

Introduction to Geometric Morphometrics

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MORPHOMETRICS

Mathematical quantification of shape.

Allows rigorous description of complex shapes.

Allows rigorous comparison among complex shapes.

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

Done properly, morphometrics will be informative as to which aspects of morphology are of interest.

- No need to decide *a priori* which features to look at.

What's The Use?

Species delimitation:

- Geographic variability (ecophenotypy).
- Biostratigraphy and correlation.
- Diversity estimates.

Species comparison:

- Disparity estimates.
- Characters for cladistic analysis.

Ontogenetic information:

- Growth patterns within species.
- Evolutionary processes (heterochrony, etc.).

Temporal trends:

- Phylogenetic/ecophenotypic morphological change.

PBDB Summer Course topics (non-morphometric):

- Paleoecology.
- Biostratigraphy.
- Speciation/extinction.
- Phylogenetics.

All rely on accurate species recognition and characterization.

- Morphometric data impinge on all branches of paleobiology!

Types of Morphometrics

- Traditional morphometrics: Length measurements, angles, etc.
- Outline-based morphometrics: Open or closed curves.
- Geometric morphometrics: Landmark configurations.

All have their uses! [All can be inappropriate for certain studies!]

Landmarks

Point of correspondence on each object that matches between and within 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.
- Coordinates contain positional information.

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:

- 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 points on the organism is important.

- *The configuration is data.*

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:

- Space between landmarks is unsampled.
- Hard to identify landmarks along curves.

Hard to graphically represent 3-D data.

WITH NIGEL: DAYS 1 AND 2

Imaging specimens.

Landmarks:

- Landmark types.
- Landmark selection.
- How to digitize landmarks.

Constructing landmark data files.

Size and shape.

- Centroid size.

Superimposition methods:

- Bookstein registration.
- Sliding baseline registration.
- Procrustes superimposition.

Comparison of shape:

- Variation in shape (disparity).
- Difference in shape:
 - Amount of shape difference between forms.
 - Procrustes distance.
 - Type of shape difference between forms.
 - Vectors on landmarks.
 - TPS deformation grids.

Comparison of shape:

- Do two samples differ in shape?
 - Hotelling's T^2 test.
 - Goodall's F -test.
 - Resampling tests.
- Do more than two samples differ in shape?
 - CVA.

All *static* shape comparisons.

- Potential correlation between size and shape not considered.

WITH ME: DAYS 3-5

Mechanics of morphometrics:

- Critical concept of shape space.
- More details on previously-introduced methods.

Allometric shape change:

- Identifying allometry.
- Dynamic comparison of pattern of shape change.
- Removing the effects of allometry.

With Me: Topic 1 - THE THEORY OF SHAPE:

What is shape?

Comparing shapes.

- Removing non-shape features.
- Procrustes distance.

Shape spaces:

- Configuration space.
- Shape space.
- Kendall's shape space.
- Tangent space.

With Me: Topic 2 - DIFFERENCE IN SHAPE BETWEEN CONFIGURATIONS:

Superimposition methods.

Visualizing difference in shape.

Variation in shape.

Statistical testing of difference in shape.

With Me: Topic 3 - 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.

Removing the effects of allometry.

Case study #1: *Olenellus chiefensis*.

With Me: Topic 4 - COMPARING ONTOGENETIC TRAJECTORIES:

Trajectories of shape change.

Comparing trajectories of shape change:

- "Angle between ontogenies".
- Rates of shape change.

Removing the effects of allometry (revisited):

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

Case Study #2: Simple ontogenies, two species.

- *Olenellus gilberti* versus *Olenellus chiefensis*.

Case Study #3: Simple ontogenies, unknown number of species.

With Me: Topic 5 - ANSWERING EVOLUTIONARY QUESTIONS WITH GEOMETRIC MORPHOMETRICS.

Evolutionary modifications to ontogeny.

Topic 1: The Theory Of Shape

THE THEORY OF SHAPE

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 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.

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

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

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

Centering the Centroid

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

$$\begin{aligned}\sum_{1 \text{ to } k} x_k &= 0 \\ \sum_{1 \text{ to } k} y_k &= 0\end{aligned}$$

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 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 X =

$$\sqrt{\sum_{i=1 \text{ to } K} \sum_{j=1 \text{ to } M} (X_{ij} - C_j)^2}$$

...for K landmarks in M dimensions, where X is the matrix of landmark coordinates of the configuration and C_j is the centroid 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 that it is mathematically independent of shape.
 - Does not induce a correlation between size and shape.
- 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:

$$\sqrt{\sum_{i=1 \text{ to } K} \sum_{j=1 \text{ to } M} (X_{ij} - C_j)^2} = 1$$

Which simplifies to:

$$\sum_{i=1 \text{ to } K} \sum_{j=1 \text{ to } M} (X_{ij} - C_j)^2 = 1$$

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 landmarks can be rotated so that they show minimal offset in location of homologous landmarks.

- “Least squares” fit.

To find the angle of rotation (\emptyset) to bring the target into optimal alignment with the reference:

$$\emptyset = \arctangent \frac{\sum_{j=1 \text{ to } K} Y_{Rj} X_{Tj} - X_{Rj} Y_{Tj}}{\sum_{j=1 \text{ to } K} X_{Rj} X_{Tj} + Y_{Rj} Y_{Tj}}$$

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 GLS Procrustes superimposition.

Configurations now differ *only* in shape.

Partial Procrustes Distance (D_p)

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

Represents the amount of difference in shape between two configurations.

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.
- Kendall’s shape space (most refined).

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 morphospace.

Shape Space

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.
 - Configurations have been placed in GLS Procrustes superimposition.

Technically...

- 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 optimal superimposition) are represented by points on that surface.

Dimensionality of Shape Space

The dimensionality of 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 (GLS) fit.

The location of any shape in shape space is specified by $KM - M - \{M(M-1)/2\}$ components.

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

Distance Between Shapes

The distance over the surface of the hypersphere between any two points is the *Procrustes distance* (p) between the shapes specified by those points.

- Equal to the angle 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 p = angle between radii.
 - Values for p range from 0 to π .

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 GLS Procrustes superimposition.

Shape Space For Triangles

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

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 (GLS) fit.

Triangles in shape space have only two degrees of freedom.

Intuitively...

Knowing the displacement at one landmark, and the configuration of one triangle, it is possible to calculate the shape of the second triangle.

- Under the constraint that all triangles lie in shape space:
 - Are centered.
 - Have unit centroid size.
 - Are rotated to optimal superimposition (GLS).

Knowing the coordinates of shape A, plus vector V1, V2, or V3, all coordinates of shape B can be calculated.

Only two parameters need to be specified to fully describe the differences between any two triangles in shape space:

- Length and direction of V1, V2, or V3.
- The displacement at a given landmark in the x-coordinate, and the displacement at that landmark in the y-coordinate.

Therefore triangles in shape space have only two degrees of freedom.

Shape Space For Triangles

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

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

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

- 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 ball.

- The location of each shape relative to a reference shape is given by two parameters (degrees of freedom).

More On Procrustes Distances

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

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

- Minimized when the centroid size of one form is 1 and that of the other is $\cos(\mathbf{p})$.
- This is the full Procrustes distance (D_F).

Procrustes Distances (\mathbf{p} , D_p , D_F)

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

- Procrustes distance (\mathbf{p}).
- Partial Procrustes distance (D_p).
- Full Procrustes distance (D_F).

Fortunately, the three measures are related:

- $D_p = \text{chord of arc } \mathbf{p} = 2\sin(\mathbf{p}/2)$
- $D_F = \sin(\mathbf{p})$.

Kendall's Shape Space

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

- Centroid size of reference form = 1.
- Centroid size of every other form = $\cos(\mathbf{p})$, where \mathbf{p} = Procrustes distance from reference form.

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

- This is Kendall's shape space.

Shape space:

- Centered location, unit centroid size, rotation to minimize partial Procrustes distance (D_p) [and therefore Procrustes distance (\mathbf{p})].

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 shape space.

Shape Spaces and Tangent Space

Shape space (and Kendall's shape space) is curved (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.

Topic 2: Difference In Shape**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.

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: $x' = x - x_A$

New y-coordinate: $y' = y - y_A$

Rotation

New x-coordinate: $x'' = x' \cos \theta + y' \sin \theta$

New y-coordinate: $y'' = y' \cos \theta - x' \sin \theta$

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

Rescaling

New x-coordinate: $x''' = x''/d$

New y-coordinate: $y''' = y''/d$

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. Choice of baseline affects scatter.

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 [p]).
- 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 (GLS) 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

Visualizing Shape Difference

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.

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

Based on the classic D'Arcy Thompson transformation 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 are available).

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.

Thin-plate Spline

Landmarks are the 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.

A Simplistic Summary of How the Thin-plate Spline is Drawn

One configuration is designated as the “reference form”.

- The other (“target”) configuration will be compared to this.

The target configuration is placed in GLS Procrustes superimposition with the reference form.

- All non-shape differences are removed.
- Configurations lie in shape space.

Vectors of displacement are calculated at each landmark, joining the reference to the target form.

An orthogonal grid is fit over the reference form, “pinned” to it at the landmarks.

The landmarks are moved along their respective vectors until the configuration matches that of the target form.

- The grid is “pulled along” at the landmarks and therefore is deformed (bent).
- Bending of the grid is minimized by the curve-smoothing spline function.
- 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.

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.

Variance in shape =

$$\frac{\sum(X - \bar{X})^2}{n - 1}$$

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

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!

What Is Being Tested?

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

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

Statistical Tests

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

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

The two should match!

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 or Wilk’s lambda.

Remember to exclude the fixed baseline landmarks prior to analysis!

Remember that some of the difference is attributable to non-shape!

*Sample size must be at least $2K - 4$ for 2-D data!

Procrustes Coordinates

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

- Cannot use Procrustes coordinates as the variables.

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

- Measures deviations from means as sums of squared partial Procrustes distances in shape space.
- Calculates ratio of explained:unexplained variation in this measure as a function of group.
- Does the amount of variation in shape within groups exceed that between groups?

Warp Scores

Warp scores are produced through the mathematical decomposition of the thin-plate spline deformation grid.

- Reduces 2K variables (Procrustes coordinates) into 2K - 4 warp scores.
- The number of variables then matches the degrees of freedom.
- Standard statistical procedures can then be applied.

The Thin-Plate Spline and Warp Scores: A Brief Summary

An Analogy

The location of any point relative to another in Cartesian space can always be described by knowing:

- The independent “styles” of offset in location that are possible.
 - It is possible for points to differ in location along the x-axis.
 - It is possible for points to differ in location along the y-axis.
- The magnitude to which each of those independent “styles” of offset in location is actually expressed.
 - How much does point A differ from point B along the x-axis?
 - How much does point A differ from point B along the y-axis?

The Relevance

Similarly, the shape of one landmark configuration relative to another can always be described by knowing:

- The independent styles of offset in landmark location that are possible.
- The magnitude to which each of those independent styles of offset in landmark location is actually expressed.

The Reality

For a given reference form, there are a limited number of orthogonal “styles” of deformation.

- A given “style” of deformation displaces all landmarks in the configuration, but not necessarily to the same degree.

By following these “styles” of deformation to different degrees, it is possible to deform that reference form into any other configuration of the same number of landmarks.

- Total deformation = Deformation Style A multiplied by some coefficient + deformation style B multiplied by some coefficient +

The Problem

The problem lies in the computation of:

- The range of deformation styles that are possible for a given reference configuration.
- The coefficients by which those styles are multiplied in a comparison of the reference form to any given target form.

This involves very heavy matrix algebra, so only a brief summary is given here!

How Can Shapes Differ?

The range of deformation styles that are possible for a given reference form are a function of the number and spacing of landmarks on that reference form.

- The styles of deformation are known even before the target form is specified.

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.

Of the six uniform transformations, four are accounted for by placing the reference and target forms into shape space (Procrustes superimposition):

- Translation in x, translation in y, rotation, rescaling.

Shear and compression/dilation are left as two components of shape difference.

- These components form the “uniform warp”.
- They are treated as if they were equivalent to the x- and y-components of non-uniform transformations.

Non-Uniform Transformations

These styles of deformation are called “partial warps” (or “principal warps”).

They are obtained by a geometric decomposition of the landmarks of the reference form.

- Each partial warp describes a pattern of relative landmark displacement, based on the spacing and location of landmarks in the reference form.
- Each partial warp is at a progressively smaller spatial scale.

Partial Warps

Eigenanalysis of a K by K “bending energy matrix” results in K eigenvectors.

- Each eigenvector describes an independent style of deformation.
- The eigenvalue for a particular eigenvector represents the bending energy required to achieve a given amount of shape change (i.e., one unit of Procrustes distance) in that particular style of deformation.

Three of the eigenvalues of the bending energy matrix are zero (i.e., these modes of deformation require no bending).

- This is because of non-independence of entries in the bending energy matrix (based on interlandmark distances).
- Because each has an x- and a y-component, this gives six coefficients, accounting for the six uniform components of the deformation.

The remaining K - 3 eigenvectors with non-zero eigenvalues correspond to localized components of shape change.

- These are the partial warps (= principal warps).
- In any given comparison, each has an x- and a y-component.
- These are the partial warp scores.

How Many Styles?

There are K - 2 styles of deformation (warps) for any given reference form.

- 1 uniform warp.
- K - 3 partial warps.

Difference in shape of any target form from the reference form will be described by 2K - 4 variables.

- 1 uniform warp (2 components).
- K - 3 partial warps (= 2K - 6 components).

The number of variables now equals the number of degrees of freedom.

- Standard statistical tests can be applied.

Using Warp Scores

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

- Hotelling's T^2 test.
 - But this has limited power in comparison to Goodall's F-test.
- CVA.
 - Can groups be statistically distinguished by their warp scores?

Critical Point # 1

In any analysis, 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 styles of deformation.
 - The warp scores for configurations A and B are not homologous.
 - They don't refer to the same styles of deformation.

Critical Point # 2

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

- 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!

Topic 3: Allometry

Why Do Individuals Differ In Shape?

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

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 shapes because they are different species, or because they are different 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 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 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!)

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.

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.

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 Topic 5.)

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.

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!

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 “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.

Advantage 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.

Critical Point #1

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

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

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.
- No allowance for increasing variance with size.
 - Could lead to overestimation of variance when standardized to a small size, or underestimation of variance when standardized to a large size.

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 (or as well as) centroid size.
- Describe deformations in terms of anatomy, not landmarks.

CASE STUDY # 1: THE ONTOGENY OF THE TRILOBITE *OLENELLUS CHIEFENSIS*

The aim of this case study is to familiarize you with the basic techniques of morphometric analysis:

- Data collection.
- Data formatting.
- Data exploration.
- Data analysis:
 - Visualizing shape and shape change.
 - Testing for allometry.
 - Removing allometry.
 - Describing shape and shape change.

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).

Data Analysis

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

- 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.

You will need to use the following programs:

- NIH Image or Scion Image
- CoordGen
- PCAGen
- Regress
- Standard
- DisparityBox
- tpsSmall
- Excel
- Statistics package

DIGITIZING 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 landmark coordinates are extracted should be the best possible for that specimen.

LANDMARK SELECTION

Landmarks will show you where interesting things are happening *after* running the analyses.

Landmarks should be selected to offer a comprehensive summary of morphology.

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

Landmarks should:

- Be homologous anatomical loci.
- Have conserved topological positions relative to other landmarks.
 - No heterotopy!
- Provide an adequate summary of overall shape.
- 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.

Landmarks

Landmarks provide the only empirical “anchor points” from which TPS analysis will interpolate patterns of integrated shape change in regions between the landmarks.

Shape change within regions not sampled by landmarks cannot be detected.

Digitizing Replicability

Error can result from:

- Inconsistent orientation of specimens relative to plane of digitization.
 - “Tilting”.
- Non-coplanarity of landmarks.
- Difficulty in pinpointing the landmark locus.
 - Biological “vagueness”.
 - Differences in illumination.
 - Differences in focus.
- Pixelation or distortion on screen.

Topic 4: Comparing Ontogenetic Trajectories

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 shape and size, comparison of patterns of shape change will help determine whether they differ in shape *because* they differ in size.
- Comparison of patterns of ontogenetic shape change between species can give insight into evolutionary processes.

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 trajectory of shape change must be described by $2K - 4$ variables:

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

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

This is a multivariate regression.

Bivariate Regression

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

$$Y = mX + c + \text{error}$$

Multivariate Regression

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

$$Y = mX + c + \text{error}$$

...only now Y is a multivariate shape descriptor [$Y_1, Y_2, \dots, Y_{2K-4}$], and m, c, and the error term have $2K - 4$ components.

COMPARING TRAJECTORIES OF SHAPE CHANGE

If two samples share the same pattern of shape change, then their ontogenetic trajectories will be parallel (or maybe even identical) 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 0° 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 ($2K - 4$), 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 = $\ln CS$, group, constant
 - Categorical variable = group
 - Interaction term = $\ln cs$ 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.

COMPARISONS AT STANDARD SIZE

Size Standardization

The regression of shape on size allows prediction of shape at any size.

Specimens within a group can be “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.

Advantage of Size Standardization

Size standardization removes the effect of difference in shape resulting from difference in size within a group.

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.

Size Standardization

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 may 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 far beyond the observed size range.

This must apply to all groups!

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.
- No allowance for increasing variance with size.
 - Could lead to overestimation of variance when standardized to a small size, or underestimation of variance when standardized to a large size.

This could apply to all groups!

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}, ..., PC₂, PC₁

CVAGen:

CVA score output files have the following arrangement:

CV₁, CV₂, ..., 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)

MORPHOMETRICS BOOKS:

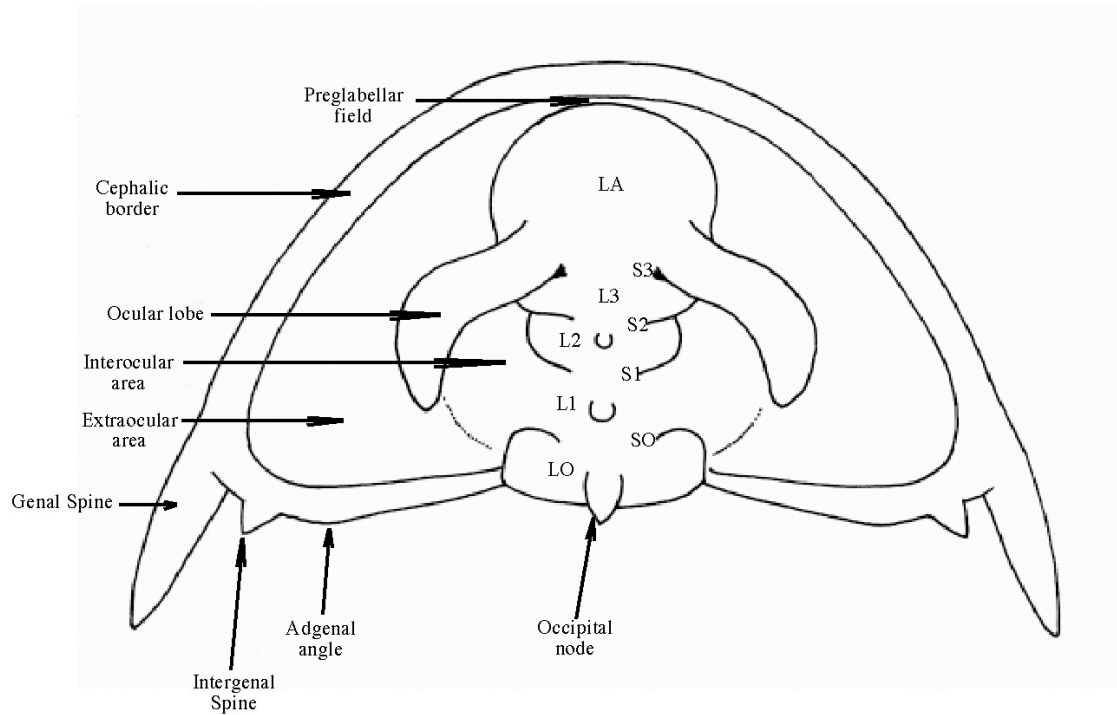
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The Head Shield (Cephalon) of an Olenelloid Trilobite



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