



Removing Allometric Effects of Body Size in Morphological Analysis

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(Received on 10 June 1999, Accepted in revised form on 14 March 2000)

In the present paper, a normalization technique to scale data that exhibit an allometric growth is presented and the way it has to be used is described. It is shown how the method has been derived from the theoretical equations of allometric growth. Consequently, the method completely removes all the information related to size, not only scaling all individuals to the same size, but also adjusting their shape to that they would have in the new size according to allometry. In the particular case of isometry when the measures are of identical dimension, this normalization coincides with ratios (one of the most popular methods but only valid in this particular case). This procedure is a theoretical generalization of the technique used by Thorpe (1975, *Biol. J. Linn. Soc.* **7**, 27–43; 1976, *Biol. Rev.* **51**, 407–452) which was recorded as one of the most efficient methods in the empirical evaluation done by Reist (1985, *Can. J. Zool.* **63**, 1429–1439).

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Introduction

Most of the variability in a set of multivariate morphometric data from natural populations is due to individual size. In morphometrics, size must be considered as a contingent source of variability since it is associated with individual growth and the aim of such studies is usually focused on shape that must be size-free.

In the general case of allometric growth (one type of ontogenetic variation), there is a variation in shape related to variation in size. The influence of size due to allometric growth may be eliminated by the appropriate statistical procedures (Gould, 1966). There are several normalization methods whose aim is to eliminate the size effect in the context of allometric growth. However, some of the most popular methods have critical shortcomings that lead to misinterpretation of

the results. Among those the ratio of every measurement to the one chosen as the independent variable effectively reduces all the individuals to the same size but does not remove the undesired size effect because they maintain their size-dependent shape due to allometry. In other words, it is only valid if growth is isometric (i.e. shape does not change with size).

Traditionally, in the studies of multivariate allometry it has generally been assumed that the first component in the principal components analysis (PCA) represents the size, because all the characters are correlated positively with this component (e.g. Jolicoeur & Mosimann, 1960; Shea, 1985). However, it has been observed that as much the size as the shape are incorporated into this first component (Mosimann, 1970; Sprent, 1972; Humphries *et al.*, 1981). Several methods have been used to correct for the size factor, e.g. regression applied a PCA (“shearing”) (Humphries *et al.*, 1981) and extraction of

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the vector of isometric size and their eigenvalue associated with a PCA (Somers, 1986), or to eliminate the size by a ratio method (Mosimann and James, 1979). The use of PCA to eliminate the influence of size in an intra-specific analysis in discriminating by shape (expressed by the second principal component) appears to be inferior to the prior elimination of this influence. The method which eliminates the influence of the ontogenetic size appears to be superior to the PCA (Thorpe, 1976).

The aim of the present paper is to present and describe a normalization technique to scale data that exhibit an allometric growth. The method is theoretically derived from the equations of allometric growth. This normalization procedure is, consequently, coherent with allometry. It completely removes all the information related to size, not only scaling all individuals to the same size, but also adjusting their shape to that they would have in the new size. In other words, this procedure is one proper way for scaling in the case of allometry. This procedure was presented empirically by Thorpe (1975, 1976) and was recorded as one of the most efficient methods in the empirical evaluation done by Reist (1985).

The Allometric Model

The general form relating any pair of magnitudes X and Y of a body is described by the equation

$$Y = \alpha X^\beta, \quad (1)$$

where α and β are parameters.

This equation is just a formal relationship between two morphometric variables. For example, if X is the diameter and Y the volume of a sphere, the parameter values are $\alpha = 2\pi/3$ and $\beta = 3$. In general, the parameter α is called the coefficient of shape, because its value only depends on the shape of the body, while β is the power fulfilling the dimensional balance (in the previous example, $\beta = 3$ because the volume is the third power of a length).

In the case of geometric bodies with no structural variability, eqn (1), with constant parameters, completely describes the relationship

between any pair of measurements taken on such a body.

The changes of shape due to body transformations involved in growth can be represented as changes in the parameter α . For example, the transformation of a cube with diagonal = 1 into a sphere of diameter = 1 could be represented by a change of the parameter α from 1.732 (= $3^{1/3}$) to 2.094 (= $2\pi/3$). Since the growth of an organism is not only an increment of its size but also a change in shape, for convenience, it could be assumed that the shape coefficient α is proportional to a size power, so that

$$\alpha = aX^\gamma, \quad (2)$$

where a and γ are constants.

Substituting eqn (2) into eqn (1) the allometric power equation stated by Huxley (1924, 1932) is obtained

$$Y = aX^b, \quad (3)$$

where both a and b are constants.

This new expression (3) is formally similar to eqn (1). It consists of a re-parameterization of eqn (1) suitable for allometry analyses. In comparison with eqn (1) here the parameters are (i) constant and (ii) have a different meaning: a is a constant factor of the shape parameter α in eqn (1) while the variability of this parameter has been transferred to the exponent, modifying it according to

$$b = \beta + \gamma. \quad (4)$$

In the particular case of isometric growth, as the shape will not change with size, the shape parameter α in eqn (1) is constant, then $a = \alpha$ and $\gamma = 0$; thus $b = \beta$, and, hence, eqns (1) and (3) are exactly the same. In such a particular case, an observer could not guess the size of an individual from a picture without a scale. However, in the general case of allometric growth where there are changes of shape with size, the parameter b would be different from the value of β expected in the dimensional balance. The shape of an individual can be expressed as a particular combination of different measurements taken over it. An

observer could say something about the size of a particular individual from a picture without a scale, if he were accustomed to seeing how shape changes with growth in that species. For example, if we were to see a picture showing child and adult silhouettes with the same height, we could guess that there were differences in their actual sizes because of our empirical knowledge of the evolution of shape with size in human growth.

It should be emphasized that the term allometric growth only refers to smooth and regular changes of shape during growth. Processes like metamorphosis, involving the appearance of new structures or disappearance of old ones, do not fit the general allometric model (3).

At this point it should also be noted that, in morphometrics, any model other than eqn (3) is usually wrong. In this respect, the linear model, $Y = A + BX$, found to be widely used, particularly in cases where X and Y have the same dimension ($\beta = 1$), is wrong for at least two reasons: (i) it cannot detect shape changes, the main aim of morphometrics, and (ii) the independent term A has no sense in morphometrics where at $X = 0$, Y must be 0.

In a morphometric context, a population can be defined as all the individuals that share the same allometric relationships. This means that the relative growth of a given pair of body magnitudes follows eqn (3) with the same parameter b for all individuals. In other words, in any individual, every given pair of body magnitudes is related to a unique parameter b in the model (3). Then the set of parameters b (one for every pair of magnitudes) constitutes a characteristic of the population.

On the other hand, we can recognize and identify particular individuals in a population, and we can do this along their life. In spite of the fact that the shape of all individuals changes with time according a fixed set of b 's, each individual keeps its particular shape allowing an observer to identify it in its different phases of growth. Since b 's have been assumed to have the same constant values for all individuals, and gather the body changes associated with size, the particular shape of an individual must be contained in the a 's, the other parameter of eqn (3). So every individual has its own set of a 's

(constant along its life) determining its individual shape.

Fitting Data

Within the frame of the previous assumptions, the morphometric relationship between a pair of body measures X and Y taken on a particular individual i of a population can be expressed as

$$Y = a_i X^b, \quad (5)$$

where the constant a_i depends on the particular individual while b is the same for all the individuals in the population. The *shape factor* a_i (see Appendix A), can be split into two factors as follows:

$$a_i = a e^{\varepsilon_i}, \quad (6)$$

where a is the *common shape factor*, shared by all individuals of the population and e^{ε_i} is the *particular shape factor* distinctive of the individual i . The relationship between X and Y may be rewritten as follows:

$$Y = a X^b e^{\varepsilon_i}, \quad (7)$$

thus now both a and b are constants for all the individuals while the shape particularity of individual i has been moved to e^{ε_i} . Now assuming that ε_i is normally distributed (see Appendix A), regression technique may be applied to obtain the parameters a and b . Note in addition that in eqn (7), if X is set to any constant value, the distribution of the corresponding Y 's would be log-normally distributed.

The particular shape factor is constant for every individual and accounts for the persistent features that make each individual not only different from others, but recognizable throughout life (it is independent of the value of X). Equation (7) may be interpreted as the particular curve for individual i relating X and Y magnitudes while the estimated regression curve,

$$Y = a X^b, \quad (8)$$

represents a theoretical mean curve of the set of all individual curves. Since the particular shape factor is a constant dependent on each individual, all particular curves of all individuals belong to the same family as the mean.

Once the parameters a and b are estimated (see Appendix A), the value of the particular shape factor for the individual i may be obtained by rearranging eqn (7). Thus, from the pair of measurement values (X_i, Y_i) made on this individual

$$e^{\varepsilon_i} = \frac{Y_i}{aX_i^b}. \quad (9)$$

hence,

$$\varepsilon_i = \ln \left[\frac{Y_i}{aX_i^b} \right]. \quad (10)$$

In this interpretation, every individual curve is represented by a single observation (X_i, Y_i) point lying upon the curve. In general, the available observations cover different values of X and it is recommended that sampling should cover systematically the entire range of variation of X in order to get good estimates of a and b . Figure 1 illustrates this interpretation.

Since the particular shape factor for the individual i is a constant, the theoretical value of Y , called Y_i^* , which this individual i would reach when $X = X_0$ is, according to (9),

$$Y_i^* = aX_0^b e^{\varepsilon_i}, \quad (11)$$

and, substituting the particular shape factor in eqn (11) by its expression (9), we obtain

$$Y_i^* = aX_0^b \frac{Y_i}{aX_i^b}, \quad (12)$$

hence,

$$Y_i^* = Y_i \left[\frac{X_0}{X_i} \right]^b \quad (13)$$

which is independent of a .

Equation (13) converts any particular observed point (X_i, Y_i) into a theoretical point (X_0, Y_i^*) in

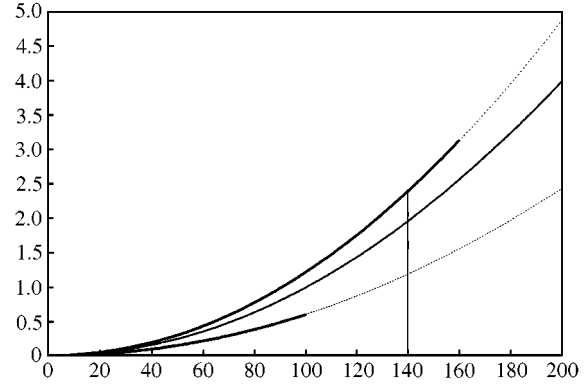


FIG. 1. Graphic illustration of the normalization procedure. The central curve (solid in all points) represents a regression curve estimated from a set of data (in arbitrary units) relating two magnitudes of a body. The lower and upper curves represent the growth trajectories of two particular individuals, one of which has a negative particular shape factor (see text), with current value for X being 100, and the other has a positive particular shape factor and its current X value has reached 160. The dashed sections indicate the trajectories assumed to be followed in the future by these two individuals. The intersection with the vertical line shows the values of Y which would have both individuals on $X_0 = 140$.

such a way that all observations taken at different values, X_i , are normalized to a unique X_0 , maintaining the particular shape factor for every individual (see the example in Appendix B). This procedure coincides with the correction proposed by Thorpe (1975). According to this author, the allometric character states have been adjusted to the values they would assume if the specimens were of mean body size.

The transformation of data proposed in eqn (13) removes all the size effects due to allometric growth. Otherwise, fundamental and permanent structures remain masked by the high amount of variance accumulated by such a contingent phenomenon. The logarithm of the particular shape factor, ε_i , computed in eqn (10) could also be used instead as a size-independent representation of a particular individual.

Discussion

In general, a morphometric data set will fit one of the two following case problems:

1. it can be assumed to be likely that all individuals follow one single allometric pattern. This

is the case in populations defined by geographical location, or sexes when no sexual dimorphism has been demonstrated, or

2. it is evident, or may be presumed, that different populations have different allometric parameters. This can happen between sexes in a clear case of sexual dimorphism, or among different species.

In the first case, the parameters a and b are estimated from all individuals, and finding an appropriate X_0 falling in the range of observed X 's, will not pose a major problem (see Appendix B).

The second case is slightly more complicated and it has been deeply analysed by Reist (1986). If the regression parameters of the different groups are similar (the shapes are not very different) an appropriate X_0 could be found. Nevertheless, two different allometric curves, relating X with two different groups, may have no common point for $X > 0$, or even if this point exists (and it could be a good candidate for X_0), it may fail in a third curve relating X to a third group.

If it becomes impossible to find a reasonable common value for X_0 or if the regression parameters of two or more groups were very different, it may imply that the comparison of such widely different organisms is meaningless.

Finally, it is considered that the method presented in this paper applies the concept following the geometric approach from a theorem of Mosimann (1970) generating a shape vector independent of size, and it generates a space of residuals instead of choosing an arbitrary variable (independent character) to use in the regression. However, Bookstein *et al.* (1985, p. 27) stated the following: "That we should not remove size from measured variables follows from a theorem of Mosimann (1970). In general, (...) residuals are still statistically dependent upon all measures of size except at most a single one not computable a priori." Obviously, these size-adjusted variables can yield satisfactory results by the above method and then it recommends a multivariate analysis to find the biological explanations in terms of the original size-free shape variables. The bivariate regression method is straightforward and useful, although there is a relatively main limitation that it assumes

growth can be adequately defined by a single independent variable (Thorpe, 1983).

Prof. P. Jolicoeur is thanked for his valuable comments on the first draft of this manuscript. Gabriel J. Torres' contribution was funded under the post-doctoral fellowship supported by the Consejo Superior de Investigaciones Científicas (CSIC), Spain. This work was supported by FAR (CICYT MAR 97/0640-C02-01).

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APPENDIX A

Stochastic for Fitting

Equation (3) is deterministic. When it is used to fit measurements taken over individuals of a population, the equation can no longer describe the actual relationship between the two variables because there are both natural variability among individuals and measurement errors. To take into account this new element implies a transformation of eqn (3) into a stochastic model to fit the data. Typically a random variable, named *error term*, should be included in the equation, which may be completed in several ways.

According to Ratkowsky (1990, p. 11), the error term can be additive or multiplicative (other possibilities will not be considered here), and its probability distribution may follow different laws. However, as few of the possible distribution laws are actually useful, only the normal-Gaussian probability distribution function will be used in this paper. Other well-known distributions

(e.g. Poisson or uniform), though used in some models, will not be discussed here. The normal-Gaussian distribution is used directly when errors are additive, while an exponential form of the normal distribution, known as log-normal, is used when the error term is multiplicative. The following equations represent both kinds of error considering the model (3) with an error for Y , and no error for X :

The additive

$$Y = aX^b + \varepsilon, \quad (\text{A.1})$$

and the multiplicative

$$Y = aX^b e^\varepsilon, \quad (\text{A.2})$$

where ε is a random variable, normally distributed with mean 0 and variance σ^2 . Hence, e^ε is log-normally distributed.

Equation (A.1) has two shortcomings in order to be adopted in morphometrics: it can produce negative values for Y which are not realistic, and also assumes the error distribution to be independent of X , while residual analysis shows frequently that the variance of Y is larger as X increases. Equation (A.2) does not have the above shortcomings and must preferably be adopted. In addition, as shown later, eqn (A.2) is more easily fitted than (A.1). If we had used eqn (A.1) instead, parameters would have to be estimated by non-linear regression, which would complicate the computations.

Equation (A.2) may be rewritten in a widely used statistical notation introducing

$$\hat{Y} = aX^b \quad (\text{A.3})$$

which is usually called the *expected value* of Y , according to the deterministic allometric model (3), while the value of Y obtained in stochastic equation (A.2) is known as the *observed value*. Thus, in terms of expected and observed values, equation (A.2) becomes

$$Y = \hat{Y} e^\varepsilon. \quad (\text{A.4})$$

Note that in eqn (A.2), error only affects the variable Y . This kind of error in stochastic

models is sometimes referred to as *process error* (Hilborn & Walters, 1992, p. 225), which means that the error term only reflects the natural variability among individuals, and measurements are error-free (no *observation error* affecting X). Francis & Shotton (1997) consider that the word “error” applied to the process variation is misleading. The term uncertainty (process uncertainty) might be more convenient since it expresses in a more accurate way that it derives from natural variation rather than error. The types of relationships in which the error term only affects one variable are called *predictive* (Ricker, 1973) since they are used to estimate Y when X is known (or X when Y is known). In the case that both variables are affected by an error term, other stochastic relationships, known as *functional* or *reduced major axis* (Teissier, 1948; Gould, 1966; Ricker, 1973), would be established. However, the *ordinary major axis* (also called the *orthogonal regression line*) of logarithmically transformed data, which assumes that both variates are affected by random fluctuations (Jolicoeur, 1975; Jolicoeur & Ducharme, 1992) also might be considered.

According to the aim of morphometrics, we can assume that the source of variability comes only from the morphological differences among individuals the measurements being error-free. Thus, a process error is assumed in the stochastic model and measurements are considered as exact. In most morphometric studies, there are many multivariate observations available to estimate the two parameters in eqn (A.2) for every pair of variables. Hence, regression techniques must be applied for fitting. According to the regression theory (Draper & Smith, 1981, Chap. 1; Ratkowsky, 1990, Chap. 1), the proper way to estimate parameters is to transform the equation in order to make the errors normal and additive (homoscedasticity). Consequently, the parameters in eqn (A.2) have to be estimated by linear regression on logarithmically transformed X and Y . The method suggested for testing the accuracy of the fitting procedure is a *posteriori* residual analysis.

After logarithmic transformation, eqn (A.2) becomes

$$\ln Y = \ln a + b \ln X + \varepsilon. \quad (\text{A.5})$$

Equation (A.5) is actually linear with additive error. Hence, the parameters can be estimated by standard linear regression procedures.

In summary, the following default assumptions on data and models are made: (i) the allometric growth equation (3) is used to compute estimates of Y ; (ii) the error term only affects the dependent variable Y , keeping the independent variable X free of error, i.e. it is a process error; and (iii) the error term is assumed to be multiplicative, with a log-normal probability distribution. Then, eqn (A.2) will properly describe the relationship between X and observed Y .

APPENDIX B

Example of Application

Packard & Boardman (1988) presented two sets of bivariate data from experiments carried out with eggs of two species of turtles (*Chelydra serpentina* and *Chrysemis picta*), each subjected to two different treatments. With the first species they found a significant difference among treatments while within the second one there was not. The goal of that paper was to show how misleading ratios could be, so they compared ANCOVA results with those using ANOVA on data scaled by ratios. Their conclusion was that ANCOVA gave the correct interpretation while ANOVA on ratios led to an erroneous conclusion in both cases (i.e. showing that the differences among treatments were significant in the second case while not in the first). These authors pointed out that the use of ratios to scale data that vary allometrically with body size is misleading. Although the reasonable field for the normalization method presented here is in multivariate analyses we will take advantage of the above bivariate example to illustrate, with a small number of data and a simple analysis, that this method works.

The data and results are presented in Tables B1–B3. Tables B1–B2 show, for each experiment, the raw data (X and Y) and the transformations: ratios (Y/X), normalized according to our method [Y^* , eqn (13)] and *particular shape* factor [$\exp(\varepsilon_i)$, eqn (9)]. The parameters a and b required for the normalization have been obtained by regression using eqn (A.5). Table B3

TABLE B1

Raw data presented by Packard & Boardman (1988) for *Chelydra serpentina* and transformed values. The regression parameters for $Y = aX^b$ are $a = 0.02825$, $b = 1.611$ and the mean X is 11.49; X_0 is set to 11.5

Egg mass (X)	Dry mass (Y)	Ratio (Y/X)	Y^* (for $X_0 = 11.5$)	$\exp(\epsilon_i)$
Group 1 ($N = 8$)				
10.223	1.184	0.1158	1.4313	0.9903
11.184	1.371	0.1226	1.4340	0.9922
12.251	1.676	0.1368	1.5136	1.0473
11.922	1.662	0.1394	1.5682	1.0851
11.485	1.509	0.1314	1.5122	1.0463
11.625	1.539	0.1324	1.5124	1.0465
11.303	1.481	0.1310	1.5228	1.0537
11.662	1.417	0.1215	1.3854	0.9586
Group 2 ($N = 8$)				
11.415	1.364	0.1195	1.3804	0.9551
11.684	1.508	0.1291	1.4699	1.0171
11.668	1.535	0.1316	1.4995	1.0376
11.322	1.387	0.1225	1.4223	0.9841
12.553	1.522	0.1212	1.3216	0.9145
12.213	1.502	0.1230	1.3633	0.9433
10.814	1.256	0.1161	1.3868	0.9596
10.493	1.230	0.1172	1.4257	0.9865

TABLE B2

Raw data presented by Packard & Boardman (1988) for *Chrysemis picta* and transformed values. The regression parameters for $Y = aX^b$ are $a = 0.705$, $b = 1.092$ and the mean X is 3.38, X_0 is set to 3.4

Egg mass (X)	Dry mass (Y)	Ratio (Y/X)	Y^* (for $X_0 = 3.4$)	$\exp(\epsilon_i)$
Group 1 ($N = 5$)				
3.050	2.349	0.7702	2.6447	0.9865
2.783	2.129	0.7650	2.6491	0.9881
2.492	1.936	0.7769	2.7176	1.0136
3.543	2.813	0.7940	2.6893	1.0031
2.495	1.908	0.7647	2.6748	0.9977
Group 2 ($N = 5$)				
4.088	3.307	0.8090	2.7044	1.0087
4.264	3.405	0.7985	2.6594	0.9919
3.200	2.528	0.7900	2.7009	1.0074
4.038	3.217	0.7967	2.6664	0.9946
3.855	3.102	0.8047	2.7046	1.0088

TABLE B3

Summary of comparative results of the statistical analyses in terms of F (Fisher) and its associated probability (P&B; Packard & Boardman, 1988)

	<i>Chelydra serpentina</i>		<i>Chrysemis picta</i>	
	F	p	F	p
P&B ANCOVA	6.10	0.029	1.33	0.29
P&B ANOVA on ratios	3.38	0.09	16.35	0.004
ANOVA on normalized data (present method)	6.59	0.022	0.52	0.48

shows the compared results of ANCOVA, ANOVA on ratios and ANOVA on the normalized data.

According to Table B3, ANCOVA and ANOVA on normalized data reveal that the two groups of *Chelydra serpentina* present significant differences ($p < 0.05$), while ANOVA on ratios does not detect them. The opposite situation happens in the case of *Chrysemis picta*. These results show that the normalization procedure presented in this paper allows to recognize the patterns,

also revealed by the ANCOVA, but misinterpreted by the ratio transformation.

As it has been said before, the method proposed here is more useful when applied to multivariate analysis, and has been success-

fully used in such analyses by Lombarte & Leonart (1993) in fish (hake) otoliths, Senar *et al.* (1994) in bird (siskins) wings and Ibañez-Aguirre & Leonart (1996) in fish (grey mullets) morphometry.