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MORPHOMETRICS AND THERIOLOGY HOMAGE TO MARCO CORTI

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To Lalla, Margherita and Marianna

ABSTRACT **-** This paper discusses the role of museum theriological collections in the context of twenty years of morphometric progresses. It also recalls twenty years of collaboration and friendship with Marco Corti, Italian theriologist and morphometrician, died on January 2007. The synthesis is addressed to the many young students that are picking up the baton and will likely contribute to the growth of the Italian school of morphometrics, to which Marco Corti dedicated most of his work at the University of Rome 'La Sapienza'.

Key words: Traditional morphometrics, geometric morphometrics, Kendall shape space, landmarks, thin plate spline

RIASSUNTO **- *Morfometria e teriologia. Omaggio a Marco Corti***. Il lavoro discute il ruolo delle collezioni teriologiche alla luce di venti anni di progressi della morfometria. Esso rappresenta anche la sintesi di un'esperienza umana e professionale, avendo l'autore condiviso molte tappe della 'rivoluzione morfometrica' degli anni ottanta con molti dei suoi protagonisti, alcuni dei quali oggi non sono più con noi. In particolare Marco Corti, teriologo e morfometrista, scomparso nel 2007. La sintesi è anche dedicata ai molti giovani studenti e ricercatori che stanno contribuendo alla crescita della scuola italiana di morfometria, cui Marco Corti ha dedicato gli ultimi venti anni di insegnamento e ricerca all'Università di Roma 'La Sapienza'.

Parole chiave: Morfomteria tradizionale, morfometria geometrica, spazio della forma di Kendall, *landmark*, *thin plate spline*

PREFACE

This article represents a compendium of both a human and professional experience. Some of the people who shared with me the rise and evolution of the new morphometrics in the last decades are no more with us. In particular, this work is dedicated to the memory of Marco Corti, theriologist and morphometrician, who died on January 2007 at the age of 56. The article is also dedicated to his wife and daughters, with the hope it will help to enlighten the important contribution of their beloved husband and father to the growth of morphometrics and theriology.

With Marco Corti I shared numerical explorations and morphometric discussions seated face to face at the Institute of Comparative Anatomy of the University of

Figure 1 - New York, 1994. Editing the proceedings of the NATO ASI 'Advances in Morphometrics' (Marcus *et al.*, 1996) at the American Museum of Natural History. From left to right: Dennis E. Slice, Marco Corti, Jim F. Rohlf, Leslie F. Marcus (standing), Gavin Naylor, and Fred L. Bookstein (picture by A. Loy).

Rome 'La Sapienza'. We attended together one of the first geometric morphometric workshop, organized by Jim Rohlf at the SUNY at Stony Brook in 1989, where we met Fred Bookstein, Dennis Slice and Leslie Marcus (1930-2002). This was the beginning of a never-ending friendship and collaboration. For almost two decades, we shared the development and growth of geometric morphometrics. Marco Corti was a leading figure in Italian morphometrics and organized many national and international workshops (Loy *et al*., 2004) (Fig. 1). In 1989, he organized with Leslie Marcus a morphometric workshop at the Vth International Theriological Congress in Rome (Marcus and Corti, 1989), followed by the NATO ASI 'Advances in Morphometrics' held in Tuscany in 1993 (Marcus *et al*., 1996), and by the workshop 'Geometric Morphometrics in Mammalogy' at the Euro-Mammal Congress in Santiago de Compostela in 1998 (Corti *et al*., 2000b). As associate professor at the University of Rome 'La Sapienza' in 2004 he proposed and taught the first Italian course in morphometrics. His scientific research was mostly dedicated to the study of the systematics and mechanisms of speciation in mammals, specially rodents (see for example, Nevo *et al.*, 1988; Corti and Thorpe, 1989; Corti *et al.*, 1989; Corti and Aguilera, 1995; Corti and Rohlf, 2001; Corti *et al*., 2001).

Before his death, on January 9, 2007, Marco still had time and strength to discuss the contents of this review. Thus, I like to think him as a co-author of this article.

INTRODUCTION

Theriological collections represent an ideal material for the study of morphological variation in time and space. In particular, mammal skulls, which form the bulk of collections, are highly informative, conservative and adaptive structures, owing the mass of information related to their multiple functions and adaptations. The skull protects the brain and the sense-organs, but it is also involved in feeding activities. It keeps trace of many no skeletal cranial tissues with which the skull is functionally, anatomically, and developmentally integrated, and of related behavioural, physiological and ecological aspects (Hanken and Hall, 1993). Thus, the skull hosts both highly conservative structures, like the brain-case, and plastic characters, like the mandible, the palate or the teeth. Till the advent of molecular investigations, the analysis of the skull represented the most powerful tool for biogeographic, phylogenetic and systematic investigations in theriology. In addition, it is still important for the study of fossil records and adaptive and functional interpretations of variation (e.g. de Beer, 1937; Simpson, 1945; Moore, 1981; Kemp, 1982, 2004; Novacek, 1993; McKenna and Bell, 1997; Hunter, 2007). This led to a precise coding of morphometric linear measurements (see for example Thomas, 1905; Fig. 2), and to a large amount of published works, including milestone references for mammal's evolution and systematics (Kemp, 1982, 2004; Novacek, 1993; McKenna and Bell, 1997).

MORPHOMETRICS AND THE THEORY OF PROBABILITY

The rise of statistical sciences and the achievement of evolutionary theories in the XIX century paved the way to the analysis of large amounts of data, joining the concepts of variability and population. Morphometrics, i.e. the quantitative study of biological shape variation (Bookstein, 1996b), has represented the main tool for the

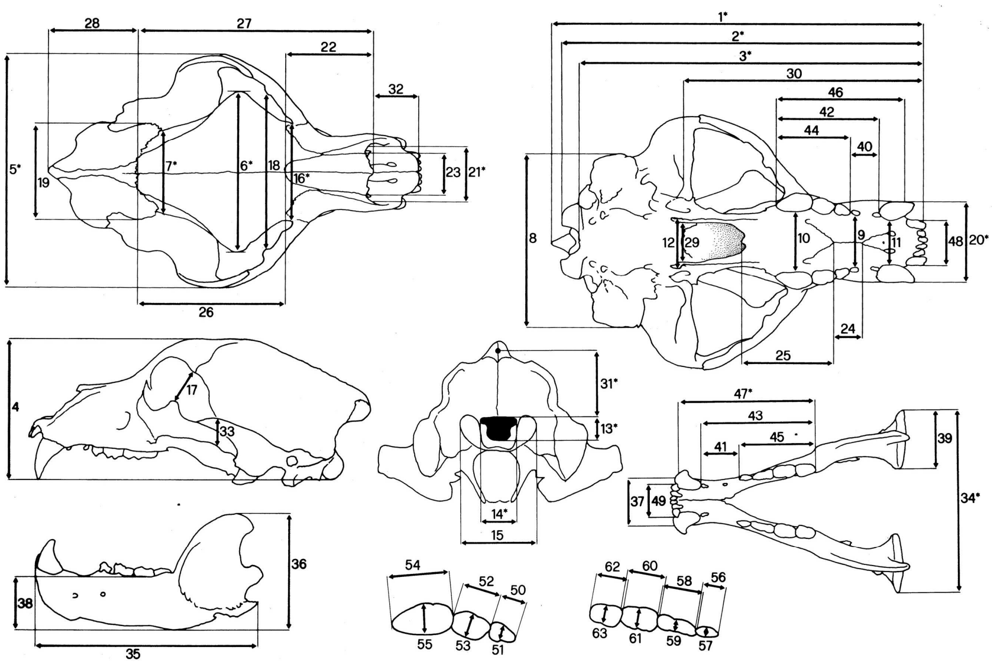


Figure 2 - Traditional linear measurements recorded on a mammalian skull (From Loy *et al.*, 2008).

investigation of variability until the advent of genetics and molecular studies. The mathematical methods used in statistics rise from the theory of probability. Since its birth, in the first half of the XVI century, this theory has received many contributions, from the discussions about the errors associated with observations, to r formula for regression, till its modern formalization by Andrey Nikolaevich Kolmogorov (1956). At the beginning of the XX century, the will to prove the existence of natural selection stirred the interest for statistical applications in biology. Fundamental contribution came from Karl Pearson, who introduced the correlation coefficient (Pearson, 1895), and from the genetist and evolutionary biologist Ronald Aylmer Fisher, who developed the methods for the statistical analysis of variance (Fisher, 1935), laying the foundations of multivariate statistics (Reyment, 1996). In 1969, Robert R. Sokal and James F. Rohlf published a milestone reference book for the analysis of numerical variation in biology, now at its third edition (Sokal and Rohlf, 2001).

TRADITIONAL MORPHOMETRICS

The term morphometrics comes from the Greek: "μορφή", meaning "shape", and "μετρώ" meaning "measurement". In mammalian studies, morphometrics has been traditionally addressed to analyse the variability and co-variability patterns of quantitative morphological characters within natural populations. The patterns of morphological variation are interpreted in terms of response to adaptive and selective forces, ontogenetic and phylogenetic constraints.

First analyses of variation of morphological characters were based on univariate or bivariate statistics applied to the analysis of one or two characters, represented by linear measurements (distances), angles, or ratios. The development of computational calculus and the exponential increase of computational power of personal computers in the second half of the XX century made possible the simultaneous analysis of large numbers of characters and samples through multivariate statistics. An introduction to multivariate statistics applied to morphometric variation in mammals was presented by Nancy Neff e Leslie Marcus in 1980, at the annual meeting of the American Society of Mammalogy (Neff and Marcus, 1980). Since then, workshops and symposia devoted to the application of morphometrics to theriological studies have been regularly organized around the world (Corti *et al*., 2000a). During the 1970s and '80s, the application of multivariate statistics to morphometrics has represented the most powerful tool of morphometric investigations (Reyment *et al*., 1984; Marcus, 1990; Reyment, 1991).

Multivariate analyses include exploratory and confirmative analyses. The first ones are aimed to summarize the information of a large number of characters and samples and to identify the pattern of variation of *taxa*. They include principal component analysis, principal coordinates analysis and factor analysis. Confirmatory analyses, including discriminant functions, canonical variate analysis and multivariate analysis of variance, are used to validate the patterns of variation identified through the exploratory techniques and to describe the phenetic relationships among *taxa* or groups defined *a priori*. Examples of applications to mammalian skull are in Jolicoeur (1959), Leamy and Bradley (1982), Thorpe *et al*. (1982), Schonewald-Cox *et al*. (1985), Pankakoski *et al*. (1987), Smith and Patton (1988), Corti and Thorpe (1989), Aguilera and Corti (1994).

The complex of these methods is now known as 'multivariate morphometrics' (Blackith and Reyment, 1971) or 'traditional morphometrics' (Marcus, 1990). The latter was coined to distinguish the use of univariate and multivariate morphometrics from new morphometrics tools that were growing in those years, now known as 'geometric morphometrics' (Rohlf and Marcus, 1993).

1. Sample size and collection labels

Sample size is a critical point in traditional morphometrics, particularly in the analysis of intraspecific geographic variation, for which several homogeneous samples are needed to detect the patterns of variation. Having to avoid the effect of non-geographic variation, such as that related to sexual dimorphism or age distribution (see for example Loy and Corti, 1986; Corti and Loy, 1985), it is fundamental to know the sex and age of museum specimens, and efforts should be addressed to fill up any missing information, likely through specific morphometric investigations.

Moreover, as neighbouring geographic localities often need to be pooled to obtain a sufficient sample size, the addition of geographic coordinates to museum labels would allow the rapid selection of samples through a GIS (Geographic Information System). Geographic coordinates also allow to integrate climatic or environmental parameters derived from digital maps in the analysis.

The diagnostic and explanatory power of traditional morphometrics is limited by the fact that it ignores the bio mathematical aspects of the original measurements (Bookstein, 1996a). Thus, it does not allow to visualize the resulting patterns of morphological variation in terms of specific changes in the shape of the analysed structures, nor it allows to distinguish between the contribution of size *vs.* shape variation. This distinction is particularly relevant when exploring allometric trajectories, among or within lineages (Huxley, 1932; Jolicoeur, 1963; Klingeberg, 1996, 1998). Many authors made an attempt to identify a 'size factor' in the traditional sets of morphometric measurements and to correct their data to obtain 'size independent' or 'size free' new data matrices (Burnaby, 1966; Mosiman, 1970; Reyment *et al*., 1984; Thorpe, 1988; Sundberg, 1989; Jungers *et al*., 1995; Cadima and Jolliffe, 1996). But these transformations fail to retain the information on the original characters, thus preventing functional or adaptive interpretations of the resulting patterns. The linear measurements of the skull can also fail to detect differences in shape, as it is shown in Figure 3.

GEOMETRIC MORPHOMETRICS AND THE MORPHOMETRIC 'REVOLUTION'

The rise of new morphometric tools in the 1980s, combining multivariate statistics, non-Euclidean geometry and computer graphics, allowed to overcome these limits and led to a morphometric revolution (Bookstein,1991; Rohlf and Marcus, 1993; Corti, 1993).

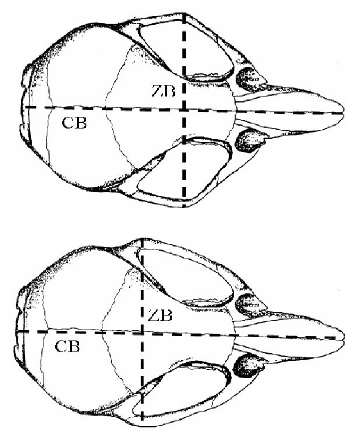


Figure 3 - An example of shape differences not detectable from linear measurements (ZB – zygomathic breadth; CB: condylo-basal length) in two imaginary rodent skulls (original drawing by Marco Corti).

The morphometric workshop held at the Fifth International Theriological Congress of Rome in 1989 (Marcus and Corti, 1989) was the last one focused on traditional morphometrics. Thereafter, international morphometric symposia and workshops, particularly those organized in theriological contexts, have been devoted to the new geometric methods, and many have been organized in Italy (Marcus *et al*., 1996; Corti *et al*., 2000a; Loy *et al*., 2004). Most of these meetings produced proceeding volumes that are traditionally indicated by the colour of their front cover: the *orange book* (Bookstein, 1991), the *blue book* (Rohlf and Bookstein, 1990), the *black book* (Marcus *et al*., 1993), and the *white book* (Marcus *et al*., 1996). These volumes also run through the genesis and development of geometric morphometrics during the last twenty years. Comprehensive manuals and textbooks have recently joined the series of the coloured books (Zelditch *et al*., 2004; Slice, 2005). The geometric morphometrics community has soon revealed as a very active and stimulating group, interacting in a very dynamic way, both through the newsletter MORPHMET, moderated by Dennis E. Slice (http://morphometrics.org/morphmet.html), and the website http://life.bio.sunysb.edu/morph, by Jim Rohlf. These online resources keep the community informed and updated about softwares (including all free-downloadable packages), meetings, literature, methodologies, statistics, job opportunities and the morphometricians network around the world.

The term 'geometric morphometrics' has been used for the first time by Leslie Marcus (Rohlf and Marcus, 1993). It refers to a group of methods that uses new data sets, such as landmarks, outlines curves or, more recently, semilandmarks and surfaces, to capture the geometric information of biological structures, and to preserve it throughout the analyses, including the multivariate treatments of data (Bookstein, 1998; Adams *et al*., 2004). These methods represent the integration of different methodological and conceptual frameworks, mainly developed during the 1980s and '90s (Kendall, 1984; 1985; Bookstein, 1986; 1989a,b; 1991; 1996a,b; 1998).

Landmarks are described by either two (x,y) or three (x,y,z) cartesian coordinates of homologous points (Fig. 4). The whole set of landmarks registered on an object corresponds to a configuration (Bookstein, 1991; Zelditch *et al*., 2004).

The analysis of outlines is useful when no homologous landmarks can be iden-

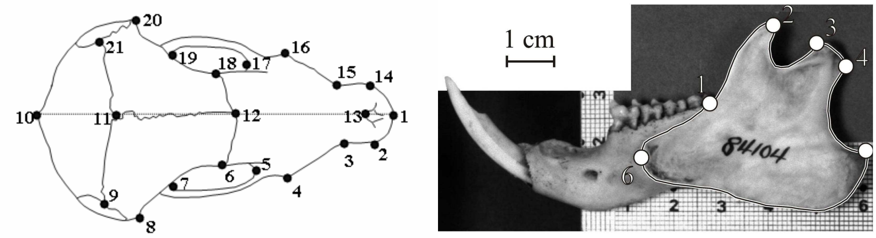


Figure 4 - Left: Landmarks on a mole skull (modified from Rohlf *et al*., 1996). Right: outline recorded from a marmot mandible (from Cardini and Slice, 2004).

tified along the contour or within the specimens, e.g. for the mandible and the human braincase. Outlines are analysed with various methods implying the fitting of functions to the curves and the use of their descriptive parameters as shape variables for successive statistical analyses (Rohlf, 1986; Rohlf, 1990a; Lohmann, 1983; Lohmann and Schweitzer, 1990; Adams *et al*., 2004). Other methods are based on differences in distances or interior angles between landmarks, like EDMA (Lele and Richtsmeier, 1991) or finite element scaling analysis (FESA, Lewis *et al*., 1980). These methods have the advantage that are invariant to location, orientation or reflection. But they are not invariant to differences in size (Rohlf, 2000b; Zelditch *et al*., 1989; Straney, 1990). Advantages and limitations of these methods are discussed by Rohlf (2000a, 2003).

1. Form, shape and size

In the context of geometric morphometrics it is essential to distinguish between the terms “form” and “shape”. Form refers to the whole morphology of an object, i.e. includes information on both size and shape. Shape refers to the component of morphological variation that is independent from size variation, i.e. to the geometric properties of an object that are invariant to location, scale and orientation (Slice *et al*., 1998; Slice, 2005).

The partition of the total variation of raw coordinates into shape and non-shape components is achieved by rotating, traslating, superimposing all the configurations within a common reference system, and scaling them to a common size (Slice, 2005; Fig. 5). This is achieved through a General Procrustes Analysis (GPA hereafter) (Rohlf, 1999). But while the effects of position and orientation must be removed permanently from the analysis, the scale factor, i.e. the size of the object, carries important biological information. Thus, it has to be measured, extracted from the data, and filed for further analyses, particularly for the study of allometry (see for example Klingenberg, 1996). One of the most elegant and effective measures of size from a set of landmarks is the centroid size (Bookstein, 1986), i.e. the square root of the sum of the squared distances of all landmarks from the centroid of the object (Bookstein, 1991). An important property of this size measure (Bookstein, 1998) is that it represents the appropriate standardization for projecting a configuration of landmarks in Kendall’s shape space (see below).

2. The thin plate spline

Deformation grids have been used since the renaissance and applied to the description of

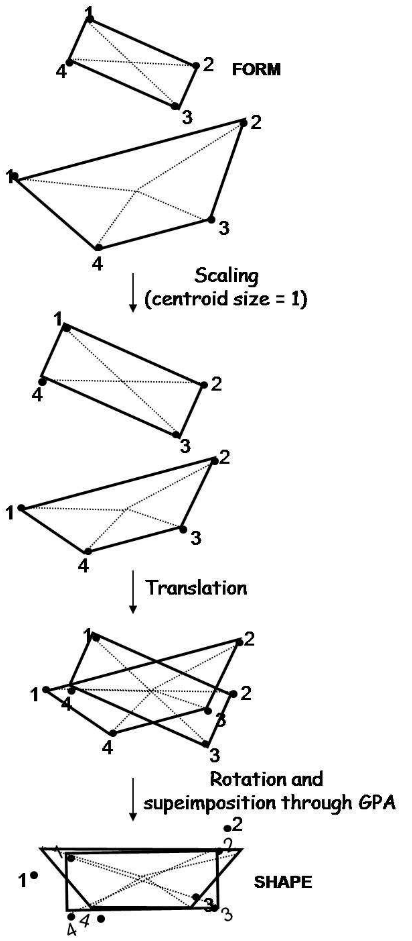


Figure 5 - Transformation of two *forms* in *shape*s through scaling, translation, rotation and optimal superimposition through GPA. Numbers refer to homologous points (landmarks) describing two configurations. Internal trajectories identify the centroids (from original drawings by Marco Corti and Anna Loy).

shape changes among biological forms by D'Arcy Wentworth Thompson in his famous book '*On growth and form*' (Thompson, 1917). Thompson explored the degree to which differences in the forms of related animals can be described by means of relatively simple mathematical transformations, and visualized through localized deformation of a cell grid underlying the objects to be compared (Fig. 6). Despite this sharp intuition, Thompson did not articulate his insights in the form of experimental hypotheses that can be tested. In the early 1980s, Fred Bookstein rediscovered the Thompson intuition, and formalized it through the elegant mathematical framework of the thin plate spline (Bookstein, 1989b, 1991). The name thin plate spline refers to a physical analogy involving the bending of a thin sheet of metal. It consists of an interpolation function originally developed for computational surface theory and computer graphics that allows to visualize the *warping* of one object into another as a Cartesian deformation (Bookstein, 1991).

In the physical setting, the deflection of the thin plate is in the z direction, orthogonal to the plane. In order to apply this idea to the problem of coordinate transformation, one interprets the lifting of the plate as a displacement of the x or y coordinates within the plane. Variation is expressed in terms of variance in the parameters of the fitted function. This is expressed relative to a *bending energy* matrix based on the coordinates of the landmarks of a convenient *reference* configuration. To produce the map the spline is solving an optimization problem as it has minimum energy of all deformations consistent with the change of landmark shape involved (Bookstein, 1996b). Given an object (configuration) described by p landmarks, the elements of the $n \times 2p$-3 partial warps (*weight matrix*) describe each of the n configurations as a linear combination of the *principal warps* computed from the reference configuration. At least three of the eigenvalues of the bending energy matrix will be equal to zero. These zero eigenvalues and their corresponding eigenvectors represent the affine, or linear or uniform components of shape variation (translation, rotation, and dilatation), that

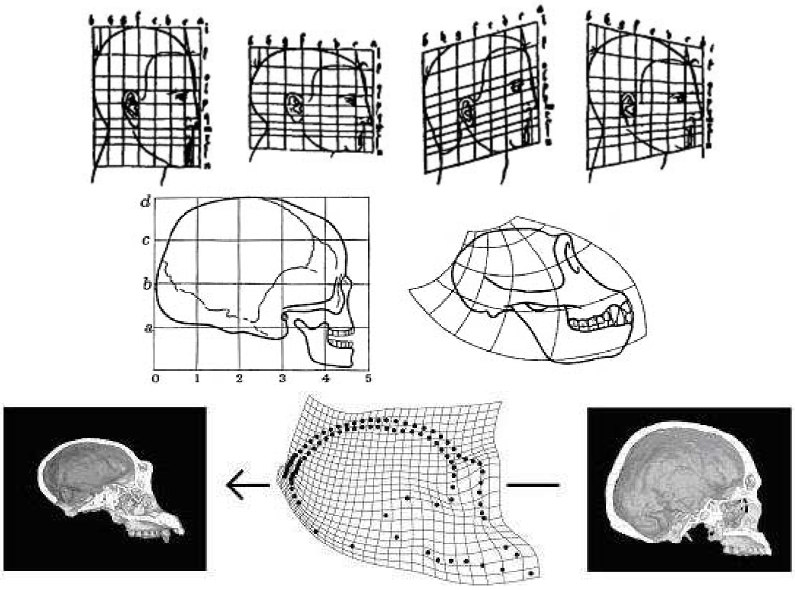


Figure 6 - Top: deformation grids used by Dürer (1528): a head with a reference grid of equal cells (left) and three transformations in specific facial types, obtained through the modifications of the cell proportions in the reference grid. Centre: cartesian transformation by D'Arcy Thompson showing the transformation from human onto chimpanzee skull (from Thompson, 1917). Bottom: thin plate spline deformation grids for the profiles of both a human and a chimpanzee skull created by Nora Dibowski e Gerhard Weber (Austria Dept of Anthorpology - Univerisity of Wien, http://www.virtual-anthropology.com/virtual-anthropology/geometric-morphometrics/thin-plate-splines).

can be analyzed separately or appended to the weight matrix of non affine or non-uniform components before performing multivariate tests for shape differences. The principal warps are the eigenvectors of the bending energy matrix, whose eigenvalues are inversely related to scale. Large eigenvalues correspond to eigenvectors that describe small-scale features, i.e. to the deformation of landmarks that are close together (as more bending energy is required to bend the thin plate). In a relative warps analysis (principal component analysis of the partial warp scores), α is the exponent used to rescale partial warps before computing their principal components, the relative warps. If $\alpha = 1$, more weight is given to large- than to small-scale variation (Rohlf, 1993, 1996). Setting $\alpha = 0$ equal weight is given to the principal warps. This last is the appropriate choice for taxonomic and exploratory studies, for which variation is not expected to be particularly important at a given scale (Rohlf, 1993). The chosen value for α will have no effect on multivariate statistical analyses of the weight matrix, such as MANOVA, discriminant functions, canonical variate analyses, or multiple regression analysis (Rohlf, 1996).

It must also be reminded that the partial warps do not have a direct relation with the biological nature of the object, as they are defined from the optimization of the *bending energy* required to *warp* the grid, which, in its turn, is a function of the relative distance among the landmarks in the reference configuration (Bookstein, 1989b). As a consequence, it is not recommended to analyze the distribution of objects in the bivariate space defined by each individual partial warp (Bookstein, 1996b). In contrast, the principal components vectors, i.e. the relative warps, of the whole matrix of partial warp scores (weight matrix) plus the uniform components are used to describe the major trends in shape variation among specimens (Adams *et al*., 2004).

As the partial warp scores (weight matrix) are scaled projections of the *x* and *y*-coordinates of the deviations of *n* configurations from the reference onto the principal warps, the choice of the reference is important. The reference is usually the mean configuration after the alignment, but it can also be represented by an earlier developmental stage or a hypothetical common ancestor (Rohlf, 1993). When both uniform and non uniform components are considered and α is set to 0, relative warps corresponds to a principal components analysis of shape changes in the geometry of Kendall's shape space. This represents the best procedure for explorative studies (Bookstein, 1996b; Small, 1996; Slice, 2005).

Thus, splines can visualize the results of an analysis of shape variation and the coefficients of these splines (partial warps plus the uniform components) can be used to perform statistical analyses in the Eucledean space tangent to the Kendall's shape space (see below) (Bookstein, 1991, 1996b; Bookstein *et al.*, 2003; Zelditch *et al*., 2004) (Fig. 7).

3 The Kendall shape space and the new synthesis

The landmark-based morphometrics and of the thin plate spline visualization by Fred Bookstein, and the contemporaneous development of the Procrustes-based theory of the analysis of shape have investigated by Kendall (1984, 1985, 1989), led to a new 'morphometric synthesis' (Dryden and Mardia, 1998; Bookstein, 1996a). This synthesis allowed the integration of the Euclidean multivariate analysis of shape with the non Euclidean shape space known as 'Kendall's shape space' that has revealed as the most rigorous theoretical and statistical framework for the description of shape and shape variation (Kendall, 1984, 1985; Small, 1996; Kent, 1994 Rohlf, 2000a,b). Kendall was able to fully describe the geometry of a space, which contains the distances, as distances of the cord or Procrustes distances, between all shapes described by the same number of landmarks (Kendall, 1984, 1985). The projection of these configurations in the tangent Euclidean space corresponds to a multivariate ordination of the shape variables obtained from data superimposed through GPA (Rohlf, 1999) (Fig. 7). It must be reminded that only the distances between individual specimens and the consensus equal the Procrustes distance in the shape space, whilst the distances between specimens do not (Slice, 2005).

Procrustes distances correspond to the difference between one configuration and the mean configuration. The results of principal components performed on the partial warp scores plus the uniform components computed from landmark configurations superimposed through GPA corresponds to the projection of these configurations on the Euclidean space tangent onto the underlying curved shape space. Differences in this tangent space are a good approximation of 'true' Procrustes distances when comparing shapes of phylogenetic related *taxa*. For example Marcus and colleagues have shown that this approximation is still good for a variety of mammal skulls, from shrews to elephants (Marcus *et al*., 2000).

THE GEOMETRIC MORPHOMETRIC PROCESS

1. Landmarks choice

Landmarks are the most extensively used data in geometric morphometric studies.

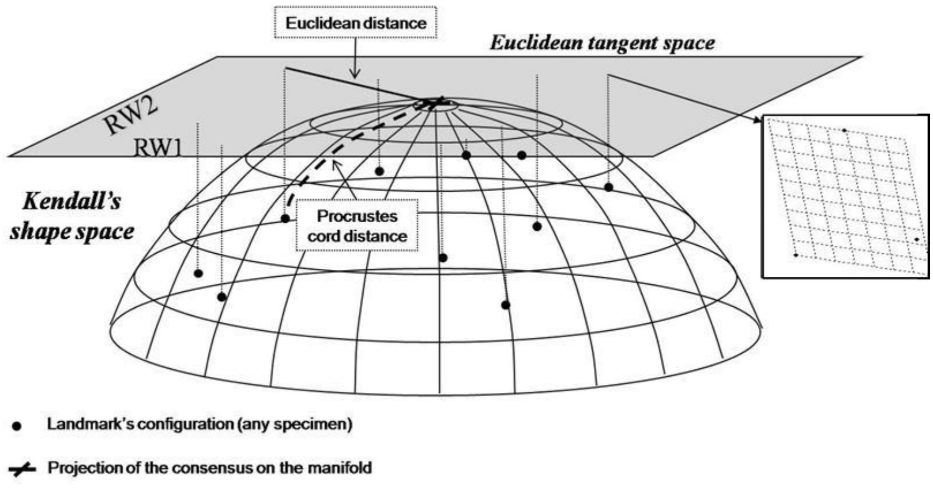


Figure 7 - Visualization of the Euclidean tangent space into Kendall's shape space for triangles. The Procrustes distance between one configuration and the mean configuration (equilateral triangle), and its relative projection in the tangent space (as defined by relative warp analysis of the partial warps and the uniform components), are shown (redrawn and modified from Zelditch *et al*., 2004). The thin plate spline deformation grid on the right is derived from the superimposition of one configuration into the other in the tangent plane.

This is partly due to the large number of analytical and visualization tools available for these data sets, particularly for two dimensional landmarks. The choice of landmarks rises from methodological and conceptual considerations.

Landmarks must detect the shape of biological significant structures, but they should also capture the whole shape (contour) of the object to allow a correct interpretation of localized changes in relation to the whole shape. Moreover, only landmarks recorded along the outline allow to measure a centroid size that reflects the size of the whole object and, likely, of the organism.

Landmarks should also be clearly identifiable across the whole sample to avoid measurement errors and subjectivity. Bookstein (1991) classifies landmarks of type I, II or III according to the precision of their location. Moreover, they should be preferably equally spaced and their number should be proportional to sample size. As a matter of fact, each landmark will lead to two o three shape variables (corresponding to the x, y, and z coordinates), and the total number of variables can easily exceed the number of specimens, thus compromising the multivariate treatment of data, especially in confirmative analyses.

Two dimensional landmarks are usually recorded from digital pictures of various projections of the object. These pictures can be stored and used for landmark recording later on, thus minimizing the time spent on the collection. Pictures are taken either directly by digital cameras, or through microscopes connected to digital cameras or to systems for image analysis.

Three dimensional landmarks retain all the geometric information of the object descriptors (Bookstein, 1991; Dean 1996), and this is particularly true for a typical three dimensional structure as the skull. Nevertheless this advantage goes along with some methodological and logistic constraints. Although some procedures allow to reconstruct 3D data from 2D ones (see for example Fadda *et al*., 1997; Fadda and Corti, 2000), three dimensional coordinates must be usually recorded directly on the specimen by the means of electromagnetic, laser light and acoustic digitizers, or by rigid and servo mechanism arms (Dean, 1996). This expands the time spent on the collection and limits the possibility of getting again through the specimens to correct errors or to record new landmarks. Even if three dimensional coordinates can also be recorded from three dimensional images taken through 3D digitizers, CT (Computer Tomography) and MR (Magnetic Resonance) scanners, these devices are still expensive and are not often available where the collection is hosted.

Last but not least, software for the visualization of shape transformations have still not been implemented, also as a consequence of the two dimensional nature of most published papers (Adams *et al.*, 2004), as only on-line publications would allow to visualize 3D motion figures.

2. Landmark coordinate recording from images

Two dimensional coordinates are digitized from digital pictures through specific software. Among the free software available at the site http://life.bio.sunysb.edu/morph are tpsDig2 (Rohlf, 2006a), COO (Dujardin, 2006), running also on Linux, and MacMorph by Mark Spencer for Apple Macintosh computers. Many packages of 3D visualizations have an option to collect 3D coordinates of points (landmarks) (see for example Dean, 1996). Edgewarp by Bill Green (see Bookstein, 1998, 2003), reads CT and other medical images in Dicom format and produces a grayscale volume which can be navigated and landmarked.

3. Data transformation

The non-shape variation due to position, orientation and scaling must be mathematically removed to obtain a matrix of shape variables (Fig. 6). Two point registration (Bookstein, 1986, 1991) and GPA represent the two focal methods of optimal superimposition (Adams *et al*., 2004).

Two-point registration is a particularly simple superimposition method which laid the foundations for much of Bookstein's development of shape theory in the late 1980's. Orientation, location and size are defined by a baseline, i.e. the length of a segment between two specific landmarks, usually located at two extremes of the object (Bookstein, 1991). Unfortunately, it is not easy to extend these Bookstein's shape coordinates to 3D data (Slice, 2005).

Using GPA, landmark configurations are superimposed using least-squares estimates for translation and rotation parameters, and configurations are scaled to a common, unit centroid size (Bookstein, 1986). The configurations are then optimally rotated to minimize the squared differences between corresponding landmarks (Gower, 1975; Bookstein, 1986; Rohlf and Slice, 1990). The process is iterated to compute the mean shape, called the *consensus* configuration. But meaningful mean coordinates cannot be computed prior to superimposition, which, in its turn, requires knowledge of the mean configuration. Thus, the mean or reference configuration is derived from an iterative process in which any specimen is initially selected to stand for the mean (Slice, 2005). GPA can be performed in 2D or 3D using the software Morpheus et al. (Slice, 1998).

When much of the shape variation is limited to just a few landmarks, generalized resistant-fit (GRF) may be used to visualize this pattern of variation (Rohlf and Slice, 1990; Slice, 1996). GRF estimates superimposition parameters as medians, rather than least-squares estimates. The rotation angle and scale are found as medians of medians across subsets of landmarks, and the translation is a simple coordinate-wise median. As in GPA, this procedure is iterated to allow a sample of specimens to be superimposed. In contrast to the use of GPA, the use of GRF does not lead to further statistical analyses (Adams *et al*., 2004).

4 Simmetrization

Symmetric objects like skulls cause a problem of redundancy, which can affect the dimension of the matrix, whilst fluctuating asymmetry can affect the interpretation of the results when it is not the phenomenon to investigate. Various techniques have been then proposed to 'simmetrize' the configurations, and obtain representative half configurations without losing information on both sides (Mardia *et al.*, 2000; Klingenberg *et al*., 2002; Giri and Loy, in press).

5 Multivariate analysis and visualization of shape changes

Shape variables may be used to statistically compare samples, and graphical representations of shape changes associated to the pattern of variation may be generated for comparison through the thin plate spline deformation grids. When parametric multivariate methods are applied to shape variables derived from GPA, a linear, Euclidean space, is assumed. An orthogonal projection of GPA coordinates to a linear space tangent at the sample mean, such as principal component analysis and regression, seems to better preserve the distances between specimens (Slice, 2005). Alternatively, non parametric, randomization tests can be used (Bookstein, 1997). In contrast, a relative warp analysis performed on the weight matrix and uniform components corresponds to a principal components analysis of shape changes in the geometry of Kendall's shape space (Bookstein, 1996b). Relative warp analysis can be performed using the software tpsRelw (Rohlf, 2006b) or Morpheus et al. (Slice, 1998). The ordination of specimens along the relative warp axes can be integrated by the visualization on shape changes through deformation grids corresponding to any point within the space (see for example Corti *et al*., 2001) (Fig. 8).

Other programs of the tps series performing various analyses on shape variables are tpsPLS, tpsSmall, tps Regr, tpsTree (Rohlf, 2007), all downloadable form the website http://life.bio.sunysb.edu/morph/. TpsTree fit and visualize thin-plate splines on trees. It performs a least-squares orthogonal generalized Procrustes analysis to obtain a reference and then estimates the splines for each group (internal node) on a tree. The estimated shapes can be visualized with estimated images in the background (see for example Macholan, 2006). TpsRegr performs a multivariate multiple regression of shape (as captured by partial warp scores and the uniform shape component) onto one or more independent variables, including the centroid size for studying allometry, or the regression on canonical variate scores performed on the weight matrix. TpsPLS performs a two-block partial least-squares analysis and it is used to explore the covariance between shape variables and another set of variables (Rohlf and Corti, 2000). TpsSmall is used to test whether the variation in shape among a set of specimens is too large to apply the statistical methods based on the tangent space approximation (e.g., thin-plate spline methods). The program can process both 2D and 3D data files. Other comprehensive software using landmarks and shape variables are NTSYSpc by Jim Rohlf (Exeter Software), Morpheus et al. (Slice, 1998), Morphologika by Paul O'Higgins, specially for the analysis of 3D coordinate data, and Mophometrika for MacOS by Jeffrey Walzer.

GEOMETRIC MORPHOMETRICS, IMAGE BANKS AND MUSEUM COLLECTIONS

Museums hosting theriological collections can play an important role in the new geometric morphometric studies. The creation of image databanks of the specimens stored in museum collections would greatly improve and speed up the morphometric analyses of large samples, contributing to minimize the damages due to specimens handling by researchers.

Digital imaging has undergone an exponential development in the last decade, allowing access to high resolution low-cost devices. For morphometric analyses images must be taken following some basic rules, as imaging systems include artefacts related to acquisition, storing and display processes (MacLeod, 1990; Fink, 1990; Rohlf, 1990b; Becerra *et al.*, 1993; Garcia-Valdecasas, 1996; Zelditch *et al.*, 2004). A high quality optical equipment and some cautions in specimens position and distance from the camera will help to reduce the effect of these artefacts. For example, Zelditch *et al*. (2004) suggest to place a piece of graph paper in the field to highlight the so-called rainbow effect, keep the object as more as possible in the centre of the image, and place the camera at such a distance that distortion effects do not occur at image margins. The resulting image is a compromise between detail accuracy and file size. If colour is important, the most economical format is JPEG. The lighting of the object is another important aspect, as shadows and the reflecting surfaces of the object (bone surfaces often reflect a lot) may impede to see important features such as skull sutures. When knowing in advance what structures must be examined, light can be adequately balanced in advance, otherwise the lighting that maximize the visibility of the whole object is recommended. Images should always include a scale factor and, possibly, a label with codes referred to the locality (geographic coordinates), sex and age of the specimen, which would allow the quick grouping of homogenous samples for sequential landmark recording. Examples of online image banks are the project Morphbank (O'Leary and Kaufman, 2007), Digital Morphology (University of Texas, www.digimorph.org), and ORSA – Open Research Scan Archive (University of Pennsylvania, http://grape.anthro.upenn.edu/~lab-/pennct/). Despite these resources were not created for geometric morphometric studies, they can be easily implemented by incorporating a coordinate digitizing software, or by offering the possibility of downloading the image files.

1. New perspectives

One limitation of landmark based morphometrics is that a sufficient number of landmarks may not be available to describe the shape of a structure. Many structure are often better described by curves where only one coordinate, either x or y, is clearly and unambiguously detectable. To solve this problem Bookstein (1997, 1998) proposed the semilandmark method, that represents an extension of the standard Procrustes superimposition, and allows to extend the mathematical formalization of the geometry of landmarks to two dimensional contours (curves) or surfaces. In addition to optimally translating, scaling and rotating the landmarks, semilandmark points are slid along an outline curve until they match as well as possible the position of the corresponding points along an outline in a reference configuration. Most of the geometric morphometric digitizing software, such as tpsDig2 (Rohlf, 2007) allow to collect outlines and sliding landmarks.

As the growing use of medical imaging has allowed the production of 3D images of extant and fossil specimens, the analysis and visualization of 3D data and the combination of landmarks, semilandmarks, outlines and surfaces are expected to yield a better description of changes in biological complexes (Fig. 9). 3D analyses are

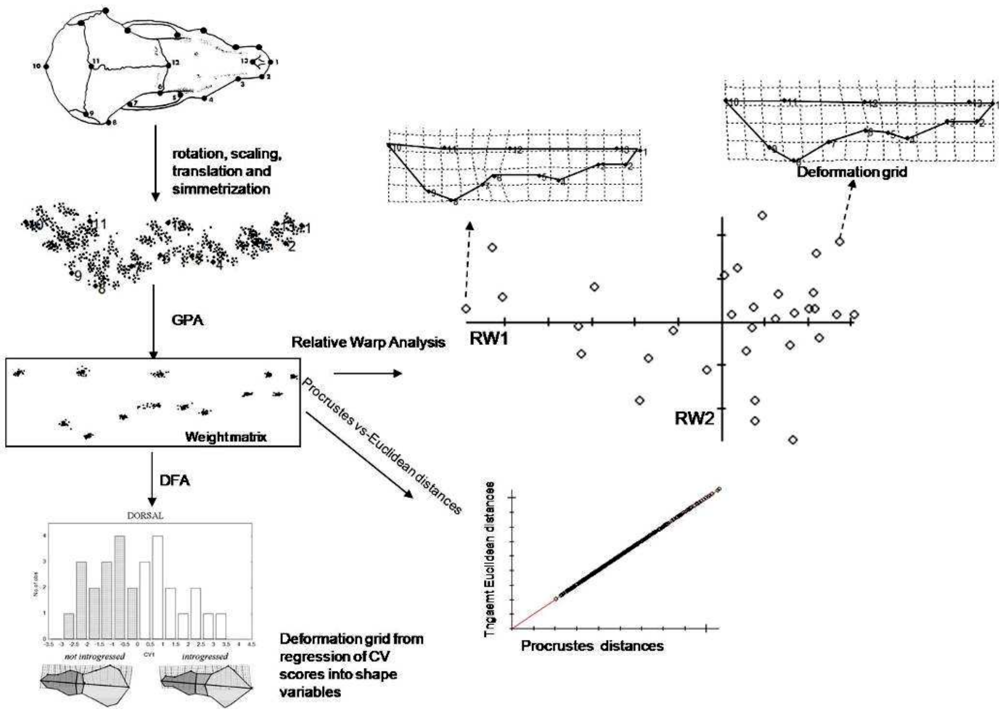
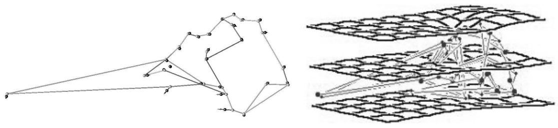
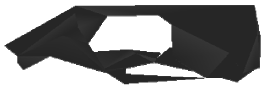


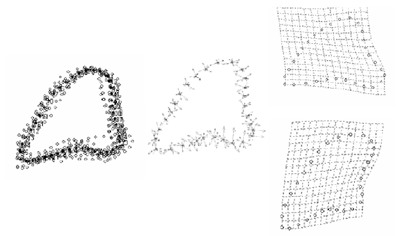
Figure 8 - Schematic set of procedures in a two-dimensional landmark based morphometric study of a mammalian skull. The original landmarks recorded on both sides of the skull are rotated, translated, scaled, and subjected to a simmetrization procedure that reduces the number of variables and the redundancy of the data set. Data are then superimposed through GPA, while values for the centroid size are saved and kept for further analyses. The residuals from superimposition through GPA (weight matrix) can be processed through an exploratory multivariate Relative Warp Analysis. In this new space, it is possible to visualize the shape changes related to each specimen by means of thin plate spline deformation grids. The weight matrix can also be exported and analyzed through confirmative analyses (e.g. DFA). The coefficients for each axis can be regressed into shape variables and shape changes of significant linear relations can be shown through thin plate spline deformation grids related to the extremes of variation along the axis (from Loy *et al*., 2001). Euclidean distances derived from multivariate analysis of shape variables can be compared to Procrustes distances to verify their significant linear relationships.



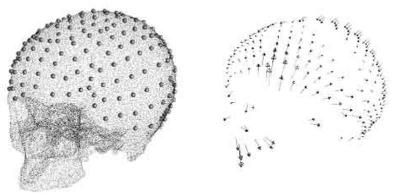
A



B



C



D

Figure 9 - Some examples of visualization of the results of three-dimensional landmarks analyses. A. Comparison of *Stenella coeruleoalba* skulls. The direction of changes is visualized either by vectors of landmark displacements (left) or through deformation grids at specific two-dimensional tangent planes (right) (original data from Anna Loy, Arianna Tamburelli, and Dennis E. Slice*)*. B. Surface rendering of the landmark configuration describing an early ontogenetic stage of a marmot skull (courtesy of Andrea Cardini, from Cardini and Thorington, 2006). C. Analysis of semilandmark recoreded on the outline of P_3 anteroconid of fossil lagormorphs of the genus *Prolagus* (original data from Chiara Angelone and Anna Loy*)*. D. Sliding semilandmark quantifying the surface of a skull (from Adam *et al*., 2004).

affording several new perspectives in the field of human paleontology and physical anthropology (see for example Gunz *et al*., 2005; Gunz and Harvati, 2007), and soon they will probably do the same for other mammal *taxa*. Progress is also expected about the study of covariation between subsets of landmarks (Bookstein *et al*., 2003), the extension of landmark based morphometrics to the analysis of articulated structures (Adams, 1999), and the fitting morphometric data to phylo-genetic inferences (Felsenstein, 2002; Rohlf, 2002).

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