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APPLICATION OF ALLOMETRIC SHAPE ANALYSIS TO FISH GROWTH WITH GEOMETRICAL NOTES

Stanislav Katina

ABSTRACT. For analysis of shape changes, i.e., affine and nonaffine deformations, one can use several known methods, such as thin-plate splines, bending energy, partial and principal warps. In the paper we show—with geometrical notes—that these methods can be effectively used for allometric problems of biomedical statistics, especially for description of growth changes from juvenile to adult individuals.

1. Introduction

Traditional morphometrics has been concerned with the traditional caliper distances or length measurements of organisms and includes some shapes measurements such as angles or selects ratios of distances between landmarks or angles. Distances may be constructed in two or three dimensions, *but the reverse is not true.* This approach has been called multivariate morphometrics. Typical applications of these *distance-based methods* include the taxonomy such as classification of plants, animals or anthropological objects. Another large part of work is allometry, i.e., differences in shape associated with size. There are many situations where these techniques can be very powerful. However, sometimes the interpretation of the important linear combinations of ratios of length or angles can be difficult. It is always easier to interpret pictures in the original space, so we consider methods where the geometry of the object is conserved. The new morphometrics (geometric morphometrics or statistical shape analysis) analyzes the shape of biological organisms which are studied using points coordinates in two or three dimensions—*landmarks.* Shape analysis is concerned with geometrical aspects of analysing objects, their variability and changes through the time,

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where location, orientation and possibly scale information can be ignored. We shall describe some techniques, especially concerning visualization—*coordinate-base methods*. We apply geometrical allometry to analysis of growth changes of the spirlin (*Alburnoides bipunctatus*) a species of fish. We use shape analysis methodology from Bookstein (1991), Dryden and Mardia (1999); thin-plate spline methods from Bookstein (1989), Green and Silverman (1994).

This part provides an introduction to the topic of shape analysis, introduces notions as shape, size-and-shape, landmarks, baseline size, Bookstein coordinates, Helmertized landmarks, pre-shape, then full Procrustes distance and the mean shape.

Definition 1. *Shape* is all the geometrical information that remains when location, scale and rotational effects are filtered out from an object.

Definition 2. *Size-and-shape* is all the geometrical information that remains when location and rotational effects are filtered out from an object.

Definition 3. A *landmark* is a point of correspondence on each object that matches between and within populations. An *anatomical landmark* is a point assigned by an expert that corresponds between organisms in some biologically meaningful way. *Mathematical landmarks* are points located on an object according to some mathematical or geometrical property of the figure. *Pseudo-landmarks* are constructed points on an organism, located either around the outline or in between anatomical and mathematical landmarks.

Definition 4. The *configuration* is the set of landmarks on an particular objects. The *configuration matrix* $\mathbf{X}_{k\times 2} = \left[\mathbf{x}^{(1)}, \mathbf{x}^{(2)}\right]$ is the matrix of Cartesian coordinates of the k landmarks in 2 dimensions. The *configuration space* is the space of all possible landmark coordinates.

Definition 5. *Baseline size* is the length between landmarks 1 and 2

$$D_{12}(X) = \|X_2 - X_1\| = \sqrt{\left(x_2^{(1)} - x_1^{(1)}\right)^2 + \left(x_2^{(2)} - x_1^{(2)}\right)^2}\,,$$
$$\text{where} \quad X_j = \left[x_j^{(1)}, x_j^{(2)}\right], \qquad j = 1, 2\,.$$

Definition 6. *Bookstein coordinates* $B_j = \left[b_j^{(1)},\ b_j^{(2)}\right]$, $j = 3, \dots, k$ are the remaining coordinates of an object after translating, rotating and rescaling the baseline to $B_1 = \left[b_1^{(1)},\, b_1^{(2)}\right] = \left[-\frac{1}{2}, 0\right]$ and $B_2 = \left[b_2^{(1)},\, b_2^{(2)}\right] = \left[\frac{1}{2}, 0\right]$ so that

$$b_j^{(1)} = \frac{\left[\left(x_2^{(1)} - x_1^{(1)}\right)\left(x_j^{(1)} - x_1^{(1)}\right) + \left(x_2^{(2)} - x_1^{(2)}\right)\left(x_j^{(2)} - x_1^{(2)}\right)\right]}{D_{12}^2} - \frac{1}{2}, \tag{1}$$

$$b_j^{(2)} = \frac{\left(x_2^{(1)} - x_1^{(1)}\right)\left(x_j^{(2)} - x_1^{(2)}\right) - \left(x_2^{(2)} - x_1^{(2)}\right)\left(x_j^{(1)} - x_1^{(1)}\right)}{D_{12}^2}. \tag{2}$$

If the baseline is taken as $[0,0]$ and $[1,0]$, then there is no $-\frac{1}{2}$ in Equation (1). If we omit coordinates $b_1^{(1)}$, $b_2^{(1)}$, $b_1^{(2)}$, $b_2^{(2)}$ we have $(2k-4)$-vector of Bookstein coordinates $B = \left(b_3^{(1)}, \dots, b_k^{(1)},\, b_3^{(2)}, \dots, b_k^{(2)}\right)^T$ and $b_j^{(1)}, b_j^{(2)} \in \mathbb{R}$.

Definition 7. The *Helmertized landmarks* X_H are given by $X_H = HX \in \mathbb{R}^{(k-1)2} \backslash \{0\}$, where H is the Helmert sub-matrix.

Definition 8. The *pre-shape* of configuration matrix X is given by $Z = \frac{X_H}{\|X_H\|} = \frac{HX}{\|HX\|}$, which is invariant under the translation and scaling of the original configuration.

Definition 9. The *full Procrustes distance* between X_1 and X_2 is

$$d_F(X_1, X_2) = \inf_{\Gamma \in SO(2),\, \beta \in \mathbb{R}} \|Z_2 - \beta Z_1 \Gamma\|,$$

where pre-shape $Z_r = \frac{HX_r}{\|HX_r\|}$, $r = 1, 2$, Γ is rotation matrix, β is the scale, $SO(2)$ is the special orthogonal group of rotations.

Theorem 10. *The full Procrustes distance in 2 dimensions is*

$$d_F(X_1, X_2) = \left[1 - \left(\sum_{i=1}^{2} \lambda_i\right)^2\right]^{1/2}, \tag{3}$$

where $0 \le d_F \le 1$, $\lambda_1 \ge |\lambda_2|$, are the square roots of the eigenvalues of $Z_1^T Z_2 Z_2^T Z_1$, and the smallest value λ_2 is the negative square root if $\det(Z_1^T Z_2) < 0$. The minimizing rotation is given by $\widehat{\Gamma} = UV^T$, where $U, V \in SO(2)$ and $Z_2^T Z_1 = V \Lambda U^T$ with $\Lambda = \operatorname{diag}(\lambda_1, \lambda_2)$. The minimizing scale is $\widehat{\beta} = \sum_{i=1}^{2} \lambda_i$.

Proof. See Dryden, Mardia (1999). □

If we obtain an estimate of the mean shape of the configuration by taking the arithmetic average of the Bookstein coordinates, it is the *Bookstein mean shape*, i.e., $[\widehat{\mu}] = \overline{B} = \frac{1}{n} \sum_{i=1}^{n} B(i)$, where $[\widehat{\mu}] = \left[\widehat{\mu}^{(1)}, \widehat{\mu}^{(2)}\right]$, n is number of objects in a sample. $B \sim N_2\big([\widehat{\mu}], \Sigma\big)$.

Note: The original landmarks ($k \geq 3$) in $\mathbb{R}^2$ are i.i.d. as bivariate normal with identical means and covariate matrix proportional to the identity, i.e., $\left[X_j^{(1)}, X_j^{(2)}\right]^T \sim N_2\Big(\left[\mu^{(1)}, \mu^{(2)}\right]^T, \sigma^2 I_2\Big)$, $j = 1, 2, \dots, k$, where $[\mu] = \left[\mu^{(1)}, \mu^{(2)}\right]$. Configuration matrix of original landmarks $\operatorname{vec}(X) = \left[X_1^{(1)}, \dots, X_k^{(1)}, X_1^{(2)}, \dots, X_k^{(2)}\right]^T$ is distributed as $N_{2k}\big(\mu_{2k\times 1}, \Omega_{2k\times 2k}\big)$. The distribution of Helmertized landmarks (see Definition 7) $\operatorname{vec}(HX)$ is $N_{2k-2}\big(\mathbf{0}, \sigma^2 I_{2k-2}\big)$, $f\big(\operatorname{vec}(HX)\big) = (2\pi\sigma^2)^{1-k} \exp\big(-\frac{1}{2\sigma^2}\big\|\operatorname{vec}(HX)\big\|^2\big)$.

2. Geometry of shape changes

The main interests of this part are affine and nonaffine transformations. The transformation gives us information about the local and global shape differences. *Affine transformation* is a transformation for which parallel lines remain parallel. *Nonaffine transformation* is studied by thin-plate splines, bending energy, partial and principal warps.

In biomedical sciences we are interested in describing the difference in shape or size-and-shape between two or more objects. The biological goal in many taxonomical or ontogenetical studies is to describe the morphological changes of organisms. The taxonomical goal is to describe intraspecific or interspecific differences, the ontogenetical goal is to depict changes of a juvenile growing into an adult.

Definition 11. By *global differences* we mean large scale trends, such as an overall affine transformation. *Local differences* are on a smaller scale. Global differences are smooth changes between the figures, whereas local changes are the remainder of the components of a deformation and are less smooth.

Consider two k landmark configuration matrices in $\mathbb{R}^2$, $X_{k\times 2} = (x_1, \dots, x_k)^T$ and $Y_{k\times 2} = (y_1, \dots, y_k)^T$, both the matrices of Bookstein coordinates and we wish to deform X into Y (the source into the target), where $x_j = \left[x_j^{(1)}, x_j^{(2)}\right]$, $y_j = \left[y_j^{(1)}, y_j^{(2)}\right]$, $j = 1, \dots, k$; $x_j, y_j \in \mathbb{R}^2$.

Definition 12. A *deformation* is a mapping from $x \in \mathbb{R}^2$ to $y \in \mathbb{R}^2$ defined by the transformation

$$y = \Phi(x) = \big(\Phi_1(x), \Phi_2(x)\big)^T.$$

The multivariate function $\Phi(x)$ would be (everything is not often true) continuous, smooth, bijective, not prone to gross distortions, equivariant under relative location, scale and rotation of the objects, an interpolant ($y_j = \Phi_j(x)$, for $j = 1, \dots, k$).

The thin-plate splines and bending energy matrix

The thin-plate spline minimizes the amount of bending in transforming between two configurations, a roughness penalty. We concentrate on the $m = 2$ dimensional case, with deformation given by

$$y = \Phi(x) = \big(\Phi_1(x), \Phi_2(x)\big)^T .$$

Having $k \times 2$ matrices X and Y which consist of 2×1 landmarks; i.e., there are $2k$ interpolation constraints

$$(y_j)_r = \Phi_r(x_j)\,, \qquad r = 1, 2, \quad j = 1, \dots, k \tag{4}$$

and we can write $\Phi(x_j) = \big(\Phi_1(x_j), \Phi_2(x_j)\big)^T$, $j = 1, \dots, k$.

Definition 13. A *pair of thin-plate splines* (PTPS) is given by the bivariate function

$$\Phi(x) = \big(\Phi_1(x), \Phi_2(x)\big)^T = c + Ax + W^T s(x)\,, \tag{5}$$

where $c_{2\times1}$, $A_{2\times2}$, $x_{2\times1}$, $W_{k\times2}$, $s(x)_{k\times1} = \big[\sigma(x - x_1), \dots, \sigma(x - x_k)\big]^T$ and

$$\sigma(h) = \begin{cases} \|h\|^2 \log\big(\|h\|^2\big)\,, & \|h\| > 0\,, \\ 0\,, & \|h\| = 0\,, \end{cases}$$

with $2k+6$ parameters c, A and W, $\sigma(h)$ is called *fundamental solution of the biharmonic equation* $\triangle^2\sigma = \left(\frac{\partial^2}{\partial x^{(1)}} + \frac{\partial^2}{\partial x^{(2)}}\right)^2 \sigma = 0$. Let

$$1_k^T W = 0\,, \quad X^T W = 0\,. \tag{6}$$

The PTPS with validity of Equation (6) are called *natural thin-plate splines.*

Equations (5) and (6) can be rewritten to matrix form

$$\Gamma_{(k+3)\times(k+3)} \mathbf{B}_{(k+3)\times2} = \begin{pmatrix} S & 1 & X \\ 1^T & 0 & 0 \\ X^T & 0 & 0 \end{pmatrix} \begin{pmatrix} W \\ c^T \\ A^T \end{pmatrix} = \begin{pmatrix} Y \\ 0 \\ 0 \end{pmatrix} = \mathbf{Y}_{(k+3)\times2}\,,$$

where S is regular, $(S)_{ij} = \sigma(x_i - x_j)$, Γ is symmetric positive definite. The inverse of Γ exists $\Rightarrow$ the inverse of S exists. Then $\mathbf{B} = \Gamma^{-1}\mathbf{Y}$, where

$$\Gamma^{-1} = \begin{pmatrix} \Gamma^{11} & \Gamma^{12} \\ \Gamma^{21} & \Gamma^{22} \end{pmatrix}\,,$$

$\Gamma^{11}_{k\times k}$, $\Gamma^{21} = (\Gamma^{12})^T$. From this $W = \Gamma^{11}Y$, $\begin{pmatrix} c^T \\ A^T \end{pmatrix} = \Gamma^{21}Y = (\widehat{\beta}_1, \widehat{\beta}_2)$. $\Gamma = \mathbf{G}\Lambda\mathbf{G}^T$, where $\mathbf{G}$ is an orthogonal matrix whose columns are standardized eigenvectors of Γ, $\Lambda = \operatorname{diag}(\lambda_1, \dots, \lambda_k)$, $\lambda_1, \dots, \lambda_k$ are eigenvalues of Λ. If inverse of S exists, then $\Gamma^{-1} = \mathbf{G}\Lambda^{-1}\mathbf{G}^T$, $\Lambda^{-1} = \operatorname{diag}(\lambda_1^{-1}, \dots, \lambda_k^{-1})$, $\lambda_1^{-1}, \dots, \lambda_k^{-1}$ are eigenvalues of Λ^{-1}. Γ^{11} is an upper left submatrix of Γ^{-1}, $\Gamma^{12}_{k\times 3} = (\Gamma^{21})^T$ is an upper right submatrix of Γ^{-1} and Γ^{22} is a lower right submatrix of Γ^{-1}, $\widehat{\beta}_1, \widehat{\beta}_2$ are least square estimators and $\operatorname{cov}\big[(\widehat{\beta}_1, \widehat{\beta}_2)^T\big] = -\Gamma^{22}$.

Definition 14. The $k \times k$ matrix B_e is called the *bending energy matrix*, where $B_e = \Gamma^{11}$, $1_k^T B_e = 0$, $X^T B_e = 0$ and $r(B_e) = k - 3$.

Theorem 15. *Transformation of Equation* (5) *minimizes the total bending energy* (TBE, *integral quadratic variation, integral bending norm, the roughness penalty*) *of all possible interpolating functions mapping from X to Y, where* TBE *is given by*

$$J(\Phi) = \sum_{j=1}^{2} \iint\limits_{\mathbb{R}^2} \left(\frac{\partial^2 \Phi_j}{\partial \big(x^{(1)}\big)^2}\right)^2 + 2\left(\frac{\partial^2 \Phi_j}{\partial x^{(1)} \partial x^{(2)}}\right)^2 + \left(\frac{\partial^2 \Phi_j}{\partial (x^{(2)})^2}\right)^2 dx\, dy\,. \tag{7}$$

The minimized TBE *is given by*

$$J(\Phi) = \frac{1}{8\pi} \operatorname{trace}(W^T S W) = \frac{1}{8\pi} \operatorname{trace}(W^T Y) = \frac{1}{8\pi} \operatorname{trace}(Y^T \Gamma^{11} Y)\,.$$

Proof. See Kent and Mardia (1994a) □

Geometrical interpretation. The PTPS is used to produce a transformation grid—from a regular square grid on the first figure to a deformed grid on the second figure through transformation $y_i = \Phi(x_i)$, $i = 1, \dots, n_{\mathrm{cp}}$, where n_{cp} (index cp = crossing points) is the number of junctions on the grid.

Each square in the deformation is being deformed into a quadrilateral, the PTPS minimizes the local variation of these quadrilaterals with respect to their neighbours. PTPS consists of an affine and non-affine part, where only the non-affine part contributes to the bending energy and the affine part has zero TBE. PTPS determines the minimum energy shape of an infinite metal plate under certain constraints. PTPS is usually 1–1 bijective; equivariant under location, rotation and scale.

Principal and partial warps

The principal and partial warps are used to decompose the thin-plate spline transformations into a large and small scale components.

Definition 16. Consider the pair of thin-plate splines deformation from $x \in \mathbb{R}^2$ to $y \in \mathbb{R}^2$, which inerpolates the k points $X_{k\times 2}$ to $Y_{k\times 2}$. An eigen-decomposition of the $k \times k$ bending energy matrix B_e from Definition 14 has non-zero eigenvalues $\lambda_1 \le \lambda_2 \le \cdots \le \lambda_{k-3}$ (*bending energies*, BE) with corresponding eigenvectors $\gamma_1, \gamma_2, \ldots, \gamma_{k-3}$ (*principal warp eigenvectors*). The functions

$$P_j(x) = \gamma_j^T s(x)\,, \qquad j = 1, \ldots, k-3\,, \tag{8}$$

are the *principal warps* (PRW), where $s(x)$ is from Equation 5.

Definition 17. The *partial warps* (PW) are defined as the set of $k-3$ bivariate functions

$$R_j(x) = Y^T \lambda_j \gamma_j P_j(x) = Y^T \lambda_j \gamma_j \gamma_j^T s(x)\,, \qquad j = 1, \ldots, k-3\,. \tag{9}$$

The *partial warp score* for Y is given by

$$(p_{j1}, p_{j2})^T = Y^T \gamma_j\,, \qquad j = 1, \ldots, k-3\,. \tag{10}$$

Geometrical interpretation. The PRW deformations are univariate functions of x and they can be displayed as a surface above the plane or as a transformation grid from x to $y = x + \big(c_1 P_j(x), c_2 P_j(x)\big)^T$ for each j, for particular values of c_1 and c_2. The PRW do not depend on the second figure Y. From PRW we construct an orthogonal basis for re-expressing the TPS transformations. The non-affine part of the PTPS deformation can be decomposed into the sum of PW: $W^T s(x) = \sum_{j=1}^{k-3} R_j(x)$. The jth PW corresponds to the movement of the most highly weighed landmarks in the jth PRW. The jth PW scores mean the contribution of the jth PRW to the deformation $X \to Y$ in the Cartesian axes. The PW are bivariate functions and they can be displayed in a plot as deformations of the square grid placed over X, where the grid (for jth PW) is deformed to $y = x + R_j(x)$. The first PRW and PW represent an overall large scale bending of the figure (large scale global deformation). The last PRW and PW represent small scale local deformations (the landmarks are closed together). The affine component of the transformation is called *zeroth-order warp* (ZOW), the affine scores are called *zeroth partial warp scores* (ZPW scores). The affine transformation is displayed as a deformed grid as its non-affine counterpart. Let the source X is a Bookstein coordinates matrix $X = [x_1, \ldots, x_k]^T$, where $x_j = \big[x_j^{(1)}, x_j^{(2)}\big]$, $j = 1, \ldots, k$; $\sum_{j=1}^{k} x_j^{(1)} x_j^{(2)} = 0$, $a = \sum_{j=1}^{k} \big(x_j^{(1)}\big)^2$, $b = \sum_{i=1}^{k} \big(x_j^{(2)}\big)^2$. Then

$$\alpha_1 = \big(a x_1^{(2)}, \ldots, a x_k^{(2)}\,,\ b x_1^{(1)}, \ldots, b x_k^{(1)}\big)^T / \,(ab)^{1/2}, \tag{11}$$

$$\alpha_2 = \big(-b x_1^{(1)}, \ldots, -b x_k^{(1)}\,,\ a x_1^{(2)}, \ldots, a x_k^{(2)}\big)^T / \,(ab)^{1/2}, \tag{12}$$

where α_1, α_2 are orthogonal to X and block diagonal matrix $\mathrm{diag}(B_e, B_e)$. Then the ZPW scores are $({}_a p_1, {}_a p_2)^T = (\alpha_1, \alpha_2)^T \mathrm{vec}(Y)$. We can decompose transformation $X \to Y$ to affine and non-affine part (AP and NAP) through squared full Procrustes distance between the shapes (see Equation (3)). This is given by

$$d_F^2(Y, X) = AP + NAP = AP + d_F^2(X, \widehat{X}), \quad \widehat{X} = X + \sum_{j=1}^{k-3} R_j(x). \tag{13}$$

Then $P(\mathrm{NAP}) = \dfrac{d_F^2(X, \widehat{X})}{d_F^2(Y, X)}$, $P(\mathrm{AP}) = 1 - \dfrac{d_F^2(X, \widehat{X})}{d_F^2(Y, X)}$.

TBE models the power of deformation; measures the overall roughness or "wiggliness" of Φ; it measures rapid variation in Φ and departure from local linearity or flatness. TBE will be large if the function of Φ exhibits high local curvature (because this will result in large second derivatives). TBE is zero if Φ is linear (the computed spline is $\Phi(x) = c + Ax$). Suppose that an infinite elastic flat thin plate is deformed to shape of the function $\varepsilon\Phi$ for small ε. Then the BE of the TPS is proportional to TBE.

Example. **Geometrical Allometry in Analysis of fish Growth**

We use Equations from previous parts. For the analysis we constructed program routines in *S-Plus*, using some of routines from Prof. Dryden (pers. communication). We analyse the growth changes of spirlin ($n = 181$). We made digital photographs, then the $\left[x^{(1)}, x^{(2)}\right]$-coordinates were measured in *Impor* PRO 32. We divided standard length SL (in cm) to 6 parts: stage 1 (SL$\in \langle 0, 2\rangle$, $n_1 = 11$), stage 2 (SL$\in \langle 2, 4\rangle$, $n_2 = 40$), stage 3 (SL$\in \langle 4, 5\rangle$, $n_3 = 41$), stage 4 (SL$\in \langle 5, 6\rangle$, $n_4 = 37$), stage 5 (SL$\in \langle 6, 8\rangle$, $n_5 = 38$) and stage 6 (SL more than $8, n_6 = 14$). In this article we use only stage 1 and stage 6. There are $n_{\mathrm{TPS\,grid}} = 33 \times 32 = 1056$ junctions for TPS-grid, $n_{\mathrm{PW\,grid}} = 21 \times 22 = 462$ junctions for PW-grid, $k = 17$ landmarks, baseline is taken as $\left[-\frac{1}{2}, 0\right]$ and $\left[\frac{1}{2}, 0\right]$, it is transformed SL (see Fig.1). Both grids have $[x_{\mathrm{begin}}, y_{\mathrm{begin}}] = [-0.600, -0.800]$ in bottom left corner, width $x_{\mathrm{width}} = 1.500$. The PRW as a surfaces have $[x_{\mathrm{begin}}, y_{\mathrm{begin}}] = [-0.550, -0.700]$, width $x_{\mathrm{width}} = 1.500$.

TPS-grid is formed by transformation of mean shape of the stage 1 to the mean shape of the stage 6 (see Fig. 2). The four (from the fourteen) PW deformation grids from the mean shape of the stage 1 to the mean shape of the stage 6 are shown in Fig. 3 together with the affine grid. The affine component explains 15.440 % of the squared Procrustes distance between the mean shape of the stage 1 and the mean shape of the stage 6 (it stretchs body of the fish sideward up and down). Then percentage of the pooled variability explained by the affine component is 74.930 % (15.403 % for the $x^{(1)}$-axis, 59.524 % for

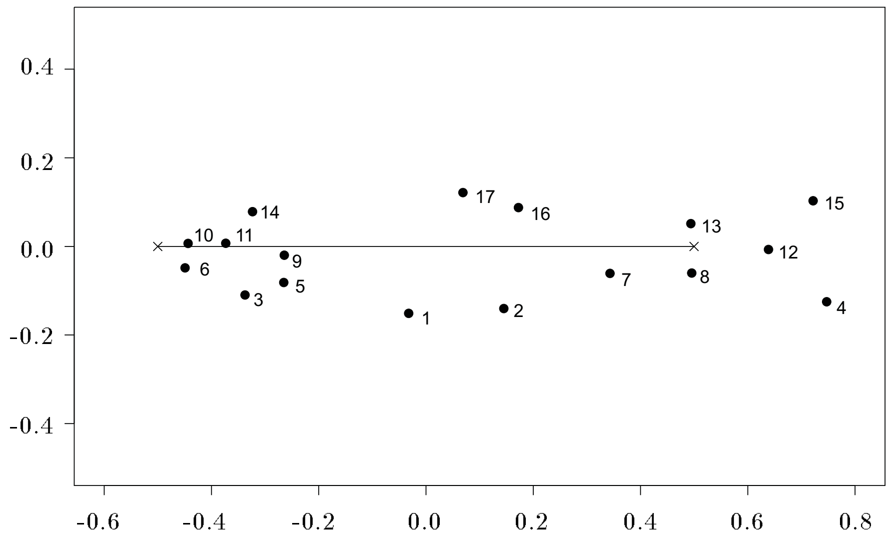

FIGURE 1. Baseline and Bookstein coordinates of landmarks.

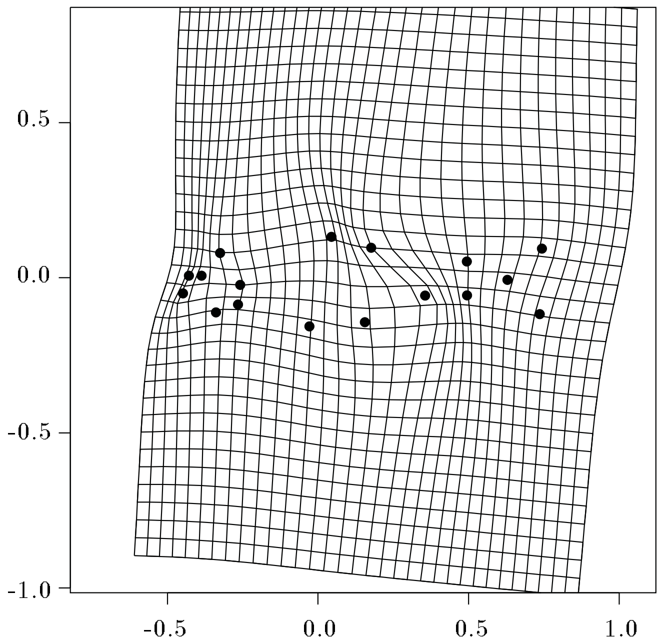

FIGURE 2. Thin-plate spline (TPS) grid, from stage 1 to stage 6.

the $x^{(2)}$-axis), and for the most important PW are 4.261 % (for PW5), 3.827 (for PW9), 2.693 % (for PW10), 1.670 % (for PW7), etc.. It is quite clear that there are the most important shape changes between landmarks 10 and 11, 16 and 17, 5 and 1, 2 and 7. In PW 5 landmarks 5 and 1 are going outwards, in partial warp PW 7 landmarks 16 and 17 become closer together, in PW 9

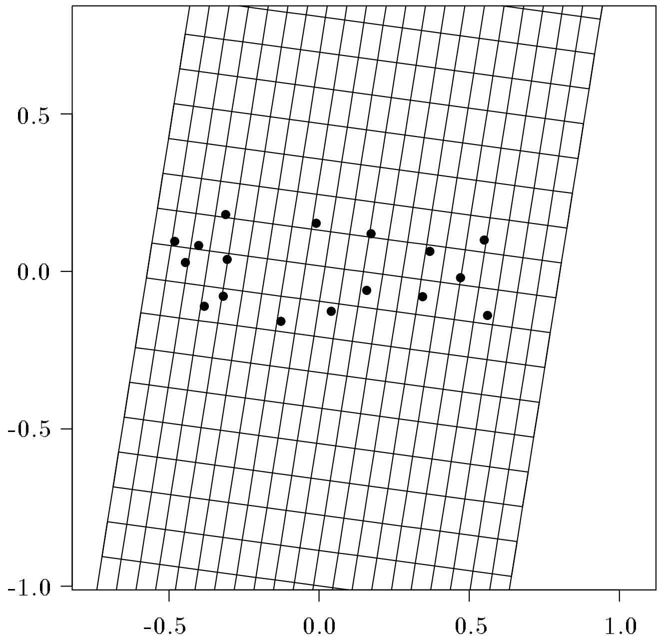

FIGURE 3A. Partial warp grid (PW), affine part, from stage 1 to stage 6.

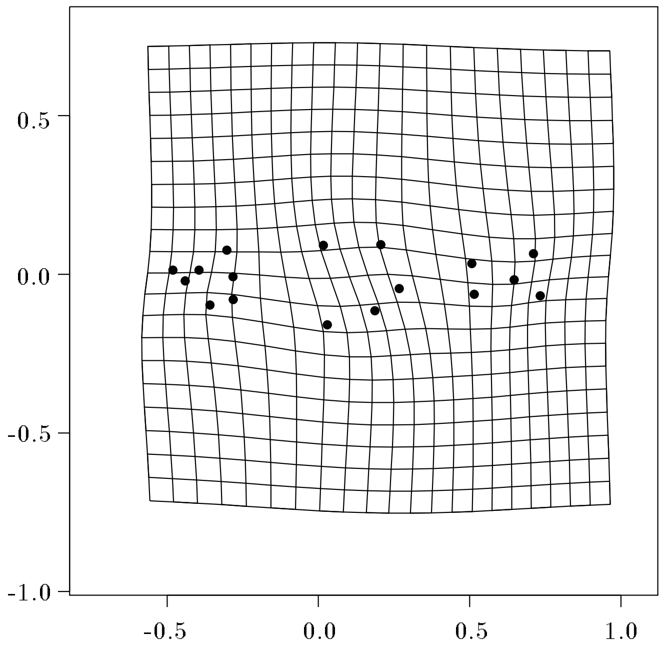

FIGURE 3B. Partial warp grid 5 (PW5), nonaffine part, from stage 1 to stage 6.

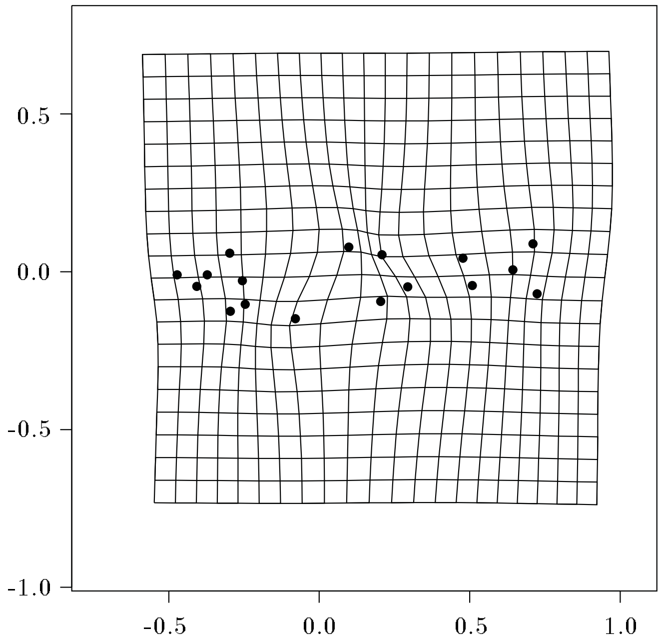

FIGURE 3C. Partial warp grid 7 (PW7), nonaffine part, from stage 1 to stage 6.

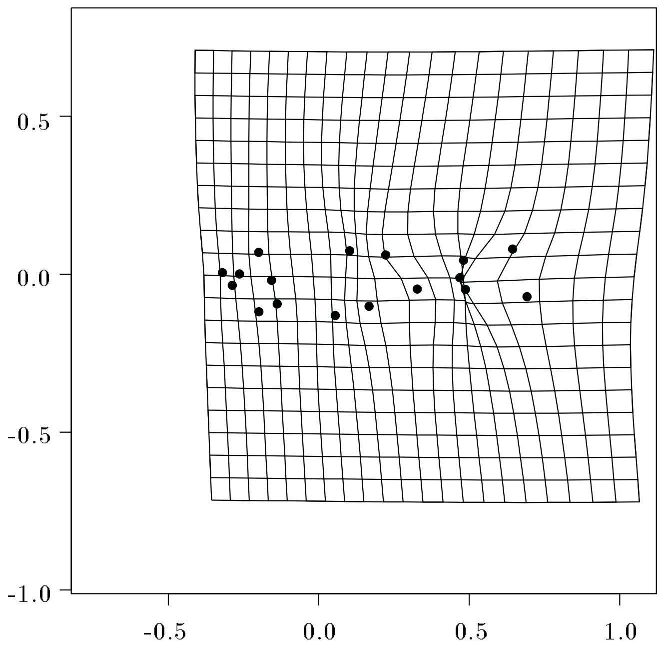

FIGURE 3D. Partial warp grid 9 (PW9), nonaffine part, from stage 1 to stage 6.

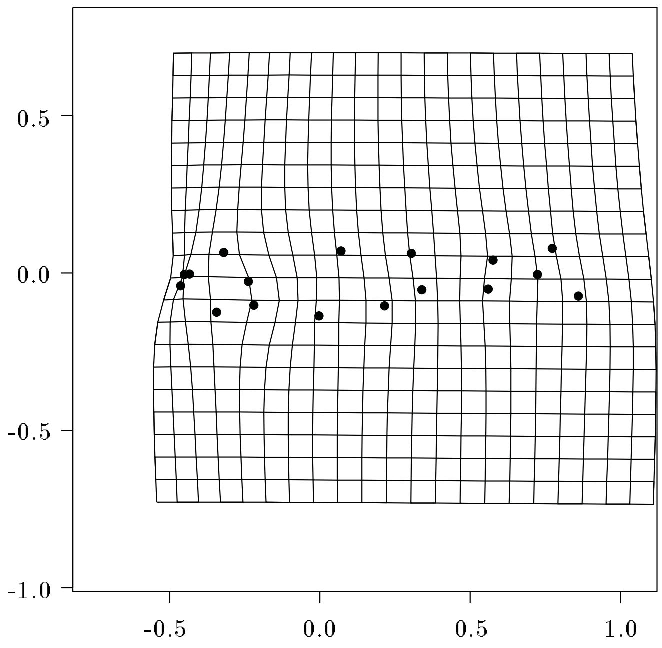

FIGURE 3E. Partial warp grid 10 (PW10), nonaffine part, from stage 1 to stage 6.

landmarks 2 and 7 are going outwards, in PW 10 landmarks 10 and 11 become closer together.

It is found that the two eigenvectors of the bending energy matrix (stage 1) are

$$\gamma_1^T = (0.27, -0.014, 0.307, 0.001, -0.318, -0.681, 0.005, 0.000, 0.182, \\ 0.215, 0.468, 0.000, 0.001, -0.202, -0.001, -0.004, 0.015),$$

$$\gamma_2^T = (0.051, -0.016, 0.269, -0.003, -0.611, 0.246, 0.001, -0.002, 0.537, \\ -0.003, -0.450, -0.004, 0.001, -0.015, 0.009, 0.010, -0.019),$$

with corresponding eigenvalues $\lambda_1 = 147.613$ and $\lambda_2 = 108.964$. We can inspect the loadings of each eigenvectors to see which coefficients are large, and hence interpret the effect. Alternatively we can plot the principal warps as a surface. There are two PRW from the mean of the first stage in Fig. 4. It is clear that the first few PRW are smoother and spread over a large scale than last few PRW which are the less smooth and smaller scale.

3. Conclusion

The main power of using mathematical statistical methods in biomedicine (both classical and geometrical methods together, both univariate and multi-

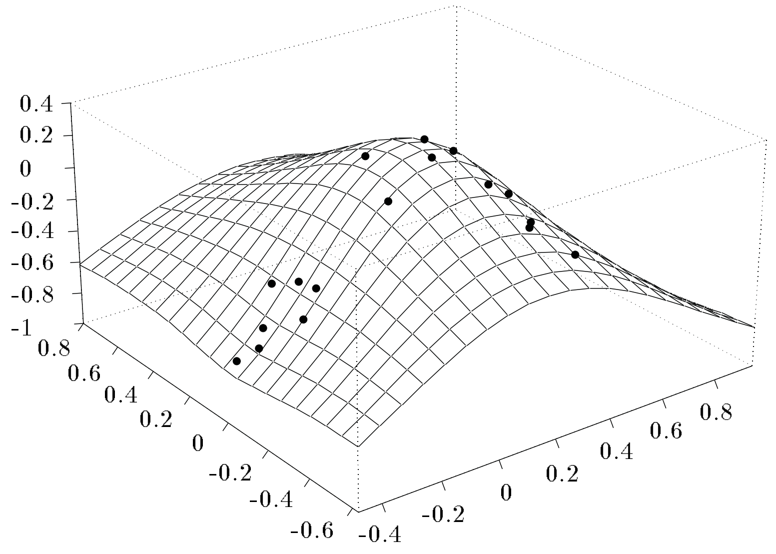

Figure 4a. Principal warp (PRW) 1, stage 1.

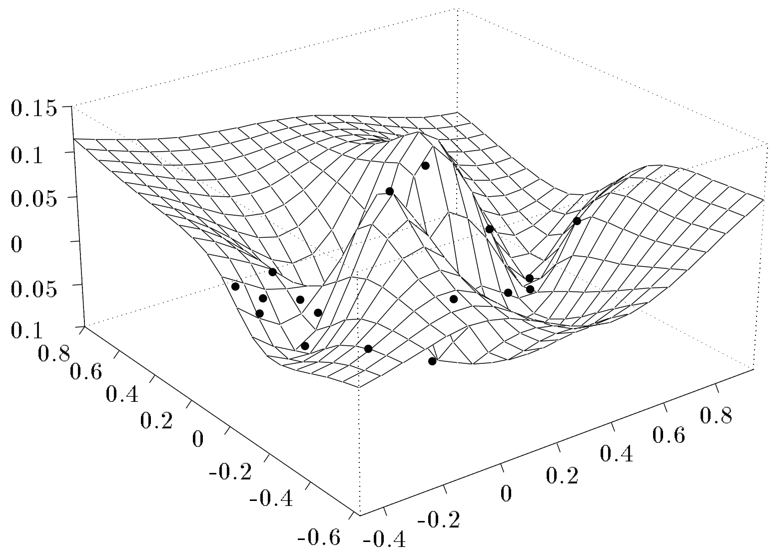

Figure 4b. Principal warp (PRW) 7, stage 1.

variate methods), is the possibility to discriminate and classify species using shape, to describe shape variability, shape changes during growth and ontogeny, shape changes during evolution, shape relationships to size, as well as to other covariates, such as sex, age or environmental conditions, diseases, etc. They are strong to reveal new relationships, which remain hidden when only one of these methods is used.

Advantages and disadvantages of using of *shape analysis* in allometry: *Advantages*—complex and understandable description of growth changes; rapid evaluation of the changes; power of deformation techniques—TPS and PW; generalization of multivariate techniques; rotation, translation and rescaling of the object to unit size; comparison of objects by the geometry of shapes, not only by measurements, is possible. *Disadvantage*—necessity to use a tool to identify groups to be tested.

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Department of Probability and Statistics
Faculty of Mathematics, Physics and Informatics
Comenius University
Mlynská dolina
SK–842 48 Bratislava
SLOVAKIA

E-mail: katina@fmph.uniba.sk