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On the comparison of the strength of morphological integration across morphometric datasets

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Evolutionary morphologists frequently wish to understand the extent to which organisms are integrated, and whether the strength of morphological integration among subsets of phenotypic variables differ among taxa or other groups. However, comparisons of the strength of integration across datasets are difficult, in part because the summary measures that characterize these patterns (RV coefficient and r_{PLS}) are dependent both on sample size and on the number of variables. As a solution to this issue, we propose a standardized test statistic (a *z*-score) for measuring the degree of morphological integration between sets of variables. The approach is based on a partial least squares analysis of trait covariation, and its permutation-based sampling distribution. Under the null hypothesis of a random association of variables, the method displays a constant expected value and confidence intervals for datasets of differing sample sizes and variable number, thereby providing a consistent measure of integration suitable for comparisons across datasets. A two-sample test is also proposed to statistically determine whether levels of integration differ between datasets, and an empirical example examining cranial shape integration in Mediterranean wall lizards illustrates its use. Some extensions of the procedure are also discussed.

KEY WORDS: **Geometric morphometrics, Morphological evolution, morphological integration.**

Over the past several decades, evaluating the degree to which morphological traits covary (*morphological integration*: sensu Olson and Miller 1958) has become a prominent subject in evolutionary biology (Cheverud 1982, 1996; Bookstein et al. 2003; Pigliucci 2003; Klingenberg 2008; Goswami and Polly 2010). Myriad studies have characterized patterns of morphological integration in a variety of organisms, in an effort to decipher the genetic, developmental, and functional mechanisms that generate such patterns (e.g., Hallgrímsson et al. 2002; Mitteroecker et al. 2004; Monteiro et al. 2005; Young and Badyaev 2006; Gómez-Robles and Polly 2012). These empirical studies have been facilitated in part by the development of quantitative approaches for characterizing patterns of morphological integration in high-dimensional data (e.g., Magwene 2001; Bookstein et al. 2003; Mitteroecker and Bookstein 2007; Márquez 2008; Klingenberg 2009; Adams and Felice 2014; Bookstein 2015). In particular, methods that evaluate covariance patterns across a priori subsets of variables have received considerable attention.

In the field of geometric morphometrics, a number of approaches are used for characterizing the integration among subsets of variables. One approach is based on Escoffier's (1973) RV coefficient (Klingenberg 2009), which is a ratio describing the degree of covariation between sets of variables relative to the variation and covariation within sets of variables. The RV coefficient ranges between zero and one, and larger values describe greater covariation between sets of variables relative to within them, which may provide evidence that there is higher integration among subsets than expected by chance. An alternative measure is based on partial least squares (PLS), where a singular value decomposition of the covariance matrix between two sets of variables ($\mathbf{S_{12}}$) is used to

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describe the maximal covariation between them (Bookstein et al. 2003; Mitteroecker and Bookstein 2007). The dominant singular value of $\mathbf{S_{12}}$ explains the maximal covariation between the two sets of variables, whose pattern of covariation is described by the first set of linear combinations (singular vectors) in each of the two datasets (Bookstein et al. 2003). Scores projected on these axes are routinely used to estimate the maximal correlation among sets of variables (r_{PLS}: Rohlf and Corti 2000), with higher correlations indicating a greater level of covariation. For both the RV and PLS approaches, statistical evaluation of the observed pattern is accomplished using permutation, where the rows (individuals) are shuffled in one subset of variables while leaving the rows in the other subset constant, thereby disassociating the covariation between subsets and generating a distribution of possible outcomes under the null hypothesis of no association between variable subsets. The observed statistic is then compared to a distribution of random statistics obtained from this procedure to evaluate its significance (see Rohlf and Corti 2000; Bookstein et al. 2003; Klingenberg 2009).

Recently, there has been increased interest in understanding the extent to which patterns of morphological integration are consistent across levels of biological organization, and whether levels of integration change over evolutionary time (Armbruster et al. 2014; Goswami et al. 2014; Klingenberg 2014). To this end, researchers have characterized levels of integration across traits and species using one or more of the methods mentioned above for subsequent qualitative or quantitative comparison (for recent examples see: Drake and Klingenberg 2010; Goswami et al. 2014; Lazic et al. 2015; Martin-Serra et al. 2015; Neaux et al. 2015). However, for such comparisons to be meaningful requires that the evaluated test measures are unaffected by other attributes of the data. Unfortunately this is not the case. For instance, the RV coefficient has been shown to be sensitive to both the sample size (n) and the number of variables examined (p), rendering comparisons of RV measures across datasets uninformative (Adams 2016; also: Smilde et al. 2009; Fruciano et al. 2013; for an extended critique of the RV coefficient see: Bookstein 2016). Additionally, as shown in part by Mitteroecker and Bookstein (2007), and comprehensively below, the PLS correlation coefficient (r_{PLS}) suffers from the same inherent issues. The objective of the current article is to provide a standardized test statistic (a z-score) for measuring the degree of morphological integration between sets of variables. Our procedure is developed for and is used on a PLS correlation of among-partition trait covariation. However, it is sufficiently flexible that it may be applied to any meaningful measure that captures the degree of integration in a dataset, and is thus a useful approach for comparing the degree of integration as new analytical approaches are developed (see Discussion).

Sample Size and Variable Dependency of r_{PLS}

To understand the properties of r_{PLS}, we conducted simulations similar to those of Adams (2016). Specifically, simulated datasets were obtained by generating random variables drawn from a normal distribution $\sim N(0,1)$, and variables were randomly assigned to one of two subsets with the constraint that the number of variables was the same in each subset. Thus, each simulated dataset represented what was expected under the null hypothesis of a random association of variables. Using this procedure, we generated 100 datasets for differing levels of sample size (n), where the total number of variables was the same ($p = 30$). Next, we performed the reciprocal simulation where all datasets contained the same number of specimens ($n = 100$), but where the total number of variables differed. From each simulated dataset, r_{PLS} was estimated, and at each level of n and p, the mean and 95% confidence intervals across the 100 datasets were calculated. All simulations were performed in R 3.2.0 (R Core Team 2015).

As is clear from Figure 1, values of r_{PLS} vary between zero and one, with larger values attained under smaller sample sizes, as well as with a larger number of variables (Fig. 1A and B: see also Mitteroecker and Bookstein 2007). Thus, like the RV coefficient, estimates of morphological integration using PLS are also sensitive to n and p, rendering comparisons of these values across datasets challenging (see also Mitteroecker and Bookstein 2007). Thus, for this purpose an alternative estimate of the degree of integration across sets of variables is required.

The z-Score for Comparing the Strength of Integration

Although studies of integration rarely state a null hypothesis in terms of the parameters tested, the permutation-based procedure described above evaluates the observed measure against a distribution of values obtained under a null hypothesis of no association between subsets of variables. Thus, some generalization of the Pearson product-moment null hypothesis, $\rho = 0$, could be implied. However, whereas Pearson's r, as an estimate of ρ, has an expected value of 0 under the null hypothesis for univariate tests, r_{PLS} has a lower limit of zero and an expected value that varies with n and p (Fig. 1 A and B). For single-sample hypotheses, stating the null hypothesis as "no association" between matrices is sufficient; the expected value is simply the mean of the sampling distribution of r_{PLS} from the permutation procedure (as described above) and the percentile of the observed r_{PLS} value is the estimate of the P-value. To either qualitatively compare or actually test the dissimilarity of two measures of integration from two samples requires calculation of effect sizes in relation to

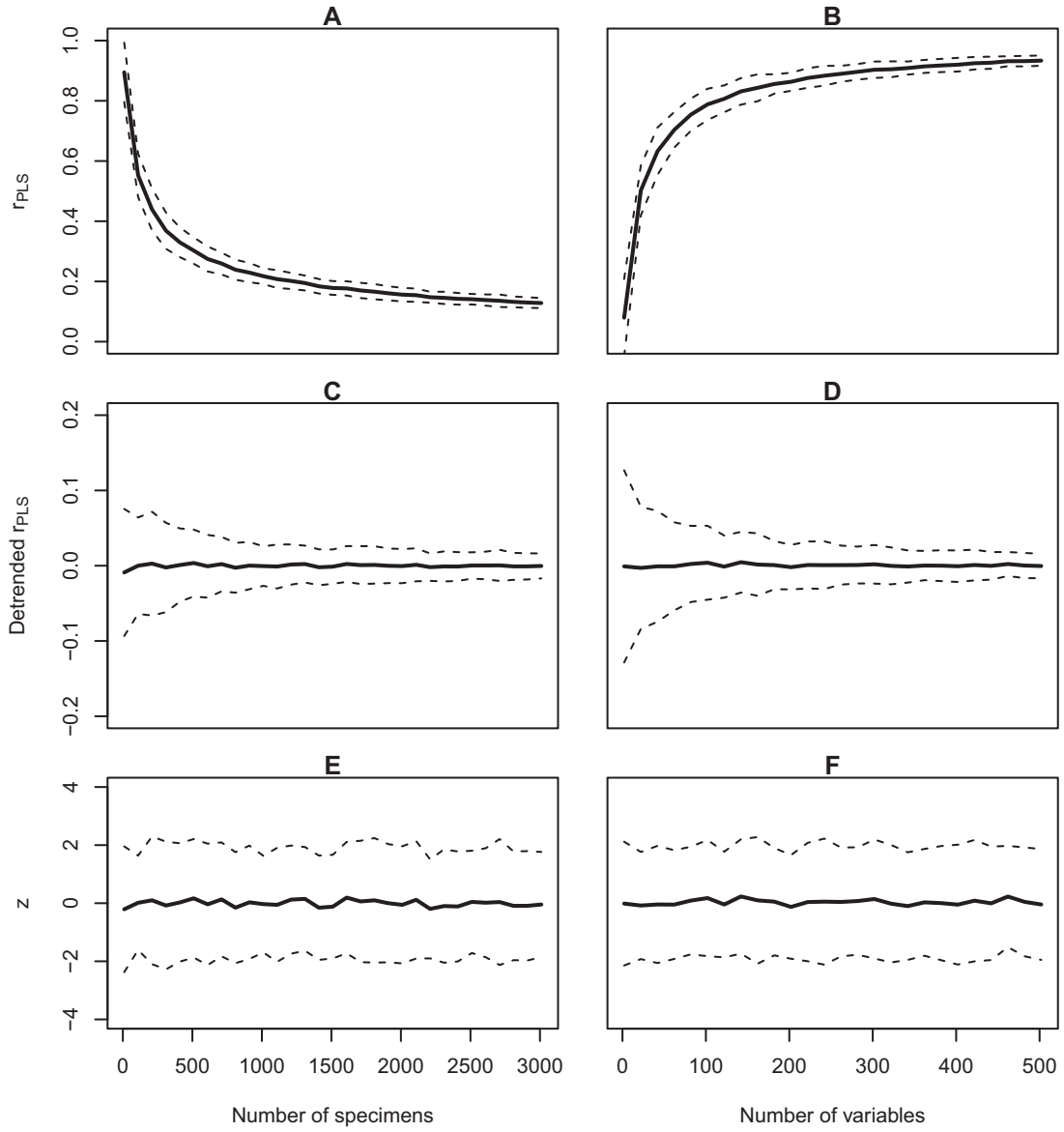


Figure 1. **Evaluation of r_{PLS} under the hypothesis of random associations of variables. Mean and 95% confidence intervals of RV values obtained from (A) 100 datasets simulated across a range of sample sizes, and from (B) 100 datasets simulated across a range of variable number. Mean and 95% confidence intervals of detrended r_{PLS} for the same simulations of (C) 100 datasets simulated across a range of sample sizes, and from (D) 100 datasets simulated across a range of variable number. Mean and 95% confidence intervals of *z*-scores for the same simulations of (E) 100 datasets simulated across a range of sample sizes, and from (F) 100 datasets simulated across a range of variable number.**

expected values under the null hypothesis of no integration (e.g., Collyer et al. 2015), especially if the two samples have different expected values. This can be accomplished by calculating the standard deviates (effect sizes) of r_{PLS} for the different samples,

$$\hat{z} = \frac{r - \hat{\mu}_r}{\hat{\sigma}_r}, \qquad (1)$$

where $\hat{\mu}_r$ is the estimated expected value of r_{PLS} under the null hypothesis, found as the mean of the sampling distribution, and $\hat{\sigma}_r$ is the SD of the sampling distribution (i.e., SE of the mean). Calculating effect sizes this way assumes that the sampling distribution is normally distributed, a property we demonstrate via simulation (below).

At first glance, the numerator of the standard deviate calculation might also seem sufficient for calculating a statistic that allows integration to be compared between sets of variables. Indeed, the numerator of the standard deviate calculation detrends r_{PLS} values that might have different expectations under the null hypothesis (Fig. 1C and D). However, there are two important concerns with using detrended r_{PLS} as a comparable statistic.

First, although the statistic is no longer *n*- or *p*-dependent, the SE (and thus, confidence interval) of the statistic varies across *n* and *p* for the same number of random permutations, decreasing with either increased *n* or *p* (Fig. 1C and D). Second, the value itself has little meaning as a correlation coefficient. For example, negative values do not indicate negative covariation, but rather less covariation than expected under the null hypothesis. Furthermore, because low *n* or high *p* can produce large expected r_{PLS} values (Fig. 1A and B), a veritable strong correlation between two sets of variables with large sample size will have a small detrended value, as a maximum r_{PLS} value of 1.0 precludes large detrended values for large samples. Therefore, standardization—dividing by the SD of the sampling (null) distribution—is needed to generate a test statistic (z) that has a constant expected value and same variance, SD, or confidence interval across the entire spectrum of sample sizes and variable number (Fig. 1E and F).

We wish to reiterate that other than concern for *n*- or *p*-dependency for comparisons of integration across multiple datasets, r_{PLS} is sufficient as a single-sample test statistic. The effect size calculation in equation (1) is merely descriptive, but allows qualitative comparison between two measures of integration that have different expected values. (Inferring the probability of the effect size from a standard normal distribution is not necessary, as it is already accomplished from the resampling experiment of the PLS analysis, as shown below.) For a statistical treatment of the comparisons of two or more datasets, however, equation (1) can be modified to calculate the effect size of the difference between two integration effect sizes as:

$$\hat{z}_{12} = \frac{|(r_1 - \hat{\mu}_1) - (r_2 - \hat{\mu}_2)|}{\sqrt{\hat{\sigma}^2_{r_1} + \hat{\sigma}^2_{r_2}}}. \quad (2)$$

The probability of $\hat{z}_{12}$ under the null hypothesis of equal integration (*P*-value) can be estimated from a standard normal distribution, provided the within-sample random r_{PLS} values are approximately normally distributed. The numerator of equation (2) can be rewritten as $(r_1 - r_2) - (\hat{\mu}_1 - \hat{\mu}_2)$, which characterizes the effect size as a difference in levels of integration compared to their expected difference. We assert that a null hypothesis test for comparing levels of integration is naturally a two-tailed test (hence the absolute value in the numerator of eq. 2), as the direction of the difference between detrended r_{PLS} scores has no appreciable meaning, especially in the absence of standardization. For example, a smaller value of $(r_2 - \hat{\mu}_2)$ than $(r_1 - \hat{\mu}_1)$ might produce a positive difference, $(r_1 - \hat{\mu}_1) - (r_2 - \hat{\mu}_2)$, even if $\hat{z}_2$ is larger than $\hat{z}_1$, because of a smaller SE in the second sample (perhaps because of a larger expected value from the sampling distribution). Therefore, only the magnitude of the effect size of the integration difference is valuable as a test statistic, rendering the hypothesis inherently two-tailed. Likewise, a confidence interval for the effect size can be estimated as:

$$(1 - \alpha)\,100\%\,\mathrm{CI} = |(r_1 - \hat{\mu}_1) - (r_2 - \hat{\mu}_2)| \pm z_{\alpha/2}\sqrt{\hat{\sigma}^2_{r_1} + \hat{\sigma}^2_{r_2}}, \quad (3)$$

where $z_{\alpha/2}$ is the quantile from a standard normal distribution corresponding to the two-tailed probability for the level of significance, α, and $\sqrt{\hat{\sigma}^2_{r_1} + \hat{\sigma}^2_{r_2}}$ is the pooled SE. The null hypothesis is rejected with $(1 - \alpha)100\%$ confidence, if the confidence interval does not contain zero, the expected difference in effect sizes under the null hypothesis. Thus, concerning hypothesis tests, confidence intervals and the two-sample z-score are test statistics for the difference in r_{PLS} between different datasets, relative to their expected difference based on *n*- or *p*-dependency. The effect sizes (eq. 1) allow one to infer which sample has greater morphological integration, when a significant difference is observed.

Statistical Properties

To determine if this test of difference in the strength of integration among samples has appropriate statistical properties (type I and type II error rates, statistical power), we performed a simulation experiment. This experiment consisted of 100 runs of generating two "populations" of data, each with $N = 10{,}000$ individuals, where each individual comprised a vector of random values sampled from a normal distribution, $\sim N(0,1)$, for a matrix, **X**, comprising 10 variables and matrix, **Y**, also comprising 10 variables. In one population, **X** and **Y** were simulated with no linear association (the range of "true" PLS correlations was 0.0459–0.0709 over 100 runs). In the other population, **X** and **Y** were simulated with a fairly strong linear association (the range of true PLS correlations was 0.8239–0.8340 over 100 runs). Within each simulation run, two sampling frames of $n = 30$ specimen numbers were randomly generated, for 200 permutations. The first sampling frame was used to sample individuals from each of the "no effect" population (no linear association) and "effect" population (linear association). In each sample, r_{PLS} values were calculated and sampling distributions for r_{PLS} were generated, based on 1000 random resampling permutations for the PLS analysis; $\hat{z}_{12}$ was calculated between samples, as in equation (2). For this comparison, we expected the null hypothesis to be rejected. Therefore, the proportion of times it was rejected is a measure of statistical power for the difference in effects. (Likewise, the proportion of times it was not rejected is a measure of the type II error rate.)

The second sampling frame was used to draw a second sample from each population, and r_{PLS} values and their sampling distributions were calculated as above, but $\hat{z}_{12}$ values were calculated between samples from the same population. For these comparisons, we expected the null hypothesis to be supported, but in one case no integration (no effect) and in the other, substantial

integration (effect) should be found from samples of each population. Irrespective of the level of integration, this procedure simulated multiple runs of a null model (no expected difference in integration between populations), allowing us to ascertain whether the type I error rates—the proportion of times the null hypothesis was rejected—was consistent in spite of different levels of morphological integration. In all cases, the significance level for the null hypothesis was assigned as $\alpha = 0.05$. Type I error rates and statistical power were calculated within runs as the proportion of 200 permutations that the null hypothesis was rejected. Means and 95% confidence intervals were calculated across the 100 runs to evaluate the tendencies of type I error rates and statistical power.

Additionally, because integration tests are often performed on geometric morphometric (GM) data, we also considered the contribution of generalized Procrustes analysis (GPA) to generate inherently correlated shape variables, and to what extent this would affect results of our proposed procedure. Briefly, GPA scales anatomical landmark configurations to unit size, centers them, and rotates them via least squares superimposition to render configurations invariant is size, position, or orientation. Procrustes residuals—the aligned landmarks following GPA—are inherently correlated shape variables produced by GPA. Therefore, even generating isotropic error on points of landmark configurations will produce correlated variables within modules prior to measuring the PLS correlation between modules. We considered the GPA artifact by assigning the randomly generated data from the simulation experiment above as residuals on mean configurations. We generated 5-point configurations of two-dimensional landmarks (a vector of 10 values) in each simulation run. These configurations served as mean vectors to which the row vectors of the previously generated random **X** and **Y** matrices were added as residuals to generate individual configurations within populations. For the "no effect" cases described above, we forced the 5-point configurations to be approximately uncorrelated ($r_{PLS} < 0.1$) between the mean configurations for **X** and **Y**. For the "effect" cases, mean configurations were the same. The mean configurations were also generated to have dispersion of several orders of magnitude greater than the random within-point dispersion to ensure resulting individual landmark configurations were reasonable. In each permutation within each simulation run, GPA was performed and Procrustes residuals were used for r_{PLS} calculations. Thus, our results allowed us to evaluate both GM (Procrustes residuals) and non-GM (multivariate data) applications for consistently generated residuals, and whether GPA altered interpretations.

We repeated the entire process for five simulation runs with 20 sampling events and PLS analyses with 1000 random permutations to consider whether the sampling distributions of r_{PLS} values were normally distributed. In each sampling event, we calculated a Shapiro–Wilk *W* statistic (i.e., 100 values total for each comparison), and the ranges of these values were qualitatively evaluated to determine if sampling distributions were approximately normally distributed (i.e., a Shapiro–Wilk *W* statistic that tends toward 1.0). All simulations were performed in R 3.2.0 (R Core Team 2015).

RESULTS

The simulation experiment demonstrated that type I error rates were appropriate for both random multivariate data and Procrustes residuals (Fig. 2). For "no effect" comparisons, the mean type I error rates (0.0453 and 0.0494 for multivariate data and Procrustes residuals, respectively) were approximately the same as the nominal significance level (0.05). Interestingly, the type I error rates were lower for comparisons between equal but large effects (i.e., when comparing datasets where both exhibited marked morphological integration of similar strength). A type I error was simulated in 0 and 1 permutation of the 100×200 total permutations, for multivariate data and Procrustes residuals, respectively. The nearly nonexistent type I errors imply that when both datasets contain integration but their levels are similar, the method displays fewer false positives as compared to when comparing datasets lacking integration (Fig. 2). When comparing samples from the "no effect" and "effect" populations, the mean statistical power was 0.8133 and 0.883 for multivariate data and Procrustes residuals, respectively. Both of these values were, approximately equal to or greater than the generally desired value of 0.8 (Fig. 2), which represents a 4:1 trade-off between type II error risk and type I error risk (Cohen 1988). However, the inherently generated within-module correlations from GPA appear to slightly increase statistical power, perhaps owing to the additional correlation between mean configurations that was simulated. These results suggest that the two-sample test of integration disparity presented here behaves as expected, statistically, and GPA does not negatively impact results.

In terms of the appropriateness of the two-sample test of integration disparity for PLS analyses, we found no evidence to suggest that the sampling distributions of r_{PLS} statistics were nonnormally distributed. For multivariate data, the ranges in *W* statistics for the "no effect" case (0.9822–0.9996) and "effect" case (0.9885–0.9997) were quite similar and sufficiently close to 1.0 in each case. For the Procrustes residuals, the ranges in *W* statistics for the "no effect" case (0.9910–0.9996) and "effect" case (0.9895–0.9996) were also quite similar and sufficiently close to 1.0 in each case. Thus, whether GPA was performed had no consequence, and any PLS analysis produced sufficiently normal sampling distributions.

It should be noted that the sampling distribution of r_{PLS} values is arbitrarily based on the a priori chosen number of PLS resampling permutations. One may wish to either choose a

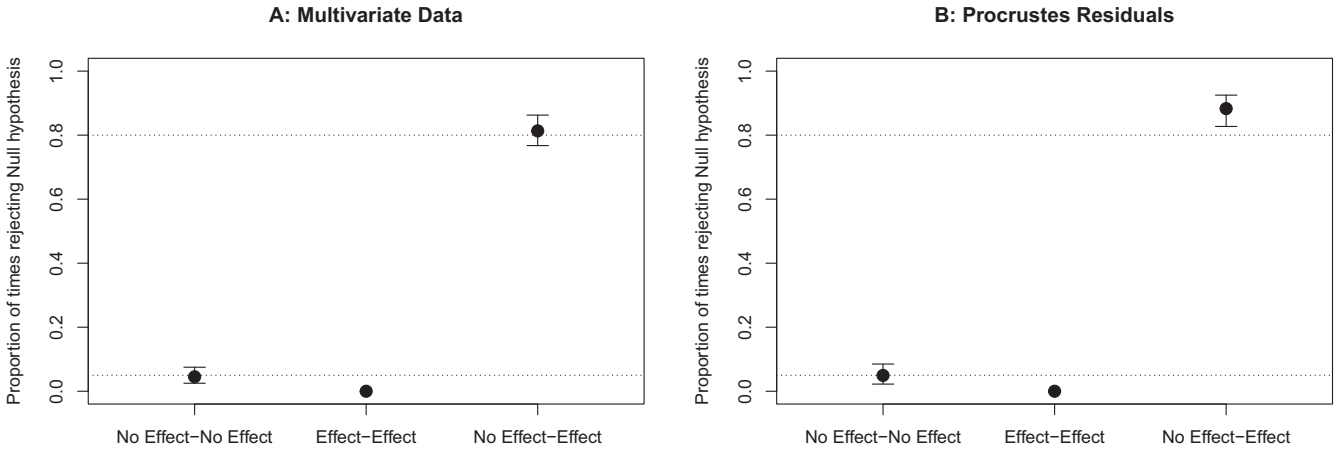


Figure 2. Proportion of times the null hypothesis was rejected in 100 runs of 200 comparisons of morphological integration for (A) multivariate data and (B) Procrustes residuals. Samples of size, $n = 30$ were obtained from populations ($N = 10{,}000$) with no integration (no effect) and substantial integration (effect). Means with 95% confidence limits are shown, unless the proportion was rather invariant, in which case, error bars are not included. Dotted lines are shown for an expected type I error rate of 0.05 or a statistical power of 0.80; within-population comparisons simulate type I errors (first two) and between-population comparisons simulate statistical power (last).

sufficiently large number of permutations or confirm that the SD of the sampling distribution remains consistent under a range of permutations. For example, we found the SD in our samples of 30 individuals in the simulation experiment was rather consistent between 200 and 10,000 random permutations, suggesting the 1000 permutations we used was adequate for measuring the difference in strength of integration among datasets. We also found that when using a small number of PLS resampling permutations (e.g., 100–200), a large effect for the observed r_{PLS}, could skew the sampling distribution (which should be normally distributed). This problem is alleviated by simply removing the observed r_{PLS} from the sampling distribution, as a small-sample bias adjustment. For sufficiently large numbers of PLS permutations (e.g., 1,000), this step was not needed, but also did not affect results. We, therefore, recommend removing the observed r_{PLS} as a procedural step to assure a more appropriate SD of sampling distributions, especially for large effect sizes.

A Biological Example

To illustrate the method described above, we conducted a comparison of levels of integration in cranial shape between rural and urban populations of the Mediterranean wall lizard *Podarcis muralis.* The data were part of a series of studies that evaluated the effects of urbanization on various aspects of phenotypic variation, including patterns of allometry, developmental stability, and integration in juvenile and adult lizards (see Lazic et al. 2015, 2016: available on Dryad and from the original authors). For this example, landmark-based geometric morphometric methods (Bookstein 1991; Mitteroecker and Gunz 2009; Adams et al. 2013) were used to characterize head shape, based on the positions of 28 homologous locations (Fig. 3A) collected from the dorsal view of 482 juvenile and 359 adult lizards from several localities. Of these, approximately half of the specimens were collected from rural locations (218 juveniles and 191 adults), and the remainder from urban sites (264 juveniles and 168 adults). For each specimen, a mirror image of their landmark locations was obtained by reflecting the coordinates about the mid-line, and a GPA was then performed to remove the effects of nonshape variation from the dataset (Fig. 3B). The symmetric component of shape was subsequently obtained by averaging landmark locations for each specimen and its mirror image. From the symmetric component of shape variation, we evaluated the degree to which the anterior and posterior regions of the head were integrated with one another. Landmarks were classified as anterior or posterior (Fig. 3A) based on the timing of ossification events during development (Lazic et al. 2015). For both juveniles and adults from rural and urban populations, the degree of integration between modules was estimated using PLS. Here, the maximal covariation between modules was characterized using the PLS correlation (r_{PLS}), which was statistically evaluated using 1000 random permutations. Additionally, z scores were obtained for all four groups, and were statistically compared to one another using the procedure described above. All analyses were performed in R 3.2.0 (R Core Team 2015) using the package *geomorph* (Adams and Otárola-Castillo 2013; Adams et al. 2016), including the function, compare.pls, which performs the method introduced here.

RESULTS

For both juvenile and adults from rural and urban lizard populations, the degree of morphological integration between anterior

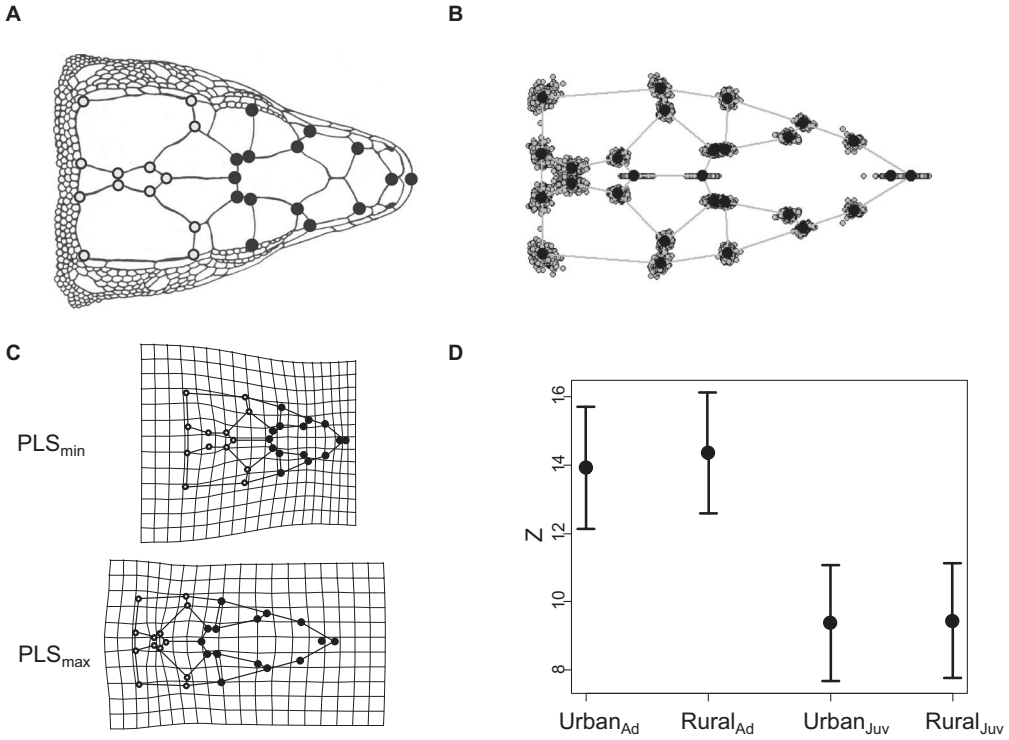


Figure 3. Graphical summary of results from the empirical example. (A) Locations of 28 landmarks on the dorsal view of a lizard head. Landmarks from the anterior module are designated by open circles, whereas those from the posterior module are designated by closed circles (from Lazic et al. 2015). (B) Procrustes superimposition of all specimens (gray) with the mean specimen shown in black. (C) Thin-plate spline deformation grids representing specimens at the extremes of the PLS axis for the adult rural population. Deformation grids are accentuated by a factor of two to facilitate visual interpretation. (D) Levels of integration in juveniles and adults from both rural and urban populations shown as *z*-scores and their 95% confidence intervals obtained from the SE of the permutation sampling distributions for each group.

and posterior regions of the head was large and highly significant (rural$_{juv}$: $r_{PLS} = 0.770$, $P_{rand} = 0.001$; rural$_{adult}$: $r_{PLS} = 0.826$, $P_{rand} = 0.001$; urban$_{juv}$: $r_{PLS} = 0.761$, $P_{rand} = 0.001$; urban$_{adult}$: $r_{PLS} = 0.858$, $P_{rand} = 0.001$). Generally, the observed integration of the frontal and distal regions of the head was represented by a relative shortening of the snout accompanied by an enlargement of the posterior area of the head (Fig. 3C); a pattern broadly observed in all groups. When converted to effect sizes, all z-score values were very large (Fig. 3D), implying that the degree of integration in each group greatly exceeded that which was expected by chance. Interestingly, when compared using equation (2) above, we found no evidence of differences in levels of integration between rural and urban juveniles or rural and urban adults, but significant differences existed in levels of integration between juveniles and adults within each population; with adults displaying significantly greater integration relative to juveniles (Table 1). Thus, while there was no evidence that environmental disturbances have affected the strength of morphological integration in urban populations, the results demonstrate that morphology is more integrated through ontogenetic time. Further, when combined with the prior observation that morphological variation among adult specimens was reduced when compared to that of juveniles (see Lazic et al. 2016), the pattern identified here is consistent with what is expected under the hypothesis of developmental canalization (Hallgrímsson et al. 2002).

Discussion

An important question in evolutionary biology is whether different taxa or traits display similar levels of morphological integration. Unfortunately, direct quantitative comparisons of the level of integration across datasets have been hampered by the fact that the measures that characterize these patterns (RV and r_{PLS}) are dependent on both sample size and the number of variables. Here, we described an unbiased effect size for quantifying the strength of morphological integration between sets of variables, using PLS analysis and its permutation sampling distribution. We demonstrated that under the null hypothesis of a random association of variables, the approach displays a constant expected value, and the same variance, SD, and confidence intervals across the entire spectrum of sample sizes and variable number. We further proposed a two-sample test to statistically evaluate the difference

Table 1. Results from empirical example comparing levels of morphological integration in juvenile and adult lizards from urban and rural populations.

Z	UR_{Ad}	RU_{Ad}	UR_{Juv}	RU_{Juv}	P	UR_{Ad}	RU_{Ad}	UR_{Juv}	RU_{Juv}
UR_{Ad}	0				UR_{Ad}	0			
RU_{Ad}	0.105	0			RU_{Ad}	0.459	0		
UR_{Juv}	2.818	2.777	0		UR_{Juv}	**0.002**	0.002	0	
RU_{Juv}	2.447	2.399	0.296	0	RU_{Juv}	0.007	**0.008**	0.383	0

(A) Matrix of pairwise differences in PLS effect sizes, and (B) their associated significance levels.

Biologically relevant focal comparisons are underlined; significant focal comparisons are shown in bold. Populations are designated as: UR = urban; RU = rural; Ad = adult; Juv = juvenile.

in effect sizes across two datasets. The approach displays appropriate type I error and statistical power, thereby providing a rigorous means of evaluating whether the degree of morphological integration differs between them. Thus, the approach provides evolutionary morphologists with a consistent means of deciphering whether levels of integration are similar to one another in two or more datasets.

Extensions to the approach developed here can be envisioned that address a wider array of empirical challenges than the ones presented. For example, if the integration among three sets of variables is of biological interest, a three-block PLS approach may be used to characterize the degree of covariation among sets of variables (see Bookstein et al. 2003). Alternatively, one may evaluate the mean of the pairwise r_{PLS} values as a general test measure, as has been proposed for evaluating hypotheses of modularity (Klingenberg 2009; Adams 2016). In either case it is important to recognize that the method developed here simply provides a quantitative estimate on the magnitude, or strength of morphological integration among sets of variables. The method provides no description of the type of integration, or *how* traits covary with one another and in what manner. For this, understanding patterns of morphological integration and the set of coordinated shape changes it embodies must still be accomplished via a thorough examination of the singular vectors from the PLS and a visual inspection of the shape changes associated with the singular vectors (see Bookstein 2016 for discussion). Thus, a proper biological understanding of patterns of morphological integration is accomplished via the combination of a quantitative characterization and statistical assessment of the magnitude of integration, along with its anatomical interpretation.

Finally, while the biological concept of morphological integration has been embraced in evolutionary biology for decades (Olson and Miller 1958), formal statistical tests of these patterns are still rather novel. As the theory of morphological integration develops, so too will the analytical methods for measuring integration, and their associated hypothesis tests (see, e.g., Bookstein 2015). An important advance made here is that for any two measures of morphological integration—irrespective of the number of variables, number of specimens studied, or expected values in a null distribution—effect sizes calculated as standard deviates in sampling distributions are values that can be compared in a general two-sample hypothesis test. We chose to use the correlation coefficient from two-block PLS as the basis for this test, but one could have likewise used maximum singular values, or vector correlations (or angles) between left and right singular vectors found through PLS, or other statistically relevant summaries. Because standard deviates can be calculated using any test statistic with a sampling distribution—essentially any statistic if resampling experiments are used—the hypothesis testing framework proposed here is merely a methodological extension of two-sample Z tests, but using resampling experiments to generate sampling distributions rather than requiring a priori knowledge of population SDs. Thus, as new analytical approaches are developed for evaluating patterns of morphological integration, the test procedure described here may be used for comparing the degree of integration observed in different datasets based on those measures. Ultimately, however, the choice of which test statistic to use in this procedure must be driven by biology. In the case of morphological integration, biological interpretations of such patterns depend on a deep understanding of how covariation patterns are embodied in terms of their singular vectors (see Bookstein 2016). Nevertheless, as the field of evolutionary morphological integration evolves, and better conceptual measures of morphological integration are developed, a hypothesis-testing framework developed here is already in place, and ready for such advances.

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LITERATURE CITED

Adams, D. C. 2016. Evaluating modularity in morphometric data: challenges with the RV coefficient and a new test measure. Methods Ecol. Evol. 7:565–572.

Adams, D. C., and R. N. Felice. 2014. Assessing trait covariation and morphological integration on phylogenies using evolutionary covariance matrices. PLoS ONE 9:e94335.

Adams, D. C., and E. Otárola-Castillo. 2013. geomorph: an R package for the collection and analysis of geometric morphometric shape data. Methods Ecol. Evol. 4:393–399.

Adams, D. C., F. J. Rohlf, and D. E. Slice. 2013. A field comes of age: geometric morphometrics in the 21st century. Hystrix 24:7–14.

Adams, D. C., M. Collyer, and E. Sherratt. 2016. geomorph 3.0.1: software for geometric morphometric analyses. R package version 3.0.1. Available at http://CRAN.R-project.org/package=geomorph.

Armbruster, W. S., C. Pelabon, G. H. Bolstad, and T. F. Hansen. 2014. Integrated phenotypes: understanding trait covariation in plants and animals. Phil. Trans. R. Soc. Lond. B 369:20130245.

Bookstein, F. L. 1991. Morphometric tools for landmark data: geometry and biology. Cambridge University Press, Cambridge, U.K.

Bookstein, F. L. 2015. Integration, disintegration, and self-similarity: characterizing the scales of shape variation in landmark data. Evol. Biol. 42:395–426.

Bookstein, F. L. 2016. The inappropriate symmetries of multivariate statistical analysis in geometric morphmetrics. Evol. Biol. 43:277–313. doi:10.1007/s11692-11016-19382-11697.

Bookstein, F. L., P. Gunz, P. Mitteroecker, H. Prossinger, K. Schaefer, and H. Seidler. 2003. Cranial integration in Homo: singular warps analysis of the midsagittal plane in ontogeny and evolution. J. Hum. Evol. 44:167–187.

Cheverud, J. M. 1982. Phenotypic, genetic, and environmental morphological integration in the cranium. Evolution 36:499–516.

Cheverud, J. M. 1996. Developmental integration and the evolution of pleiotropy. Am. Zool. 36:44–50.

Cohen, J. 1988. Statistical power analysis for the behavioral sciences. Lawrence Erlbaum, Hillsdale, NJ.

Drake, A. G., and C. P. Klingenberg. 2010. Large scale diversification of skull shape in domestic dogs: disparity and modularity. Am. Nat. 175:289–301.

Escoufier, Y. 1973. Le traitement des variables vectorielles. Biometrics 29:751–760.

Fruciano, C., P. Franchini, and A. Meyer. 2013. Resampling-based approaches to study variation in morphological modularity. PLoS ONE 8:e69376.

Gómez-Robles, A., and P. D. Polly. 2012. Morphological integration in the hominin dentition: evolutionary, developmental, and functional factors. Evolution 66:1024–1043.

Goswami, A., and P. D. Polly. 2010. Methods for studying morphological integration and modularity. Pp. 213–243 *in* J. Alroy, and G. Hunt, eds. Quantitative methods in paleobiology. The Paleontological Society, Boulder, CO.

Goswami, A., J. B. Smaers, C. Soligo, and P. D. Polly. 2014. The macroevolutionary consequences of phenotypic integration: from development to deep time. Phil. Trans. R. Soc. Lond. B 369:20130254.

Hallgrímsson, B., K. Willmore, and B. K. Hall. 2002. Canalization, developmental stability, and morphological integration in primate limbs. Am. J. Phys. Anthropol. 119:131–158.

Klingenberg, C. P. 2008. Morphological integration and developmental modularity. Ann. Rev. Ecol. Evol. Syst. 39:115–132.

Klingenberg, C. P. 2009. Morphometric integration and modularity in configurations of landmarks: tools for evaluating a priori hypotheses. Evol. Dev. 11:405–421.

Klingenberg, C. P. 2014. Studying morphological integration and modularity at multiple levels: concepts and analysis. Phil. Trans. R. Soc. Lond. B. 369:20130249.

Lazic, M. M., M. A. Carretero, J. Crnobrnja-Isailovic, and A. Kaliontzopoulou. 2015. Effects of environmental disturbance on phenotypic variation: an integrated assessment of canalization, developmental stability, modularity, and allometry in lizard head shape. Am. Nat. 185:44–58.

Lazic, M. M., M. A. Carretero, J. Crnobrnja-Isailovic, and A. Kaliontzopoulou 2016. Postnatal dynamics of developmental stability and canalization of lizard head shape under different environmental conditions. Evol. Biol. 368–379. doi:10.1007/s11692-016-9377-4.

Magwene, P. M. 2001. New tools for studying integration and modularity. Evolution 55:1734–1745.

Márquez, E. J. 2008. A statistical framework for testing modularity in multidimensional data. Evolution 62:2688–2708.

Martin-Serra, A., B. Figueirido, J. A. Perez-Claros, and P. Palmqvist. 2015. Patterns of morphological integration in the appendicular skeleton of mammalian carnivores. Evolution 69:321–340.

Mitteroecker, P., and F. L. Bookstein. 2007. The conceptual and statistical relationship between modularity and morphological integration. Syst. Biol. 56:818–836.

Mitteroecker, P., and P. Gunz. 2009. Advances in geometric morphometrics. Evol. Biol. 36:235–247.

Mitteroecker, P., P. Gunz, M. Bernhard, K. Schaefer, and F. L. Bookstein. 2004. Comparison of cranial ontogenetic trajectories among great apes and humans. J. Hum. Evol. 46:679–698.

Monteiro, L. R., V. Bonato, and S. F. D. Reis. 2005. Evolutionary integration and morphological diversification in complex morphological structures: mandible shape divergence in spiny rats (Rodentia, Echimyidae). Evol. Dev. 7:429–439.

Neaux, D., E. Gilissen, W. Coudyzer, and F. Guy. 2015. Integration between the face and the mandible of *Pongo* and the evolution of the craniofacial morphology of orangutans. Am. J. Phys. Anthropol. 158:475–486.

Olson, E. C., and R. L. Miller. 1958. Morphological Integration. Univ. of Chicago Press, Chicago, IL.

Pigliucci, M. 2003. Phenotypic integration: studying the ecology and evolution of complex phenotypes. Ecol. Lett. 6:265–272.

R Core Team. 2015. R: a language and environment for statistical computing. Version 3.2.0. R Foundation for Statistical Computing, Vienna. Available at http://cran.R-project.org.

Rohlf, F. J., and M. Corti. 2000. The use of partial least-squares to study covariation in shape. Syst. Biol. 49:740–753.

Smilde, A. K., H. A. L. Kiers, S. Biklsma, C. M. Rubingh, and M. J. vanErk. 2009. Matrix correlations for high-dimensional data: the modified RV-coefficient. Bioinformatics 25:401–405.

Young, R. L., and A. V. Badyaev. 2006. Evolutionary persistence of phenotypic integration: influence of developmental and functional relationships on complex trait evolution. Evolution 60:1291–1299.

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