GEOMETRIC MORPHOMETRICS

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Contents

Summary

The aim of this chapter is to provide a simple introduction to Geometric MorphoMetrics (GMM). GMM is the numerical study of the interaction of size and shape with covarying factors in biology. It represents the modern form of morphometrics, a discipline with a century long history, and it includes a variety of methods. GMM makes extensive use of the advances in computer technology and digital imaging, providing tools to analyze and collect data within a rigorous statistical framework. Thanks to the combination of analytical power and intuitive visualizations using computer graphics, GMM has rapidly emerged as one of the most widely applied disciplines in biology.

In this review, I will: 1) briefly summarize the history of morphometrics; 2) provide a rapid overview of the new methods; 3) describe and exemplify the analysis of anatomical landmark coordinates using Procrustes methods, the leading set of techniques in morphometrics. Specific topics, which may not be of interest for the general reader but could be of help to those who may want to learn and apply these methods, are discussed in a series of sections in Appendix 1.

1. Background

1.1. Size and Shape: What are they?

Morphometrics is the quantitative statistical description of biological variation in form. Form is composed of size and shape. Informally, size is a measure of the magnitude or scale of an object. It says something about how big or small something is. Different measures of size may be relevant to different studies and will often result in different assessments of relative size and shape among the same objects. A linear distance, such as the condylobasal distance often used to measure cranial length in mammals, might be an estimator of size. Several distances can be combined as a sum or average, and many other options (areas, volumes etc.) can provide valid alternatives depending on the study aims and the methodological framework. Once size has been defined, shape is all the geometric information which is left after removing differences in size and position (i.e., translation and rotation of the objects one relative to the other). This is an operation that our brain routinely carries out, as we readily recognize the shape of an object regardless whether it is big or small, to the right (or left) of its original position, rotated etc. (Figure 1).

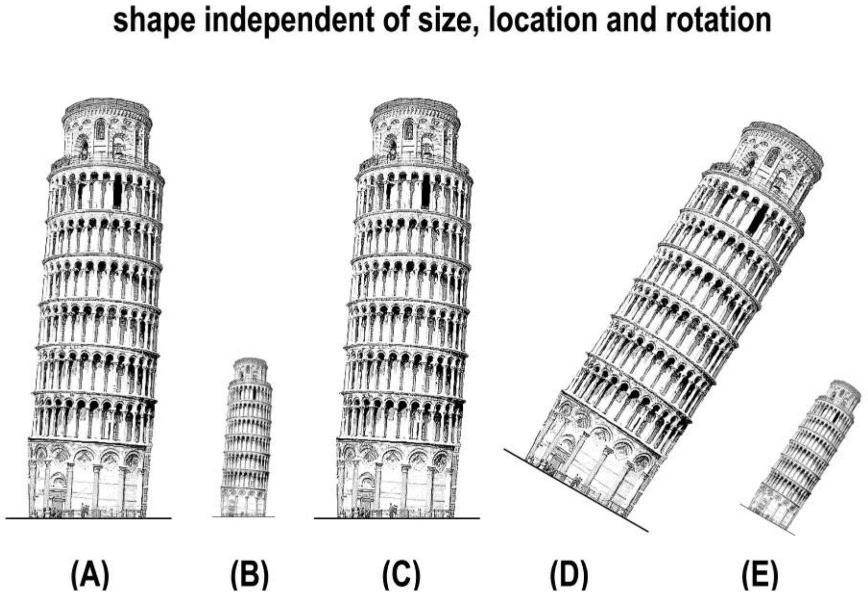

Figure 1. Size and shape: Pisa tower (A) recognizable shape despite differences in size and translation (B), translation only (C), rotation and translation (D) and rotation, translation and size (E). (Figure inspired by Chris Klingenberg's lecture in Turin, 2008; modified from a photo available at: http : / / en . wikipedia . org / wiki / File : Leaning_tower_of_pisa_2 . jpg) ..

1.2. Morphometrics 'Using Calipers'

Traditionally a morphometric analysis was achieved by taking linear distance measurements between pairs of anatomical points (landmarks) using calipers and subjecting these measurements to univariate and multivariate statistical tests. For instance, one could measure the length of femora and tibiae in adult humans and chimpanzees, assess differences and relate these to the different modes of locomotion of these two closely related species by contextualizing them biomechanically.

Geometric morphometrics extends the approaches of Traditional MorphoMetrics (TMM). This is achieved by using Cartesian coordinates, which are measurements of relative landmark locations (Figure 2), rather than using distances or angles derived from measurements based on those same landmarks. Since these relative positions represent the geometric 'essence' of shape variation, the anatomical points themselves are directly used in GMM to extract size and shape variables. These operations are performed in ways that preserve the geometry of the set (or configuration) of landmarks, and in principle allow derivation of traditional length and/or angle measurements from the landmarks.

Accuracy and statistical power are increased and results can be visualized with images and diagrams that are more intuitive than the tables of linear distances and coefficients of TMM. The differences between modern humans and chimpanzees captured by a small set of points on adult crania, for example, can be shown by rendering outlines and using transformation grids to help to visualize and interpret the main changes over the whole structure (Figure 3).

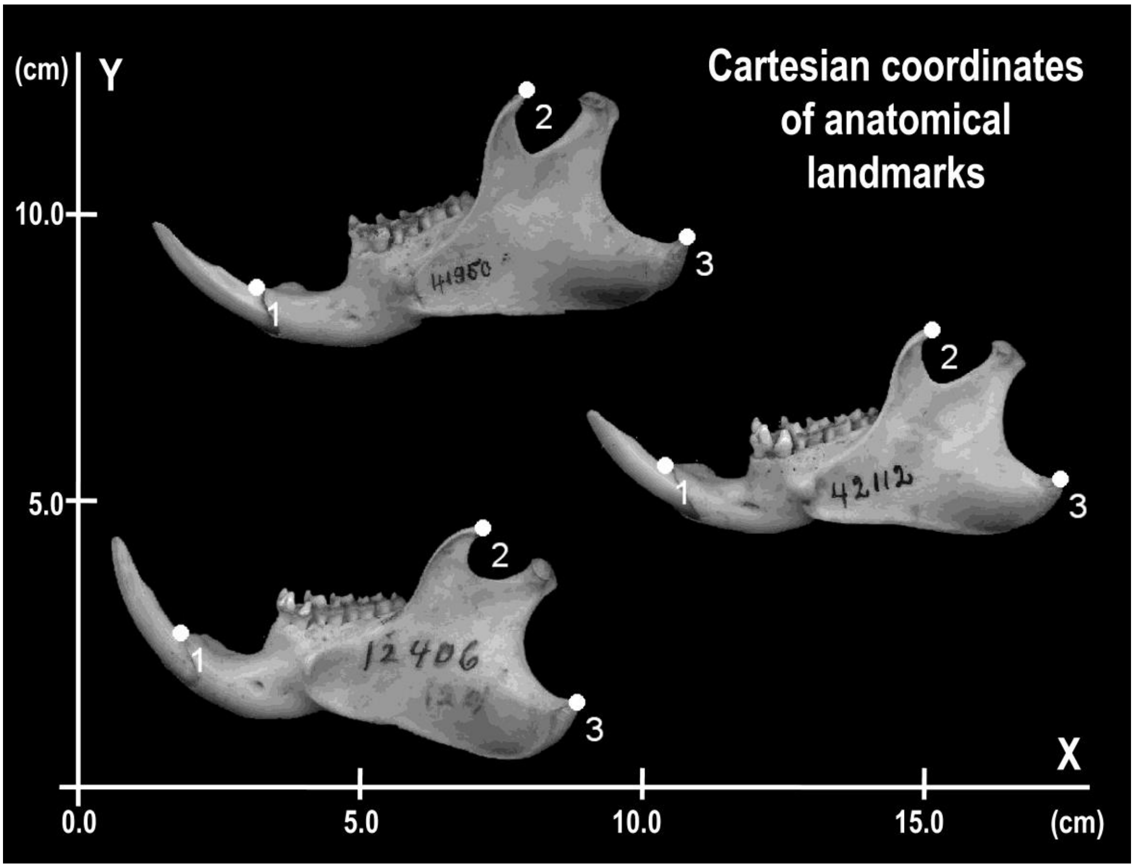

Figure 2. The form of three marmot hemimandibles is measured, in this example, using the Cartesian coordinates of three anatomical landmarks (1, tip of the alveolus; 2, tip of coronoid; 3, tip of angular process) digitized on their pictures

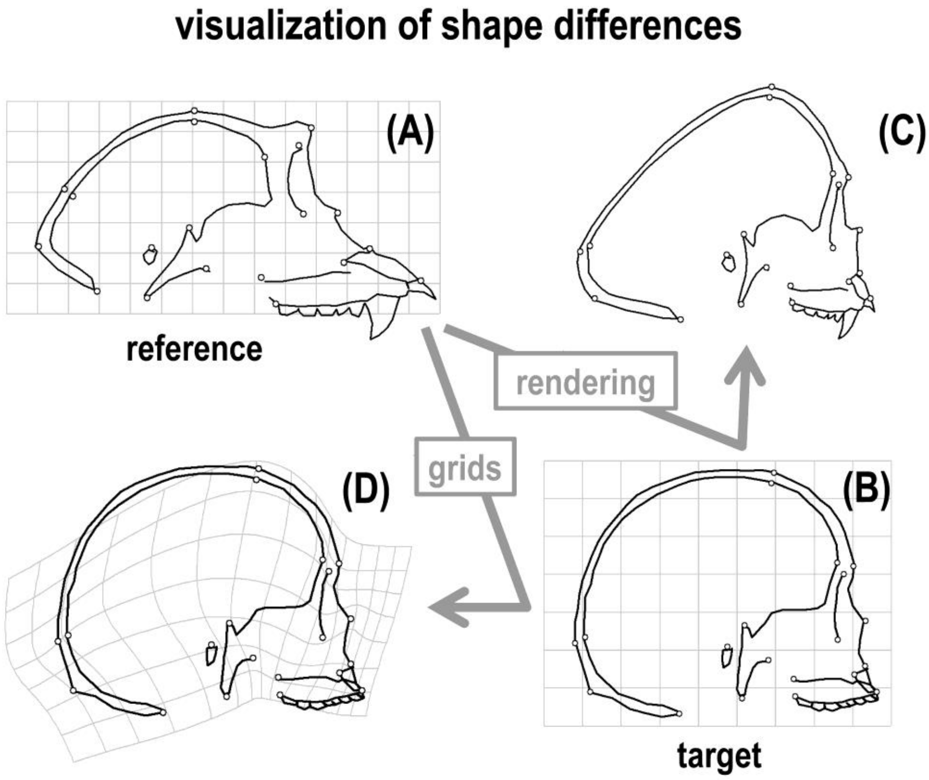

Figure 3. Visualization of shape differences. Cranial variation of chimpanzees and humans is captured by a configuration of 20 landmarks on the mid-plane. Differences are shown by warping the reference chimpanzee shape (A) into the human target (B) until the landmarks in the reference coincide with those in the target: the resulting changes in the contours (C) and the grid squares (D) help to see the main aspects of cranial variation in the comparison of these two species. ‘‘A way to think about... [contour rendering and transformation grids]... is as if one form were printed on a transparent stiff plastic sheet [together with a set of square grids] and then manipulated by bending so that its ‘shadow’ takes on the prescribed landmark positions of the second form’’ (p. 1168, Zelditch et al., 1992) (Data courtesy of D. Slice).

1.3. Calipers and Statistics: Few Words on the Early History of Morphometrics

The history of morphometrics is at least as long as that of modern science. At the end of the 19th century and the beginning of the 20th, scientists begun to extensively measure phenotypic traits and summarize their findings using sample means and other parameters. For instance, a series of papers by Bumpus is considered one of the first evolutionary studies using morphometrics (Adams et al., 2004).

Bumpus(1898 [NB: additional references, including citations in the Appendix 1, are found after the main annotated bibliography and are provided to help beginners and those interested in more specialist aspects, that go beyond the interests of general readers) took several measurements on house sparrows collected after the birds had been stranded on a north American beach during a storm. Some of them survived and other died. Based on these data, he concluded that those most likely to survive had traits closer to the sample average, which he saw as a kind of 'Darwinian optimum'. Half a century before Bumpus' work, and even before Darwin's “On the origin of the species” (1859), Morton (1839) compared human ethnic groups using estimates of cranial capacity. This was an “empirical approach, generating data by systematically measuring large numbers of actual specimens, [that] was groundbreaking” (p. 1, Lewis et al., 2011). Even earlier, in the 17^{th} century, the German anatomist Elsholtz measured human variation thus marking the beginning of the field of anthropometry. In fact, human proportions have been studied and compared by artists for millennia (Reyment, 2010; Slice, 2005).

The analytical techniques to examine measurements were, however, developed only relatively recently and provided the bases for the mathematical description of form variation in samples. A prominent role in this advancement was played by the English school of biometricians led by Galton (1822-1911) and Pearson (1857-1936). They developed fundamental methods such as the correlation coefficient, linear regression and principal component analysis, all of which are still used by contemporary scientists.

Their contributions, together with those of other biostatisticians, such as Fisher (1890-1962) and Mahalanobis (1893-1972), led to the birth of the modern discipline of statistics and produced many of the methods (analysis of variance, discriminant analysis etc.), which soon became the standard analytical tools of morphometricians. In the same decades, Huxley and Teissier (1936) coined the term allometry to describe the differential rates of growth of anatomical regions as distinct from uniform changes in the size of the organism, and formalized this concept in a simple equation, whose parameters can be estimated by regression models (Gayon, 2000).

Multivariate morphometrics, later renamed TMM (Marcus, 1990), emerged from this scientific background in the second half of the 20^{th} century. The name morphometrics appeared in the literature for the first time in the work by Blackith on the relationship between form and swarming behavior in locusts (1959).

It was used to indicate the application of statistical analysis to the study of morphological variation. In an extended presentation of his research, Blackith (1960) used a technique called linear discriminant analysis (DA) to combine measurements on locusts in a way that emphasizes group differences.

With this method he managed to find directions in the phenotypic space that best separate grasshopper morphs, demonstrating a connection between color polymorphism and external morphology.

In Appendix 1-A, additional readings are suggested that review the field of TMM and describe how the statistical tool-kit for the analysis of linear measurements became even more powerful thanks to the improvements of old methods, the development of new techniques and the design of innovative applications.

1.4. Three Main Limits of Traditional Morphometrics

TMM has offered and still offers a broad range of useful techniques to address a variety of scientific questions in biology and other fields. However, TMM has limitations that are difficult to overcome. These limitations provided the motivation to develop innovative techniques that eventually led to GMM:

i) TMM data typically consist of size measurements, such as lengths. Using multivariate analysis, variables can be 'size-corrected' to extract information on shape. There is a range of methods in TMM to separate size and shape, but it is hard to say which, if any, works best. Separating size differences due to allometry from those due to differences in overall size (or scale) of the organisms is often challenging. Studies suggesting that one or other technique is more accurate or effective are unlikely to be generalizable. 'Size-corrections' also make interpretations less straightforward, as they are often based on standardizations or combinations of the original variables. The use of ratios is not ideal either. Ratios are apparently simple, but they are ambiguous, because the same ratio can be produced for very different shapes (Figure 4).

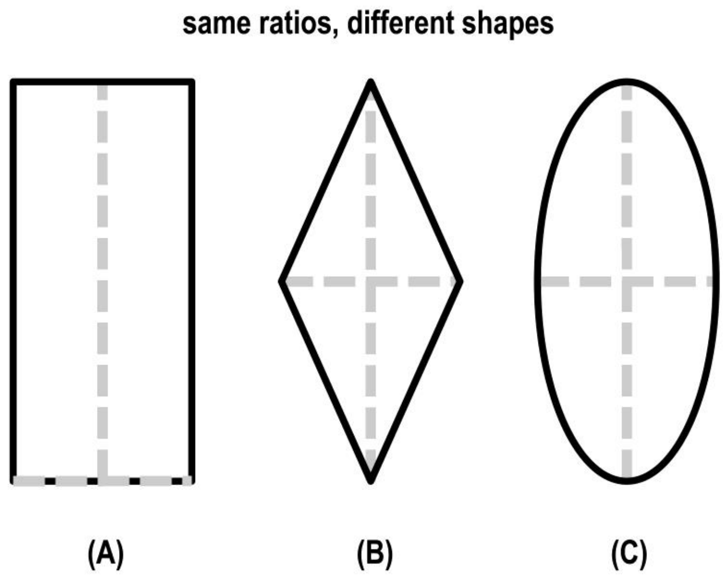

Figure 4. Same ratios different shapes: the ratio (dotted lines) between the maximum height and the maximum width of the rectangle (A), rhombus (B) and ellipse (C) is the same, but these three polygons have clearly different geometric shapes.

ii) TMM uses measurements between points, but it does not preserve their spatial relationships. In Figure 4, there is no way to know, using just the height and width of the two polygons, where the straight line representing the width intersects with line measuring the height; the orientation of one line relative to the other is also missing. It is true that by adding more information one could reconstruct the spatial arrangement of a set of anatomical landmarks, but this rapidly becomes impractical for a large number of points. Using linear measurements between pairs of a total of *q* 2D landmarks (i.e., landmarks on pictures of a study structure, such as for instance those in Figure 2), a minimum of $3+3\times(q-3)$ distances are needed to completely reconstruct the relative positions of the landmarks (Corruccini, 2006). This means that for just 30 landmarks, one needs to take no less than 84 caliper measurements for each individual and for 100 landmarks that number raises to almost 300. If landmarks were reconstructed in 3D (i.e., as if they had been measured directly on a skull or another 3D structure), the formula would be $6+4\times(q-4)$ (Slice, personal communication), and the number of linear measurements to take even bigger.

iii) TMM produces results, such as tables of measurements or coefficients (from regressions, DA etc.), that are not easily related to the original morphologies and therefore make the interpretation of findings more complicated.

1.5. From Traditional Morphometrics to Geometric Morphometrics

The history of the attempts to develop effective methods to describe morphological variation using geometric techniques is as long as that of TMM. Thompson (1917), one of the fathers of mathematical biology, showed in his seminal book "On growth and form" that grids (such as those in Figure 3) could be employed to compare shape differences in biological structures. A rigorous quantitative method for predicting how the grids bend, expand and contract in the transformation of one shape into another was not developed, however, until the end of the 1960s (Sneath, 1967) and had its successful mathematical formalization only in the second half of the 1980s (Bookstein, 1989).

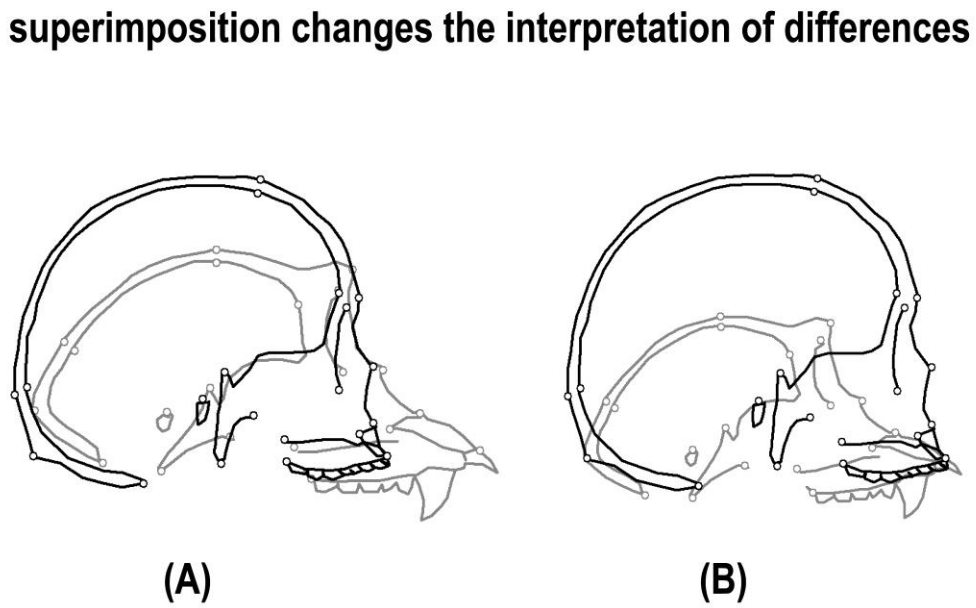

Figure 5. Chimpanzee (grey) and human (black) crania superimposed using different mathematical criteria: (A) Procrustes superimposition; (B) Bookstein baseline with inion and prosthion as the common baseline. (Data courtesy of D. Slice).

Superimposing structures as a way to measure and visualize differences (Figure 5) was also suggested more than a century ago. Despite their appeal, superimposition methods, which are also called registrations or alignments, have not been really adopted until fairly recently. Their limited success was a consequence of the arbitrariness of the choice of the superimposition.

This crucially affects the outcome of the visualization and may lead to considerably different interpretations of shape changes. This is exemplified in Figure 5 using the same data as in Figure 3 to measure cranial differences between humans and chimpanzees: although both types of superimpositions (which are explained in the next paragraph) clearly indicate the extraordinary expansion of the braincase in humans, this aspect is over-emphasized in B compared to A, whereas facial prognathism in chimpanzees is more evident in A.

The principle behind the superimposition method used in Figure 5B dates back to the work of Pearson at the beginning of the 20th century. He superimposed human facial profiles on two points to emphasize shape variation. This technique became later known as Bookstein superimposition or Bookstein baseline, and results in Bookstein coordinates. It simply involves rescaling, rotating and translating specimens until the two baseline points overlap.

This superimposition implies that the length of the baseline is the standard measure of size in the analysis, as the coordinates are normalized via division by this length. Pearson applied the baseline superimposition in order to identify the mummified head of Oliver Cromwell by matching its landmarks to the available corpus of portraits and busts (Bookstein et al., 2004). Boas, the father of American anthropology and a contemporary of Pearson, tried a similar approach for comparing skull shapes, but he decided to minimize the sum of distances between all cranial points in a pair of specimens to align them (Cole, 1996).

Boas' original intuition was later picked up by other scientists (Sneath, 1967), who elaborated the method and studied the properties of the space of shapes it generates (see the series of papers by Kendall cited in Slice, 2005, and in particular Kendall, 1977). In the 1990s, the method was further developed and became known as generalized Procrustes analysis (GPA) or simply Procrustes superimposition (Rohlf and Slice, 1990). GPA is now the most popular superimposition model because of its desirable statistical properties (most importantly perhaps being the establishment of a generalized metric of shape distance, the Procrustes distance between specimens – see below).

However, in terms of the biological interpretations of differences, GPA is as arbitrary as other superimposition methods. This is why visualizations of superimposed shapes should be examined with the greatest caution or altogether avoided. A best known example of this problem is the so called 'Pinocchio effect' illustrated in Figure 6 (see also Appendix 1-B): although the only difference between Pinocchio before (A) and after lying (B) is the length of the nose (C), superimposed shapes (D) suggest otherwise; however, this apparent difference is purely an artifact of the superimposition used to separate size and shape.

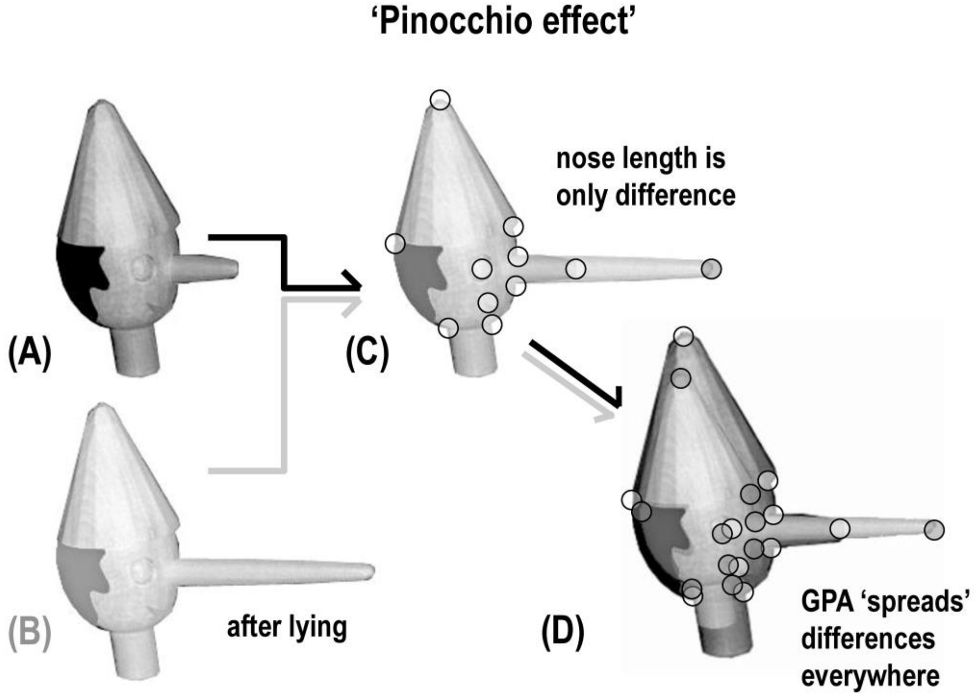

Figure 6. Pinocchio effect. Pinocchio before (A) and after (B) lying: the only difference is the nose length, captured by the dark grey landmark (C). However, after the Procrustes superimposition using the set of landmarks shown in (C), differences seem to occur all over the head. (Pictures modified from a photo available at http : // en . wikipedia . org / wiki / File:PinnocchiFirenze . jpg).

Superimposition methods rely on specific points, the landmarks, to describe the aspects of shape variation. For a meaningful comparison, landmarks must have a precise one to one correspondence. It is often said that they must be 'homologous'.

As for the whole landmark configuration itself, the kind of 'homology' of the landmarks depends on the scientific questions: "... in a study of bat and bird wings if one is interested in function, landmarks at wing tips and along the leading and trailing edges may be functionally equivalent; they might embody the question in being related to functionally relevant aspects of form.

However, these landmarks may lie on structures that are not equivalent in other ways; for a study of growth or evolution, alternative landmarks may be the most suited ones" (p. 89, Oxnard and O'Higgins, 2009). 'Homologous' landmarks, however, are often limited in number or may be missing altogether. The optic cup in the human eye is a small crater-like depression at the centre of the region where the optic nerve connects to the retina (Figure 7A).

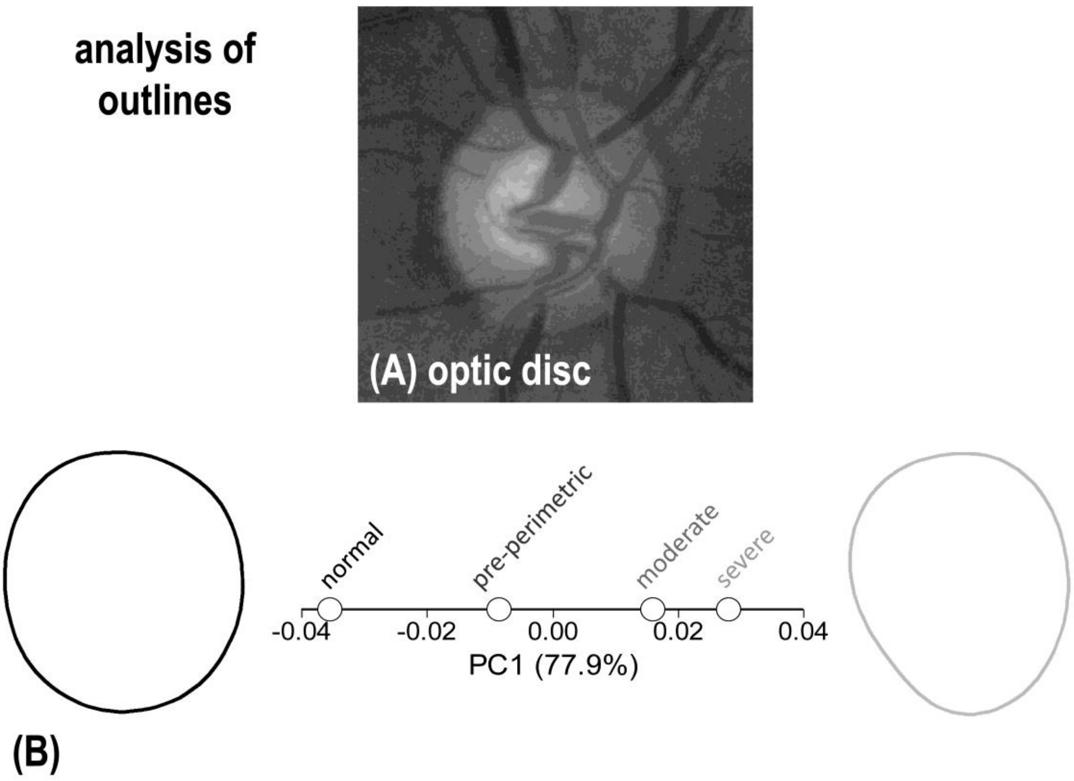

Figure 7. (A) Optic disc (modified from a photo available at http : // en . wikipedia . org / wiki / File :Retinography . jpg). (B) Analysis of outlines of the optic disc using Elliptic Fourier Analysis. PCA of mean reconstructed shapes for groups of patients with different degree of severity of glaucoma (data courtesy of Paul Sanfilippo): the main axis of change (77.9% of total shape variance) perfectly aligns with the direction set by the severity of the disease (i.e., Normal / Pre-perimetric / Moderate / Severe) as assessed by AGIS score (Advanced Glaucoma Intervention Study - AGIS Investigators, 1994). Mean reconstructed shapes for the opposite extreme of the range of variation (i.e., Normal *versus* Severe) are shown.

The cup is approximately circular and lacks well defined anatomical landmarks. In order to measure and compare anatomical structures such as the optic cup, a series of methods for the analysis of outlines was developed. Figure 7B shows the gradient of variation in the optic cup shape as a function of the degree of severity of glaucoma captured by the main axis of variation in its outlines.

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