

Quantifying Disparity

Disparity (and variation) can be empirically quantified using many types of data:

- Categorical (attribute; discrete) variables.
- Ordered categorical (ranked) variables.
- Meristic (discontinuous) variables.
- Continuous variables (measured or derived).

Different analytical techniques are required for different types of data.

- See Wills (2001) for an excellent review.

This topic focuses on quantifying disparity (and variation) using continuous morphometric data.

- Traditional morphometric data.
- Geometric morphometric data.

"Phylogenetic" Disparity

In studies conducted within an explicit phylogenetic framework, disparity is quantified with reference to cladogram topology.

- Reversals/homoplasy are treated as such.
- Variables often correspond to (discrete) cladistic characters.
    - E.g., number of state changes within a clade; patristic dissimilarity.

"Phenetic" Disparity

In phenetic studies (conducted without reference to an explicit phylogenetic framework), disparity is quantified independently of phylogenetic trajectory.

- No "penalty" for reversals/homoplasy.
- No inference regarding the causation behind similarity.
    - Could be inheritance from a common ancestor or convergence.

Phylogenetic Versus Phenetic Disparity

Important theoretical distinctions arise when interpreting "phylogenetic" versus "phenetic" disparity. The hypothesis to be tested should determine the approach taken.

Theoretical Morphospace

A theoretical morphospace is constructed by axes derived from the range of theoretically possible morphologies.

- The morphospace is delimited *prior to* data analysis.
- Addition, removal, or substitution of specimens from the sample does not change the nature of the morphospace.

Few published studies have attempted to construct theoretical morphospaces.

- E.g., Raup (1966, 1967), McGhee (1991, 1999), Thomas & Reif (1993).

Empirical Morphospace

An empirical morphospace is constructed by axes derived from the data.

- The morphospace is delimited *after* data analysis.
- Addition, removal, or substitution of specimens from the sample can change the nature of the morphospace.

Most published studies of disparity involve empirical morphospaces.

Theoretical Versus Empirical Morphospace

Whether the morphospace is delimited by theory or empirical data can be an important consideration in disparity studies.

The hypothesis to be tested should determine the approach taken.

Important Distinctions In Disparity Studies

This topic focuses on quantification of disparity in empirical morphospaces using continuous morphometric variables, without reference to a phylogenetic framework.

- The methods can easily be applied to phylogenetic studies.
- The methods can often be extended to (or analogous methods exist for similar analysis of) categorical, ordered categorical, or meristic variables.
    - See Wills (2001).

Quantifying Disparity

Disparity is typically investigated in two general ways:

- Quantification of the *amount of morphospace occupied* by a sample.
    - Area or volumetric measures.
    - A function of the periphery of the sample in morphospace.
    - Sensitive to sample size.
- Quantification of the *average dissimilarity* between forms in morphospace.
    - Distance-based measures.
    - A function of the dispersion within the sample in morphospace (typically measured as a variance).
    - Less sensitive to sample size.

Amount Of Morphospace Occupied

Sources of raw data:

- Traditional morphometric data:
    - Length measurements.
- Geometric morphometric data:
    - Interlandmark distances.
    - Landmark configurations.

Standardizing Data

If disparity of shape is the focus of the study, then size differences between specimens should be removed by rescaling variables prior to analysis.

- For traditional morphometric data:
    - Scale measurements relative to a standard length.
    - Scale measurements on each specimen to the square root of the sum of the squared variables for that specimen.
- For geometric morphometric data:
    - Scale interlandmark distances relative to unit baseline length.
    - Scale configurations to unit centroid size.

Consideration should be given as to whether the (rescaled) variables should be standardized in any way.

- Variables can be "standardized" by basing analyses on:
    - Normalized ranges.
    - Normalized variances.
    - Log-transformed variables.
    - A correlation (rather than covariance) matrix.

The hypothesis to be tested should determine the need for any standardization.

Defining A Morphospace

An empirical morphospace can be defined by:

- Scores on the original variables (rescaled and standardized if appropriate).
- PC axes, following eigenanalysis of the variance-covariance (or correlation) matrix of the original variables.
    - Data reduction possible.
- CV axes, following discriminant functions analysis or CVA of the original variables.
    - Data reduction possible.
    - But axes are then defined on the basis of variation between groups.
- Uniform and principal warp axes, following thin-plate spline analysis of landmark configurations.
    - Axes are dependent upon choice of reference form.
- Relative warp axes, following RWA of the warp scores.
    - Data reduction possible.
    - Axes are dependent upon choice of reference form.

Amount of Morphospace Occupied

The amount of morphospace occupied by a sample can be quantified as a:

- Rectangular hypervolume.
- Convex hull.

Rectangular Hypervolume

A cube or hypercube is fitted around the observed data.

- A "box-wrapping" technique.

The (hyper)volume of the (hyper)cube is a function of the ranges (or variances) on all dimensions.

- The product of all ranges (or variances).
    - Sometimes expressed as the k^{th} root (k = number of dimensions).
- The sum of all ranges (or variances).
    - (Not really a volume.)

See Wills (2001, pp. 91-93).

"Rectangular Hypervolume"

Total variance = sum of univariate variances of all dimensions.

- Proportional to the mean squared Euclidean distance among points in morphospace.
- Proportional to the mean squared distance between each point and the overall centroid of the sample.

Disadvantages of the Rectangular Hypervolume

Assumes "hypercubic" data distribution.

- Often a poor assumption.
- Misfit worsens as more dimensions are included.
- For multi-group comparison, this assumption holds for all groups.
- See Wills et al. (1994) for a modified version involving calculation of hyper-ellipsoids.

No consideration of internal data distribution.

- The "walls" of the occupied hypervolume are defined by peripheral values.
- Sensitive to sample size.
- Sensitive to outliers.

Sensitive to the number of dimensions.

Sensitive to low range (or variance) along single dimensions when calculated as the product of ranges (or variances).

- Addition of dimensions can decrease hypervolume.

Axes defining a morphospace for all specimens may lie at an angle to axes optimally representing a less inclusive group.

- Compromises comparison of hypervolumes.

Convex Hull

A multidimensional "convex hull" is fit so as to minimally enclose all the data.

- The bounds of the hull are not determined by reference to the orientation of data cloud with respect to morphospace axes.
- No concavities in the "hull".
- A "shrink-wrapping" technique.

Calculation of the (hyper)volume of the (hyper)polyhedron is computationally intense.

- See Xu et al. (1998), Foote (1999, Appendix 6).

See Bookstein and Reyment (1989), Foote (1999), Wills (2001, pp. 94-95).

Advantages of the Convex Hull

Does not assume a "hypercubic" distribution.

- Misfit is less sensitive to number of dimensions included.
- For multi-group comparison, does not assume similar shape of morphological distribution across groups.

Disadvantages of the Convex Hull

Computationally intense.

Hard to calculate the overlap in morphospace occupation between samples.

- See Foote (1999).

No consideration of internal data distribution.

- The "walls" of the occupied hypervolume are defined by peripheral values.
- Sensitive to sample size.
- Sensitive to outliers.

Reducing Sensitivity To Outliers

Hypervolumes (of rectangular hypercubes or convex hulls) can be calculated for trimmed data sets:

- E.g., use 80% of specimens falling closest to centroid.
    - Foote (1991, 1999).

The sensitivity of the calculated hypervolume to extreme values can then be examined.

Reducing Sensitivity To Sample Size

Hypervolumes (of rectangular hypercubes or convex hulls) can be calculated for randomly culled data sets:

- Rarefaction analysis.
- Jack-knifed data.

The sensitivity of the calculated hypervolume to sample size can then be examined.

- E.g., Foote (1992).

AVERAGE DISSIMILARITY

Average dissimilarity is a measure of "average scatter" within a sample.

- The internal distribution of data is considered.
    - Less sensitive to outliers.
    - Less sensitive to sample size.

Average dissimilarity can be quantified as variance in an inter-specimen distance measure:

- Variance in Euclidean or Procrustes distance of specimens from the group centroid.
- Variance in Euclidean distance between all possible pairs of specimens.

See Wills (2001, p. 94).

Euclidean Distance

The Euclidean distance d between specimens 1 and 2 (measured over p variables) is quantified as:

$$d_{12} = \sqrt{(\sum_{j=1 \text{ to } p} (x_{j1} - x_{j2})^2)}$$

Within-Group Variation

For traditional morphometric data, variation within a group can be quantified as the variance in Euclidean distance of specimens away from the mean for the group.

- See Foote (1993b).

$$\text{Variance in shape} = \frac{\sum (x_i - \bar{x})^2}{n - 1}$$

…where $x_i - \bar{x}$ is the difference between specimen *i* and the mean of the group in variable *x*.

Within-Group Variation

For geometric morphometric data, variation in shape within a group can be quantified as the variance in Procrustes distance of specimens away from the mean form for the group.

$$\text{Variance in shape} = \frac{\sum (x_i - \bar{x})^2}{n - 1}$$

…where $x_i - \bar{x}$ is the Procrustes distance between configuration x_i and the mean form of the group.

Confidence Limits on Within-Group Variation

Bootstrap resampling permits estimation of confidence limits on the observed within-group variation.

- The original data set is resampled (with replacement) to estimate the range of disparity values possible under resampling.
    - Includes assessment of uncertainty in centroid location of the group.

Statistical Comparison Of Within-Group Variation

Bootstrapping permits statistical comparison of within-group variation between groups.

- H_0: The groups show no significant differences in variation.

To conduct the test:

- Calculate the variation for each group.
- Calculate the difference in variation between groups.
- Resample each group (with replacement), and recalculate the variations and differences between variations.
- Repeat the resampling a large number of times, and calculate the 95% confidence interval for the range of differences between groups.
- If zero is *not* included within this interval, then the null hypothesis is rejected at 95% confidence.

Contributions of Individual Groups To Overall Disparity

The influence of an individual group to overall disparity of a more inclusive group (measured as average dissimilarity) can depend on where the group lies in morphospace.

- An *increase* in sampled diversity of a group lying close to the grand mean of all groups can *decrease* average dissimilarity of the whole sample.

Disparity and Partial Disparity

Foote (1993b) presented a method by which the contribution of a particular group to the total morphological disparity of a more inclusive sample could be calculated.

- This is the *partial disparity* of the group within the total sample.

Total Morphological Disparity

The morphological disparity (MD) of a sample containing more than one group can be quantified as a variance:

$$MD = \frac{\sum_{i = 1 \text{ to } g} \sum_{j = 1 \text{ to } n} d_{ij}^2}{N - 1}$$

… where *g* = number of groups; *n* = number of specimens in group *i;* N = total number of specimens; and d_{ij}^2 = squared distance of the j^{th} specimen in group *i* from the grand centroid.

Partial Disparity

The contribution of group *i* to the total morphological disparity can be quantified as its partial disparity (PD):

$$PD_i = \frac{\sum_{j=1 \text{ to } n} d_{ij}^2}{N - 1}$$

… where n = number of specimens in group i; N = total number of specimens; and d_{ij}^2 = squared distance of the j^{th} specimen in group i from the grand centroid.

- Note that the partial disparity of group *i* is *not equivalent* to within-group variation of group *i*.

It follows that the total morphological disparity within the sample is equal to the sum of partial disparities of all included groups:

$$MD = \sum_{i=1 \text{ to } g} PD_i$$

Confidence Limits on Partial Disparity

Bootstrap resampling permits estimation of confidence limits on the observed partial disparity.

- The original data set is resampled (with replacement) to determine the range of partial disparity values possible under resampling.
    - Resampling is done at the specimen level, not the group level.
    - Includes assessment of uncertainty in centroid location of the group.
    - Includes assessment of uncertainty in location of the grand centroid.

Discreteness Of Groups In Morphospace

Calculate the mean pairwise distance between specimens within each group (W).

- Sometimes divided by the total number of variables compared.
- Relatively insensitive to sample size.

Calculate the mean pairwise distance between group centroids (A).

"Discreteness" of the groups in morphospace is quantified as A/W.

"Discreteness" can also be measured as the generalized distance (D^2; the square Mahalanobis distance).

- Requires estimation of variance-covariance matrices.
- Assumes equality of these matrices across groups.

Uses Of Disparity Measures

Comparison of intraspecific variability or interspecific disparity:

- Across environments.
- Through ontogeny.
- Through geologic time.
- Across preservational modes.

Comparison of disparity versus diversity patterns.

Measuring Variation

"Standing biological variation" within a species may be more accurately quantified if:

- Ontogenetic influences are controlled.
    - E.g., use only morphologically mature specimens.
    - E.g., size standardization to remove effects of allometry.
- Environmental influences are controlled.
    - E.g., use specimens from a single lithofacies.
- Temporal influences are controlled.
    - E.g., use minimally time-averaged collections.
- Taphonomic influences are controlled.
    - E.g., use noncompacted specimens.

The hypothesis to be tested should determine the need for controlled parameters.

References and Further Reading

Bookstein, F. L. 1989. "Size and shape": A comment on semantics. *Systematic Zoology* **38**: 173-190.

Bookstein, F. L. 1991. *Morphometric Tools for Landmark Data: Geometry and Biology*. Cambridge University Press. 435 pp.

Bookstein, F. L. 1996. Standard formula for the uniform shape component in landmark data. Pp. 153-168 *in* Marcus, L. F., M. Corti, A. Loy, G. J. P. Naylor, and D. E. Slice (eds.), *Advances in Morphometrics*. NATO ASI Series A: Life Sciences Volume 284. Plenum Press, New York.

Bookstein, F. L., and R. A Reyment. 1989. Microevolution in Miocene *Brizalina* (Formainifera) studied by canonical variate analysis and analysis of landmarks. *Bulletin of Mathematical Biology* **51**: 657-679.

Carroll, J. D., and P. E. Green. 1997. *Mathematical Tools for Applied Multivariate Analysis*. Revised Edition. Academic Press, San Diego. 376 pp.

Ciampaglio, C. N., M. Kemp, and D. W. McShea. 2001. Detecting changes in morphospace occupation patterns in the fossil record: characterization and analysis of measures of disparity. *Paleobiology* **27**: 695-715.

Dryden, I. L., and K. V. Mardia. 1998. *Statistical Shape Analysis*. John Wiley and Sons.

Elewa, A. M. T. (ed.). 2004. *Morphometrics: Applications in Biology and Paleontology*. Springer, Berlin. 263 pp.

Feldmann, R. M., R. E. Chapman, and J. T. Hannibal (eds.). 1989. *Paleotechniques*. Paleontological Society Special Publication No. **4** (reprinted 1997).

Foote, M. 1989. Perimeter-based Fourier analysis: A new morphometric method applied to the trilobite cranidium. *Journal of Paleontology* **63**: 880-885.

Foote, M. 1991. Morphologic patterns of diversification: Examples from trilobites. *Palaeontology* **34**: 461-485.

Foote, M. 1992. Rarefaction analysis of morphological and taxonomic diversity. *Paleobiology* **18**: 17-29.

Foote, M. 1993a. Discordance and concordance between morphological and taxonomic diversity. *Paleobiology* **19**: 185-204.

Foote, M. 1993b. Contributions of individual taxa to overall morphological disparity. *Paleobiology* **19**: 403-419.

Foote, M. 1997. The evolution of morphological disparity. *Annual Review of Ecology and Systematics* **28**: 129-152.

Foote, M. 1999. Morphological diversity in the evolutionary radiation of Paleozoic and post-Paleozoic crinoids. *Paleobiology Memoirs* **25** [Supplement to *Paleobiology* **25** (2)]: 1-115.

Kendall, D. 1977. The diffusion of shape. *Advances in Applied Probability* **9**: 428-430.

Kim, K., H. D. Sheets, R. A. Haney, and C. E. Mitchell. 2002. Morphometric analysis of ontogeny and allometry of the Middle Ordovician trilobite *Triarthrus becki*. *Paleobiology* **28**: 364-377.

Marcus, L. F., M. Corti, A. Loy, G. J. P. Naylor, and D. E. Slice (eds.). 1996. *Advances in Morphometrics*. NATO ASI Series A: Life Sciences Volume 284. Plenum Press, New York. 587 pp.

McGhee, G. R., Jr. 1991. Theoretical morphology: The concept and its applications. Pp. 87-102 *in* N. L. Gilinsky and P. W. Signor (eds.), *Analytical Paleobiology*. Paleontological Society Short Courses in Paleontology, Number **4**.

McGhee, G. R., Jr. 1999. *Theoretical Morphology: The Concept and its Applications*. Perspectives in Paleobiology and Earth History. Columbia University Press, New York. 316 pp.

Press, W. H., B. P. Flannery, S. A. Teukolsky, and W. T. Vetterling. 1988. *Numerical Recipes in C: The Art of Scientific Computing*. Cambridge University Press.

Raup, D. M. 1966. Geometric analysis of shell coiling: general problems. *Journal of Paleontology* **40**: 1178-1190.

Raup, D. M. 1967. Geometric analysis of shell coiling: coiling in ammonoids. *Journal of Paleontology* **41**: 43-65.

Rohlf, F. J., and F. L. Bookstein (eds.). 1990. *Proceedings of the Michigan Morphometrics Workshop*. University of Michigan Museum of Zoology Special Publication Number 2, Ann Arbor, Michigan. 380 pp.

Shaw, A. B. 1956. Quantitative trilobite studies I. The statistical description of trilobites. *Journal of Paleontology* **30**: 1209-1224.

Shaw, A. B. 1957. Quantitative trilobite studies II. Measurement of the dorsal shell of non-agnostidean trilobites. *Journal of Paleontology* **31**: 193-207.

Siegel, A. F., and C. J. Morgan. 1996. *Statistics and Data Analysis: An Introduction*. Second Edition. John Wiley and Sons, New York. 635 pp.

Slice, D. E. 2001. Landmark coordinates aligned by Procrustes analysis do not lie in Kendall's shape space. *Systematic Biology* **50**: 141-149.

Slice, D. E., F. L. Bookstein, L. F. Marcus, and F. J. Rohlf. 1996. Appendix I. A glossary for geometric morphometrics. Pp. 531-551 *in* Marcus, L. F., M. Corti, A. Loy, G. J. P. Naylor, and D. E. Slice (eds.), *Advances in Morphometrics*. NATO ASI Series A: Life Sciences Volume 284. Plenum Press, New York.

Small, C. G. 1996. *The Statistical Theory of Shape*. Springer.

Sneath, P. H. A. 1967. Trend-surface analysis of transformation grids. *Journal of Zoology* **151**: 65-122.

Sokal, R. R., and F. J. Rohlf. 1995. *Biometry.* Third Edition. W. H. Freeman and Company, New York. 887 pp.

Thomas, R. D. K., and W.-E. Reif. 1993. The skeleton space: a finite set of organic designs. *Evolution* **47**: 341-360.

Thompson, D'Arcy W. 1942. *On Growth and Form: A New Edition.* Cambridge University Press.

Webster, M., H. D. Sheets, and N. C. Hughes. 2001. Allometric patterning in trilobite ontogeny: testing for heterochrony in *Nephrolenellus*. Pp. 105-144 *in* M. L. Zelditch (ed.), *Beyond Heterochrony: The Evolution of Development.* Wiley-Liss.

Webster, M., and M. L. Zelditch. 2005. Evolutionary modifications of ontogeny: heterochrony and beyond. *Paleobiology* **31**: 354-372.

Wills, M. A. 2001. Morphological disparity: A primer. Pp. 55-144 *in* J. M. Adrain, G. D. Edgecombe, and B. S. Lieberman (eds.), *Fossils, Phylogeny, and Form: An Analytical Approach*. Topics In Geobiology, Volume **19**. Kluwer Academic/Plenum Publishers, New York.

Wills, M. A., D. E. G. Briggs, and R. A. Fortey. 1994. Disparity as an evolutionary index: a comparison of Cambrian and recent arthropods. *Paleobiology* **20**: 93-130.

Xu, Z.-B., J. S. Zhang, and Y. W. Leung. 1998. An approximate algorithm for computing multidimensional convex hulls. *Applied Mathematics and Computation* **94**: 193-226.

Zelditch, M. L., D. L. Swiderski, H. D. Sheets, and W. L. Fink. 2004. *Geometric Morphometrics for Biologists: A Primer*. Elsevier Academic Press, San Diego. 443 pp.

ADDITIONAL NOTES ON IMAGE PROCESSING:

In Adobe Photoshop:

To convert the image to grayscale:

- Image → Mode → Grayscale

To rotate the image:

- Image → Rotate Canvas

To adjust the image levels:

- Image → Adjustments → Levels

To crop the image:

- Rectangular marquee tool: draw a box around relevant portion of image (including scale bar).
- Edit → Cut
- File → New (size should be that of the cut portion of the image, by default)
- Edit → Paste

To adjust the image size:

- Image → Image size (adjust document size, using percent; Constrain Proportions MUST BE SELECTED)

To filter the image:

- Filter → Sharpen (to your preference)

In NIH Image or SCION Image:

To process the image:

- Process → Smooth (or → Sharpen)
- Adjust Brightness and Contrast in Map window.

ADDITIONAL NOTES ON DATA EXTRACTION:

In NIH Image or SCION Image:

To set the scale:

- Analyze → Options (set Digits Right of Decimal Point; select Perimeter/Length; deselect other options)
- Select ruler function from tool menu.
- Draw a line of known true length (over the scale bar).
- Analyze → Set Scale (select correct units; set Known Distance)
- Save image.

To extract length measurements:

- Analyze → Options (set Digits Right of Decimal Point; select Perimeter/Length; deselect other options)
- Select ruler function from tool menu.
- Draw a line over desired variable.
- Analyze → Measure
- Repeat for all variables.
- Analyze → Show Results
- Copy and paste results window into Excel.

To extract angle measurements:

- Analyze → Options (set Digits Right of Decimal Point; select Angle; deselect other options)
- Select angle function from tool menu.
- Draw a line over desired angle, clicking at start point, at the angle, and at finish point.
- Repeat for all variables.
- Analyze: Show Results
- Copy and paste results window into Excel.

To extract landmark coordinates:

- Analyze → Options (set Digits Right of Decimal Point; select X-Y Center; deselect other options)
- Select crosshairs function from tool menu.
- Click on location of desired landmark.
- Repeat for all landmarks.
- Analyze → Show Results
- Copy and paste results window into Excel.

CONSTRUCTING DATA FILES

1. Select a baseline on the sagittal axis (cephalic length or glabella length).

2. Calculate the distance between the landmarks at the anterior (A) and the posterior (B) of the baseline. This is the length of the baseline AB.

$$\text{Distance AB} = \sqrt{(((A_x - B_x)^2) + ((A_y - B_y)^2))}$$

3. Rotate, rescale, and translate the landmark configuration on each specimen using two-point registration (with baseline AB) to obtain the Bookstein (shape) coordinates. Landmark A will end up with coordinates (0, 0) and landmark B will end up with coordinates (1, 0).

To find bcZ_x:

$$bcZ_x = \frac{(B_x - A_x) \times (Z_x - A_x) + (B_y - A_y) \times (Z_y - A_y)}{(B_x - A_x)^2 + (B_y - A_y)^2}$$

To find bcZ_y:

$$bcZ_y = \frac{(B_x - A_x) \times (Z_y - A_y) - (B_y - A_y) \times (Z_x - A_x)}{(B_x - A_x)^2 + (B_y - A_y)^2}$$

4. Rescale each configuration back to its original size by multiplying all landmark coordinates by the original baseline length (calculated in step 2). Landmark A will end up with coordinates (0, 0) and landmark B will end up with coordinates (L, 0), where L is the baseline length.

5. Reflect paired (i.e., non-axial) landmarks across the sagittal axis (baseline). This is done by taking the square root of the squared value for the x- and for the y-coordinate of each landmark.

6. Average the coordinates of paired landmarks. For configurations in which only one member of the pair is present (i.e., missing values on one side), use the single known value. The number of landmarks remaining in the configuration (n) will be [(P/2) + U], where P is the original number of paired (non-axial) landmarks and U is the number of unpaired (axial) landmarks digitized.

7. Delete all configurations for which any landmark is missing.

8. Stack all configurations in TPS format. This takes the form of two columns of data, where the first column is all of the x-coordinates and the second column is all of the y-coordinates. Each configuration must be headed with the phrase "LM=n" in the first column and a specimen identifier (e.g., "Ot7") in the second column).

9. Save this data file in a Text (Tab Delimited) format.

MORPHOMETRICS SOFTWARE

NIH Image/Scion Image:
http://rsb.info.nih.gov/nih-image/Default.html
http://www.scioncorp.com/

Jim Rohlf's software (SUNY Stonybrook):
http://life.bio.sunysb.edu/morph

Dave Sheets' software (Canisius College, NY):
http://www.canisius.edu/~sheets/morphsoft.html

Morphometrika (Jeff Walker):
http://www.usm.maine.edu/%7Ewalker/software.html

OUTPUT FILES:

CoordGen:
Coordinate output files in the X1, Y1…CS format have the following arrangement:
$LM1_x$, $LM1_y$, $LM2_x$, $LM2_y$, …, LMk_x, LMk_y, centroid size

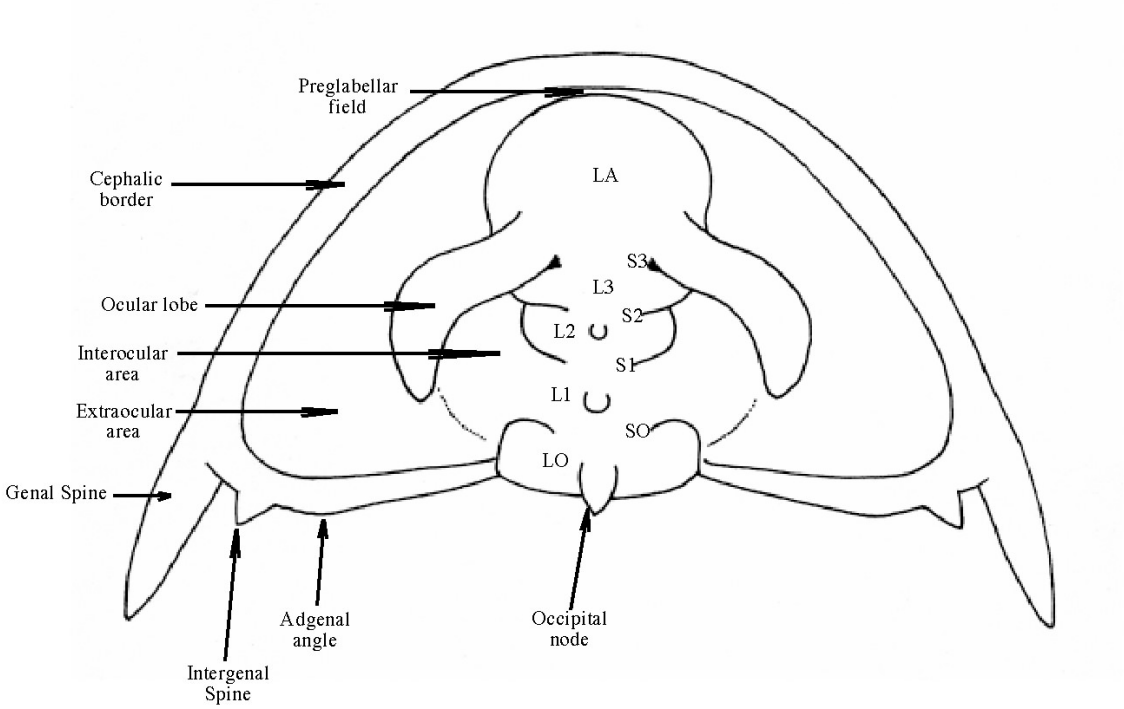
PCAGen:
PCA score output files have the following arrangement:
PC_{k-3}, PC_{k-2}, …, PC_2, PC_1

CVAGen:
CVA score output files have the following arrangement:
CV_1, CV_2, …, CV_{2k-4}

Regress:
Partial warp scores output files have the following arrangement:
PWk-3_x, PWk-3_y, PWk-2_x, PWk-2_y, …, $PW1_x$, $PW1_y$, uni_x, uni_y, centroid size

Procrustes distance output files have the following arrangement:
Procrustes distance, ln(centroid size)

The Head Shield (Cephalon) of an Olenelloid Trilobite



NOTES:
The glabella is the central, raised portion of the head shield (cephalon).
The glabella consists of five segments (labeled LO, L1, L2, L3, and LA from back to front) separated by furrows (SO, S1, S2, and S3, respectively).
The ocular lobes often merge into LA.

The presence/absence and size of the preglabellar field varies.
The size of the intergenal spine varies and may be absent on large individuals.
The adgenal angle may be very weakly developed.