

Controlling for Body Mass Effects: Is Part-Whole Correlation Important?

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Introduction

Many physiological and life-history traits scale with body mass, and this mass dependence must often be taken into account when comparing group means or when analyzing correlations between traits. Residual analysis (Bennett 1987), multiple regression (Hayes and Shonkwiler 1996), and analysis of covariance (ANCOVA, Atchley et al. 1976; Packard and Boardman 1987) have been proposed as robust techniques to reduce or eliminate mass dependence, since the use of ratios (i.e., the trait value divided by body mass) is problematic (Packard and Boardman 1987). The first of these three methods all involve regression, so problems may arise when the physiological trait of interest is the mass of an organ or tissue and the covariate is body mass. In a regression between organ mass and body mass, both variables include the mass of the organ. This tends to create a positive relationship between the two variables, even when no correlation between organ mass and the rest of body mass (i.e., body mass minus organ mass) exists. Sokal and Rohlf (1995) referred to the correlation between a part (e.g., organ mass) and the whole (e.g., body mass) as "part-whole correlation." A number of authors have addressed the issue of part-whole correlation within the context of scaling (e.g., Prothero 1986; LaBarbera 1989) and the analysis of ratios (e.g., Atchley et al. 1976; Jackson and Somers 1991 and references therein).

Descriptions of the effects of part-whole correlation in ANCOVA, multiple regression, and residual analysis, however, have been only anecdotal. For instance, Tracy and Sugar (1989) and Burness et al. (1998) described examples where removal of part-whole correlation changed the results of ANCOVA and residual analysis, respectively. A more in-depth study of the effects of part-whole correlation on regression-type analyses (e.g., multiple regression, residual analysis, and ANCOVA) is lacking, and as a result, this potential problem is often ignored. For instance,

in recent years (1994–1997) eight papers in *Physiological Zoology* have described the use of multiple regression, residuals, or ANCOVA to control for the effects of body mass on organ mass(es). Correction for part-whole correlation was mentioned in only one of these studies (Hammond et al. 1994), and in this case, it was performed for multiple regression but not for ANCOVA, even though part-whole correlation occurs in both of these techniques.

In this technical comment, I provide an explicit treatment of the effects of part-whole correlation on certain regression-type analyses (i.e., multiple regression, residual analysis, and ANCOVA). Despite my focus on analyses involving regression, I use Sokal and Rohlf's (1995) term part-whole correlation (rather than part-whole regression) to avoid the introduction of new jargon. I describe the effects of part-whole correlation (PWC) in two types of analyses frequently applied to physiological data: (1) Is the mass of organ X correlated with trait Y, controlling for body mass? and (2) Are there differences in organ mass among groups, controlling for body mass? I show that correction for PWC is not always necessary or desirable in multiple regression; deciding when to account for PWC requires careful consideration of the specific question being asked. I also describe a previously unreported effect of PWC on the interaction term of ANCOVA-like analyses.

Is the Mass of Organ X Correlated with Trait Y, Controlling for Body Mass?

Residual analysis (Bennett 1987) and multiple regression (Hayes and Shonkwiler 1996) are often used to determine if two traits are correlated after adjusting for the statistical effect of a third variable. Both of these techniques use regression, so PWC occurs when an analysis includes organ mass and body mass. Residual analysis involving ordinary least squares (OLS) regression is equivalent to OLS multiple regression, providing the degrees of freedom are handled appropriately (Hayes and Shonkwiler 1996), so only multiple regression will be considered in the following discussion.

A typical example is a test of the correlation between a measure of metabolic rate (e.g., basal metabolic rate, BMR) and the mass of an organ, controlling for body mass. Such analyses are often performed to test the hypothesis that small, highly metabolically active organs contribute disproportionately to

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BMR (e.g., Daan et al. 1990). A multiple regression model of this analysis is as follows:

$$\text{BMR}_i = \beta_0 + \beta_1 \text{organ mass}_i + \beta_2 \text{body mass}_i + \varepsilon_i, \quad (1)$$

where ε_i is a random residual. A test of whether the slope estimate β_1 is significantly different from zero (i.e., a test of the relationship between BMR and organ mass, controlling for body mass) is used to test whether the data are consistent with the hypothesis that the organ in question contributes disproportionately to BMR. The term “contributes disproportionately” means that the effect of a gram of a particular organ on BMR is different than that of a gram of the rest of the body (body mass minus organ mass, BM – OM). Phrasing the question in this way leads to an alternative analysis—that is, the model

$$\text{BMR}_i = \beta'_0 + \beta'_1 \text{organ mass}_i + \beta'_2 \text{BM} - \text{OM}_i + \varepsilon_i \quad (2)$$

—and a test of whether the slope estimate β'_1 is significantly different than β'_2 , that is, whether the data are consistent with the hypothesis that the change in BMR due to an increase of 1 g in organ mass is different than that caused by an increase of 1 g in BM – OM. It can be shown that a test of the null hypothesis $\beta'_1 = \beta'_2$ in model 2 is equivalent to a test of the null hypothesis $\beta_1 = 0$ in model 1. That is, these two approaches yield identical results. Model 2 is clearly not affected by PWC since organ mass and BM – OM do not share a term in common, and therefore PWC does not pose a problem in the test of the null hypothesis $\beta_1 = 0$ in model 1. Because OLS residual analysis is equivalent to OLS multiple regression (Hayes and Shonkwiler 1996), a test of the correlation between the residuals of BMR and the residuals of organ mass on body mass is equally valid. Therefore, correcting for PWC in this type of analysis (i.e., a test of $\beta_1 = 0$ in model 1) is not necessary or desirable. Using BM – OM rather than body mass as the covariate in multiple regression (or to produce residuals) can affect the interpretation of the results (e.g., Burness et al. 1998).

PWC may be ignored only when one is interested in the proportional contribution of an organ to some trait, as in the example described above. In other cases, BM – OM may be the appropriate covariate. For instance, in a study by Hammond et al. (1994), one analysis was of the form

$$\text{organ mass}_i = \beta_0 + \beta_1 \text{food intake}_i + \beta_2 \text{BM} - \text{OM}_i + \varepsilon_i \quad (3)$$

(see table 1 in Hammond et al. 1994; I have omitted one of the covariates for simplicity). The authors sought to determine if organ mass was affected by food intake, that is, the null hypothesis was $\beta_1 = 0$. The test of this null hypothesis is equivalent to the test of $\beta'_1 = 0$ in the model

$$\begin{aligned} \text{food intake}_i &= \beta'_0 + \beta'_1 \text{organ mass}_i \\ &+ \beta'_2 \text{BM} - \text{OM}_i + \varepsilon_i \end{aligned} \quad (4)$$

Although models 2 and 4 are of the same form, the null hypotheses are different ($\beta'_1 = \beta'_2$ in model 2 vs. $\beta'_1 = 0$ in model 4), that is, the type of question being asked is qualitatively different in these two scenarios. Because Hammond et al. (1994) were testing whether $\beta'_1 = 0$ in model 4, their use of BM – OM as the covariate (rather than body mass) was appropriate.

Are There Differences in Organ Mass among Groups, Controlling for Body Mass?

If one wishes to test for a difference in organ mass among groups after adjustment for body mass (e.g., Hammond et al. 1994), ANCOVA is an appropriate technique to use (Packard and Boardman 1987). However, because ANCOVA involves linear regression procedures (Sokal and Rohlf 1995), the use of body mass as a covariate results in PWC. In principle, BM – OM is the appropriate covariate. The effect of BM – OM on organ mass is the effect to be controlled; an apparent effect of body mass on organ mass may just be a statistical artifact.

The use of body mass, rather than BM – OM, as a covariate can lead to misleading results. PWC will alter the slope of a regression between two variables and the extent of this effect will depend in part on the strength of the relationship (i.e., the product-moment correlation coefficient) between the two variables (Fig. 1; App. A). Therefore, if the strength of the relationship between organ mass and BM – OM differs among groups, the slopes of organ mass on body mass will tend to differ, even when the slopes of organ mass on BM – OM are

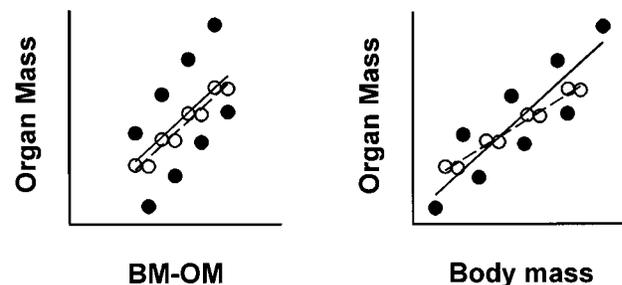


Figure 1. The effect of PWC on the slope of a regression line. The correlation coefficient between organ mass and BM – OM is greater in one group (open circles, dashed line) than the other (solid circles, solid line). The two groups have equal slopes in the regression of organ mass on BM – OM. However, in the regression of organ mass on body mass, the slopes differ.

the same for all groups. Heterogeneity in slopes will preclude a test of differences in means among groups since ANCOVA requires the slopes of all groups to be parallel (Sokal and Rohlf 1995).

An example of this effect can be found in a study of the body composition of European starlings, *Sturnus vulgaris* L. (J. K. Christians and T. D. Williams, unpublished data). We wished to test for a difference in flight-muscle mass between 2 yr, controlling for body mass. When body mass was used as a covariate, the year \times body mass interaction term was marginally significant ($P = 0.0524$; Table 1), indicating that the slope of the regression between flight-muscle mass and body mass differed between years. Because the assumption of homogeneity of slopes was not met, we could not make inferences about the main effects (i.e., year and body mass). However, when BM – OM was used as a covariate, the interaction term was not significant ($P = 0.2976$; Table 1), and therefore further analysis of the main effects was possible. Using body mass rather than BM – OM would have unnecessarily prevented a comparison of flight-muscle mass between years.

Although a significant group \times body mass interaction may reflect real differences among groups, such an interaction can be misleading when it is caused by differences in the strength of the relationship between organ mass and body mass, and not by a difference in the slopes of organ mass on BM – OM. Of course, if the strength of the relationship between organ mass and BM – OM differs among groups, an assumption of ANCOVA is violated (i.e., residuals will not have common variance; Steel and Torrie 1980). However, in some cases differences in correlation coefficients among groups will be ignored because they are not statistically significant. Using BM – OM rather than body mass as a covariate in ANCOVA avoids a possible complication of violating the assumption of homogeneity of variances.

Even if no assumptions of ANCOVA are violated (e.g., slopes are parallel and residuals have common variance), PWC can still affect the test for differences in organ mass among groups,

controlling for body mass. For example, Tracy and Sugar (1989) described an instance where PWC affected the results of an ANCOVA. No significant difference between two groups was found when PWC was not taken into account, but when BM – OM was used as the covariate, the group effect was statistically significant (Tracy and Sugar 1989). Using body mass rather than BM – OM will not always decrease the apparent significance of differences between groups. The effect of PWC on the significance of the group term in an ANCOVA will depend on the variance in organ mass among groups relative to the variance in BM – OM among groups (for further details, see App. B).

Conclusions

I have demonstrated that PWC should be taken into account in some types of analyses commonly applied to physiological data. Choosing the appropriate covariate (i.e., body mass vs. BM – OM) requires careful consideration of the question being asked. For instance, in studies of the proportional contribution of organs to metabolic rate, controlling for body mass (i.e., ignoring PWC) may be desirable. However, in other situations (e.g., Hammond et al. 1994), BM – OM may be the appropriate covariate.

Although certain authors (e.g., Hammond et al. 1994; Konarzewski and Diamond 1995; Burness et al. 1998) have recognized that PWC may affect multiple regression and residual analysis, the potential impact of PWC in ANCOVA has largely been ignored (but see Tracy and Sugar 1989). PWC may generate misleading interaction terms (i.e., significant differences among groups in the slope of the regression of organ mass on body mass) and/or change the significance of the main effects.

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Table 1: Results of ANCOVA, testing for differences in flight muscle mass among years

Source	df	Sum of Squares	F	P
With body mass as the covariate:				
Year	1	.28	5.13	.0251
Body mass	1	5.99	108.47	.0001
Year \times body mass	1	.21	3.83	.0524
Error	130	7.18
With BM – OM as the covariate:				
Year	1	.16	1.80	.1824
BM – OM	1	3.34	37.25	.0001
Year \times BM – OM	1	.10	1.09	.2976
Error	130	11.66

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Appendix A

The Effect of PWC on the Slope of a Regression

It can be shown that the slope of the regression of organ mass on body mass is equal to

$$\beta_{OB} = \frac{(\beta_{OR}^2/r_{OR}^2) + \beta_{OR}}{(\beta_{OR}^2/r_{OR}^2) + 2\beta_{OR} + 1}, \quad (A1)$$

where β_{OR} is the slope of the regression of organ mass on BM – OM and r_{OR} is the product-moment correlation coefficient between organ mass and BM – OM.

Appendix B

The Effect of PWC on the Test for Differences in Organ Mass among Groups in ANCOVA

It can be shown that the F -statistic for a test of differences in organ mass among groups, controlling for body mass ($F_{\text{body mass}}$), is equal to

$$\left(\frac{\sum R_{\text{total}}^2 \sum B_{\text{within}}^2}{\sum R_{\text{within}}^2 \sum B_{\text{total}}^2} \right) F_{\text{BM-OM}} + \left(\frac{\sum R_{\text{total}}^2 \sum B_{\text{within}}^2}{\sum R_{\text{within}}^2 \sum B_{\text{total}}^2} - 1 \right) \frac{\sum n - a - 1}{(a - 1)}, \quad (B1)$$

where $F_{\text{BM-OM}}$ is the F -statistic for a test of differences in organ mass among groups, controlling for BM – OM; $\sum R_{\text{total}}^2$ and $\sum B_{\text{total}}^2$ refer to the total sum of squares of BM – OM and body mass, respectively; $\sum R_{\text{within}}^2$ and $\sum B_{\text{within}}^2$ refer to the sums of squares of BM – OM and body mass within groups, respectively; n is the total sample size; and a is the number of groups.

Equation (B1) is of the form

$$F_{\text{body mass}} = \gamma F_{\text{BM-OM}} + (\gamma - 1) \delta,$$

where δ will always be positive. Therefore, if $\gamma > 1$, then $F_{\text{body mass}} > F_{\text{BM-OM}}$ (i.e., PWC will increase the apparent signif-

icance of differences among groups), whereas if $\gamma < 1$, then $F_{\text{body mass}} < F_{\text{BM-OM}}$. Because the sums of squares of body mass is made up of the sum of squares of BM – OM and that of organ mass, the term γ will tend to be greater than one when the variance in BM – OM among groups is greater than the variance in organ mass among groups and vice versa.

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