Electronically supplementary material

for

Size correction: comparing morphological traits among populations and environments

By

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APPENDIX I
CONSEQUENCES OF VIOLATING
ASSUMPTIONS OF ANALYSIS OF
COVARIANCE

Analysis of Covariance (ANCOVA), when applied as a method of size correction, uses a univariate descriptor of body size as a covariate to compare the sizes of morphological traits of different groups. ANCOVA uses a pooled within-group regression coefficient (i.e., estimating a common slope for multiple groups), and corrects the degrees of freedom used for comparisons because the slopes of the within-group regression lines are estimated from the same data. ANCOVA tests for differences in allometry by testing for heterogeneity of slopes. In principle, this approach should overcome the most obvious criticism of residuals analysis and shearing (which are predicated on pooled regressions).

The standard ANCOVA model is:

\[ Y_{ij} = \mu + \alpha_i + \beta(X_{ij} - \bar{X}) + \varepsilon_{ij}, \]

where the \( j \)th observation in group \( i \) of the independent variable \( Y \), is a function of four primary components: 1) the grand mean of all observations of the dependent variable \( \mu \); 2) the effect of treatments \( \alpha \); 3) the effect of the covariate \( \beta(X_{ij} - \bar{X}) \); and 4) the error \( \varepsilon_{ij} \) (Huitema 1980). Applications of this model require a number of assumptions (Huitema 1980 and Sokal and Rohlf 1995), but here we deal explicitly with two assumptions unique to ANCOVA that are important for its application in size correction: 1) the covariate and the effect of the treatment are independent, and 2) the covariate is fixed and measured without error. Although we discuss these two assumptions separately they are intimately linked and the potential for introducing bias in ANCOVA is greatest when they are both violated.

When the covariate and the effect of treatment are correlated (e.g., if treatments affect body size as well as size-corrected morphology) the interpretation of ANCOVA results can be ambiguous. In such cases, the within-group covariate means will be different from the grand covariate mean and when averaged may generate a grand covariate mean that is not (and potentially cannot be modified to be) shared by the treatment groups (Huitema 1980, Sokal and Rohlf 1995). Therefore, comparing adjusted means may be problematic because the covariate may either add or mask differences between treatment groups that may be misinterpreted as real treatment effects. Fortunately, the assumption of independence of covariate and treatments in ANCOVA can be tested by performing an analysis of variance (ANOVA) on the covariate; however it is difficult to know the degree of bias (if any) that is introduced by a significant result (Huitema 1980).

The second important assumption is that the covariate is fixed and measured without error (Huitema 1980, Sokal and Rohlf 1995). If the covariate is in fact measured with error, which is presumably true in morphological studies, then ANCOVA may lead either to positive or negative bias or to an inflated Type I error rate. The problem is that ANCOVA is based on Model I regression, which attributes all error to the independent variable. However there are three potential sources of error (the third is specific to applications for size correction):

1. error in the dependent variable;
2. measurement error in the covariate;
3. imperfect correlation between the variable being used as the covariate and the true size growth axis;

We investigated these potential sources of bias in ANCOVA for two different scenarios. First, we tested for bias when two groups were offset along the covariate but did not differ once the covariate was incorporated. Second, we tested for bias
when two groups were offset along the covariate dimension and in trait space. For each scenario we generated 500 3 dimensional data sets for each of two groups. Each data set consisted of 200 points randomly drawn from a multivariate normal distribution (see appendix III) (these data were generated using the same parameters as the data used for figures 1 and 2). For each set of simulated data we ran an ANCOVA and saved estimates of effect size. We repeated this procedure 10 times for each scenario but added error to the covariate for each successive run (the error added to the covariate incorporates sources 2 and 3 from above).

We found that adding measurement error to the covariate increased the probability of finding a difference between the two groups when there was no difference (i.e. Type I error rate was increased) (Figure A1a-c). When there was a true difference between two groups, we found that adding error to the covariate could result in underestimation, overestimation, and even a shift in the direction of effect (Figure A1d-f). These results stem from the problems

Figure A1: Results from simulations of ANCOVA analyses. Data represent means and 95% confidence intervals for estimates of effect size from 500 runs. Inset figures illustrate schematically how adding error (“Noise”) to the covariate results in a particular direction of bias. In panels a, b, and c there is no true difference in the two groups. In panels d, e, and f the true differences are indicated by the solid horizontal line. Panels a and d illustrate the estimates for trait 1, b and e illustrate trait 2 and c and f illustrate the results for trait 3. For each trait we see that non-negligible bias occurs when the error in the covariate is 1 to 10% of the error in the response variable.
associated with using a Model I regression (error only in the dependent variable) to analyze data with error in both the dependent and independent variables. Adding error to the covariate spreads the data horizontally, which reduces the slope of the within-group regression lines. For the null case this results in an inability to accept the null and in all other cases it makes interpretation of ANCOVA results ambiguous. ANCOVA does not always give misleading results, even when the covariate is measured with error. For example, when the range in the covariate was the same for both groups, we found no effects of error in the covariate (results not illustrated). For the scenarios used here (where the groups are offset along the covariate axis) it appears that ANCOVA performs well until the error in the covariate is more than 60% of the error in the response variable. Furthermore, in some systems, body size measured in mass may involve less error than linear traits. However, the problem of accurately representing the true size growth axis is not solved by precise measurement. Lack of correlation between a chosen covariate and the true body size/growth axis can also lead to biased results (source 3 above). We urge researchers who use ANCOVA as a method of size-correction to be aware of the potential biases imposed and to interpret their results cautiously.

**Literature Cited**
APPENDIX II
ESTIMATING POWER OF CPCA FOR IDENTIFYING SHARED ALLOMETRY

Houle et al. (2002) expressed concern that CPCA (as implemented by Phillips and Arnold 1999) might lack power and therefore lead to the conclusion that there are common PCs when none actually exist. For example, Houle et al. (2002) found that relatively large sample sizes were required to detect differences in the orientation of first principal components. However, the correlation structure of the data affects the validity and power of CPCA. Houle et al.'s data sets had approximately 75% of the variance explained by within-group PC1s. In contrast, in studies of multivariate allometry, within-group PC1s should explain >85% of the variance in the data to be considered a good estimate of size (Jolicoeur 1963). Indeed, in most morphological datasets, within-group PC1 often explains over 95% of the variance. If high loadings on CPC1 increase the power of CPCA to detect differences in CPC1, then Houle et al.'s concerns may not be relevant to most studies using size-correction.

Therefore, we determined how the proportion of the variance explained by PC1 affected the power of CPCA to detect a fixed divergence of two groups in PC1 with a given sample size. We constructed 3-variable variance-covariance matrices for two groups where the variance associated with each principal component decreased geometrically (e.g. 1, 0.2, 0.04) and where the first and second principal components of the second group were rotated by a specified angle relative to those of the first group. We varied the fraction of variance explained by PC1 on a log series between 0.72 and 0.98, and varied sample sizes per group in a log series between 10 and 200 (both ranges are typical for studies of morphological plasticity). For each fraction of variance/sample size combination we tested 10 angles (evenly spaced between 0 and 45 degrees), and each angle was run 500 times to determine power: we interpolated the power curve to calculate the angle between true PC1 for which we had 80% power to detect a difference in the PC1s between groups (Figure A2).

![Figure A2. Contour of the critical angle (angle for which there is 80% power to detect a difference in PC1 between groups) for α=0.05 and the specified combination of sample size and fraction of variance in PC1.](image)

Power increased with sample size and angle (i.e., the difference in the body size dimension). If 75% of the variance was associated with PC1, a 15° angle required a sample size of ~150 per group, and a sample of 20/group could not even detect a 45° displacement 80% of the time. These results confirm the results of Houle et al. (2002), who used a dataset with 75% of the variance associated with PC1. However, most morphometric studies have >90% (often >95%) of the variance loading on PC1. For 95%, a 15° angle can be reliably detected with a sample size of only 20/group. Samples of 50/group can detect displacements of <10°. Thus, we conclude that CPC is a powerful tool in morphometric analyses and that failure to detect substantial violation of common allometry is likely to be rare in morphometric studies using CPCA/BBMP for size-correction.

**Literature Cited**


APPENDIX III
PARAMETER VALUES AND THE "R" PACKAGE

We simulated data that represented a multivariate set of morphological measurements for two groups of individuals (e.g. prey exposed to predators and prey not exposed to predators). We assumed that there was some underlying allometry that determined how individuals that change in size also changed in shape as a result. We also assumed that the data have been appropriately transformed (e.g. by logging all trait values) to make them multivariate normally distributed with constant variance-covariance matrices, independent of size. To generate these data we wrote a function that created identically shaped but offset multivariate normal groups. We used the variance matrix

\[
\begin{pmatrix}
10 & 8 & 2 \\
8 & 10 & 3 \\
2 & 3 & 10
\end{pmatrix}
\]

with principal directions (eigenvectors) (-0.65, -0.67, -0.35) [eigenvalue=19.3]; (0.32, 0.18, -0.93) [eigenvalue=8.7]; and (0.68, -0.72, 0.09) [eigenvalue=1.93].

We used standard algorithms coded in the MASS package of R to draw 200 points for each group and shifted the mean of group 2 (group 1’s mean was located at the origin, (0,0,0)) by specifying distances (offsets) along the first and second principal directions. For our null cases (difference only in size but not size-corrected shape), the offset was 10 along PC1 and 0 along PC2, leading to a group 2 mean of 10 e_1 = (-6.5,-6.7,-3.5). For cases where there were differences in size but not shape, the offset was 10 along PC1 and 20 along PC2, leading to a group 2 mean of 10 e_1 + 20 e_2 = (-0.11,-3.08,-22.14).

The R Environment and Package cpcbp

Source or binary versions of the latest version of R, which is a free and open source program, can be downloaded from the R website along with documentation (http://www.r-project.org). The binary or source code for R Package "cpcbp" is available for download via the Internet at

http://www.zoo.ufl.edu/bolker/R/windows/cbpbp_0.1.2.zip
or

http://www.zoo.ufl.edu/bolker/R/src/cpcbp_0.1.2.tgz

R includes instructions for installing add-on packages across an Internet connection or from a local ZIP file. Below is a list of the functions that are available in our CPC/BBMP package (this list can be obtained during an R session by typing “package (help=cpcbp)”).

Description of package:

Package: cpcbp

Title: Common principal components and back-projection analysis

Author: Ben Bolker

Maintainer: Ben Bolker

Depends: R (>= 2.0.0)

Description: Auxiliary functions for CPC and Flury back-projection analysis
Index (function names and descriptions):

- **bp.anova** Analysis of variance incorporating back-projection error
- **bp.error** Calculate back-projection errors
- **bpmat** Burnaby's back-projection matrix
- **bp.means** Estimate back-projected means and standard deviations
- **calc.cpcerr** Calculate errors of CPC eigenvectors
- **coverfun** Calculate error coverage
- **covmat** Construct variance-covariance matrix
- **cpc.options** Set CPC calculation options
- **meancorrect** Mean-correct a data matrix
- **phillips.cpc** Run Phillips's CPC program from R
- **phillips.getpmat** Utility functions for reading output from Phillips' CPC program
- **plot.dat.theor** Plot multigroup data along with theoretical predictions
- **plot.multigrp** Plot grouped data
- **pooled.cpc** Compute CPC by mean-correcting each group
- **simdata** Simulate data for back-projection exercises
- **sim.theor** Generate theoretical values for back-projection exercises
- **strip.blanks** String utility functions
APPENDIX IV
ESTIMATING ERROR IN CPC1

To quantify and account for the error arising from estimation of the size axis (CPC1) we generated a multivariate dataset that represented morphological measurements on individuals from two groups of individuals (e.g. prey exposed to predators and prey unexposed to predators). We assume that there is some underlying allometry by which individuals that change in size will also change in shape (on an appropriate scale, e.g. log-transformed trait values). We aim to separate changes in shape caused by phenotypic plasticity from changes that are simply due to changes in size.

To do this, we calculate common principal components (CPCA) for within-group variation, back-project to eliminate the effects of the first CPC (CPC1), and perform univariate analyses of the resulting size-standardized traits separated by group. We make two assumptions here (1) within-group allometric variation in size-related traits is a good proxy for between-group variation in size, and (2) CPC1 characterizes effects of size (e.g. CPC1 has positive loadings for all traits).

The back-projection equation is:

$$X(1 - \beta_1\beta'_1)$$

where $X$ is an $n \times p$ data matrix, $I$ is a $p \times p$ identity matrix ($n$ is the total number of observations and $p$ is the number of traits/variables measured) and $\beta_1$ (following Flury’s (1988) notation, $\beta_j$ is the $j$th eigenvector, treated as a column vector and $\beta'_j$ is the $i$th element of the $j$th eigenvector) is the estimated first principal direction (eigenvector), scaled so that $\beta'_1\beta_1 = 1$. To understand this formula, break up equation 6 to see that the first multiplication $X\beta_1$ projects $X$ onto the first principal direction (calculating a scalar that is the score for CPC1). The second multiplication (multiplying by $\beta'_1$) translates this score back into the original coordinate system (Klingenberg 1996, Burnaby 1966). Given this back projection process, any error present in CPC1 is propagated into the back projected data (i.e. size-corrected trait values) (Fig. 2; main text).

To account for this error we compute the errors on the elements of the eigenvector $\beta_1$ (see Flury 1988: the following discussion up to equation 5 recapitulates pp. 74-85 and equation numbers 2.12 – 4.8). We start by computing

$$\hat{\lambda}_j = \frac{r_i^{-1}(\hat{\lambda}_j - \hat{\lambda}_{ih})^2}{\beta'_j\beta_j}$$

where $r_i = n_i/n$ (fraction of total data points in group i) and $\hat{\lambda}_j$ and $\hat{\lambda}_{ih}$ are estimates of the $j$th and $h$th eigenvalues of group $i$'s variance-covariance matrix. Given $\hat{\lambda}_j$ we can calculate a harmonic mean across $k$ groups

$$\hat{\lambda}_{jh} = \left(\sum_{i=1}^k (\hat{\lambda}_j)^{-1}\right)^{-1}$$

and find the large-sample estimate of the standard error of $\beta_{mh}$ to be

$$s(\beta_{mh}) = \left(\frac{1}{n} \sum_{j=1}^p \hat{\theta}_{jh} \beta_{mj}^2\right)^{1/2}$$

where $\beta_{mh}$ is the $m$th element of the $h$th principal component. More generally we know that the variance-covariance matrix of the elements in $\beta_1$ is:

$$\frac{1}{n} \sum_{h=2}^k \hat{\theta}_{1h} p_h p'_h$$

Suppose we have calculated the error variances $\sigma_{\beta_{ij}}$ for each component of the first eigenvector. Then the $ij$th element of the outer-product matrix $\beta_i\beta'_i$ is $b_{ij} = \beta_{ii}\beta_{jj}$. In general, the errors for two quantities can be combined by (Lyons 1991)
\[ V(f(a,b)) \approx V(a) \left( \frac{\partial f}{\partial a} \right)^2 + V(b) \left( \frac{\partial f}{\partial b} \right)^2 + 2C(a,b) \left( \frac{\partial f}{\partial a} \frac{\partial f}{\partial b} \right) \] (6)

which for \( f(a,b) = a \cdot b \) reduces to

\[ V(a)b^2 + V(b)a^2 + 2C(a,b)ab = a^2b^2 + V(a) + V(b) + 2C(a,b)ab \] (7)

The approximation in (6) is based on a Taylor series expansion; when \( f(a,b) = a \cdot b \) the only missing term is \( E \left[ (a-\bar{a})^2 \cdot (b-\bar{b})^2 \right] \) which in turn is equal to \( V(a) \cdot V(b) + C(a^2,b^2) \); we found this term to be generally negligible, as shown by our good type I error results based on (7).

Therefore the error variance of \( \beta_{ij} \) is approximately

\[ \sigma_{\beta_{ij}}^2 = \sigma_a^2 \beta_{ii} + \sigma_b^2 \beta_{jj} + 2\sigma_a \sigma_b \beta_{ij} \] (8)

where \( \sigma_{a,b} \) denotes the covariance between \( a \) and \( b \). The covariances of the elements of the back-projection matrix ( \( b_{ij} = \beta_{ii} \beta_{jj} \)) with each other must also be calculated. We derive these covariances by expanding \( E[b_{ij}b_{ik}] = E[\beta_{ii}\beta_{ij} \cdot \beta_{ii} \beta_{ik}] \) and assuming all third and fourth central moments are zero:

\[ \sigma_{\beta_{ii} \beta_{ij}} = 2 \sigma_{\beta_{ii} \beta_{ij}} + \beta_{ii} \gamma_{\beta_{ii} \beta_{ji}} + \beta_{ij} \gamma_{\beta_{ij} \beta_{ii}} + \beta_{ij} \gamma_{\beta_{ij} \beta_{ij}} \] (9)

Now, we calculate the error variance introduced into the mean of the \( i \)th variable by

\[ \sigma_{BP}^2 = \sigma_{\beta_{ii}}^2 \bar{x}_{i}^2 + \sum_{k \neq j} \bar{x}_j \bar{x}_k \sigma_{\beta_{kj}} \] (10)

and combine the back projection error and the within group variance

\[ \sigma_{\beta_{ij}}^2 = \sigma_{BP}^2 + \sigma_i^2 \cdot \sigma_j^2 \] (12)

This now provides us with an estimate of the combined variances of each group. These combined variances can then be used as the variance terms in a t-test. Alternatively, back-projection error can be incorporated into an analysis of variance by adding the back projection sums of squares to the error sum of squares. In order to calculate the overall back-projection sum of squares for use in a corrected ANOVA we substitute the sum of the absolute deviations of the trait means of each group (\( \bar{x}_{i,j} \) for trait \( i \) in group \( j \)) from the overall mean of each trait (\( \bar{x}_i \)), or

\[ \sum_j |\bar{x}_{i,j} - \bar{x}_i| \], for the mean trait values in the calculations above. This procedure is necessary because the back-projection error affects the back-projected data in every group in the same direction, not independently. (An R package that implements this algorithm is available via http://www.zoo.ufl.edu/bolker/R/windows/).

**Literature Cited**


