A Generalized *K* Statistic for Estimating Phylogenetic Signal from Shape and Other High-Dimensional Multivariate Data

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Abstract.—Phylogenetic signal is the tendency for closely related species to display similar trait values due to their common ancestry. Several methods have been developed for quantifying phylogenetic signal in univariate traits and for sets of traits treated simultaneously, and the statistical properties of these approaches have been extensively studied. However, methods for assessing phylogenetic signal in high-dimensional multivariate traits like shape are less well developed, and their statistical performance is not well characterized. In this article, I describe a generalization of the *K* statistic of Blomberg et al. that is useful for quantifying and evaluating phylogenetic signal in highly dimensional multivariate data. The method (K_{mult}) is found from the equivalency between statistical methods based on covariance matrices and those based on distance matrices. Using computer simulations based on Brownian motion, I demonstrate that the expected value of K_{mult} remains at 1.0 as trait variation among species is increased or decreased, and as the number of trait dimensions is increased. By contrast, estimates of phylogenetic signal found with a squared-change parsimony procedure for multivariate data change with increasing trait variation among species and with increasing numbers of trait dimensions, confounding biological interpretations. I also evaluate the statistical performance of hypothesis testing procedures based on K_{mult} and find that the method displays appropriate Type I error and high statistical power for detecting phylogenetic signal in high-dimensional data. Statistical properties of K_{mult} were consistent for simulations using bifurcating and random phylogenies, for simulations using different numbers of species, for simulations that varied the number of trait dimensions, and for different underlying models of trait covariance structure. Overall these findings demonstrate that K_{mult} provides a useful means of evaluating phylogenetic signal in high-dimensional multivariate traits. Finally, I illustrate the utility of the new approach by evaluating the strength of phylogenetic signal for head shape in a lineage of *Plethodon* salamanders. [Geometric morphometrics; macroevolution; morphological evolution; phylogenetic comparative method.]

Phylogenetic comparative biology is predicated on the notion that species are not independent due to their shared evolutionary history (Felsenstein 1985; Revell et al. 2008). This insight is derived largely from the observation that closely related species tend to be similar in their trait values; a pattern termed *phylogenetic signal* (Blomberg et al. 2003; Munkemuller et al. 2012). Indeed, simply having knowledge of the evolutionary relationships among species, and an expected model of trait evolution, is sufficient to analytically derive the expected degree of covariation between taxa under the evolutionary model specified (see Felsenstein 1985; Grafen 1989; Harvey and Pagel 1991; Martins and Hansen 1997; Garland and Ives 2000; Rohlf 2001; Rezende and Diniz-Filho 2012). Importantly, because this covariation is not zero, species are not evolutionarily independent, and as such statistical procedures that assess patterns in cross-species data must account for this nonindependence by including the expected covariation among taxa in the analysis (Revell 2010).

In macroevolutionary studies, one often examines the consequences of shared evolutionary history by determining the degree to which phylogenetic signal is displayed in phenotypic traits. For instance, estimating levels of phylogenetic signal in phenotypic data has been used as a guide to determine whether statistical analyses of cross-species data must take phylogeny into consideration (e.g., Abouheif 1999; Rheindt et al. 2004; Møller et al. 2008), although this practice has been questioned on theoretical grounds (Rohlf 2006;

Revell 2010). Likewise, identifying differences in the degree of phylogenetic signal exhibited across classes of traits can provide insights into which aspects of the phenotype are more evolutionarily labile than others (e.g., Blomberg et al. 2003; see also Gittleman et al. 1996). On the other hand, it is well recognized that phylogenetic signal alone is not a direct means of elucidating the evolutionary processes responsible for phenotypic diversification, because multiple processes can produce similar patterns of phylogenetic signal (see Blomberg et al. 2003; Revell et al. 2008; Ackerly 2009; (Pennell and Harmon, 2013)). Nevertheless, examining the degree to which traits exhibit phylogenetic signal is an important step in understanding how phenotypic variation is distributed across species (Klingenberg and Gidaszewski 2010; Rezende and Diniz-Filho 2012), and how such patterns associate with patterns of species diversification (Cardini and Elton 2008).

Over the past two decades, several approaches have been developed to estimate phylogenetic signal in phenotypic data sets. These methods differ primarily in how they characterize the dependency between trait covariation among species and the evolutionary relationships of those taxa. For instance, some approaches measure the fit of the phenotypic data to the phylogeny under a particular model of evolutionary change using likelihood. A parameter that scales elements of the phylogenetic covariance matrix is included during this optimization, and this parameter is then treated as an estimate of phylogenetic signal (e.g.,

Pagel 1999; Freckleton et al. 2002; Blomberg et al. 2003; Zheng et al. 2009). Other methods utilize the association between phenotypic variation among species and their phylogenetic relationships, which can be estimated from matrix correlation methods or through autoregressive and related procedures (e.g., Abouheif 1999; Cubo et al. 2005; Pavoine et al. 2008). Another common approach estimates phylogenetic signal as the ratio of observed to expected phenotypic variation, given a phylogeny and a Brownian motion model of evolution (Blomberg et al. 2003). Several studies have examined the statistical attributes of different methods, and have identified common analytical elements across procedures (see Harmon and Glor 2010; Hardy and Pavoine 2012; Munkemuller et al. 2012; Pavoine and Ricotta 2012).

Many empirical studies have estimated the strength of phylogenetic signal in single, univariate traits such as morphological measurements (e.g., Ackerly 2009; Blankers et al. 2012; Roncal et al. 2012) or behavioral variables (e.g., Gingras et al. 2013; Kamilar and Cooper 2013; see also Blomberg et al. 2003). However, organismal phenotypes are often characterized multivariately, either by a set of traits treated simultaneously or by complex, multidimensional traits such as shape. For sets of traits treated simultaneously, Pagel's λ can be used to estimate the degree of phylogenetic signal displayed in the data set (Freckleton et al. 2002; for a conceptually related approach see Zheng et al. 2009). However, an unappreciated limitation of this approach is that when the number of trait dimensions equals or exceeds the number of taxa in the phylogeny ($p \geq N$), Pagel's λ cannot be estimated, because the matrix computations used to obtain the likelihood of the model are singular. Alternatively, the degree of phylogenetic signal can be estimated for multivariate data using the Mantel correlation between phylogenetic distances and phenotypic distances (e.g., Polly 2001; Cardini and Elton 2008; Ivanovic et al. 2009; Perez et al. 2009). However, Mantel tests often display inflated Type I error rates and low statistical power when used for this purpose (Harmon and Glor 2010). Therefore, these procedures may not be optimal for assessing phylogenetic signal in high-dimensional multivariate data.

Recently, an approach was proposed for estimating the degree of phylogenetic signal in multidimensional phenotypic traits like shape (Klingenberg and Gidaszewski 2010). Multidimensional traits are phenotypic attributes that require more than one number to encode. Examples of multidimensional traits include function-valued traits that characterize ontogenetic trajectories, growth curves, and reaction norms (sensu Kirkpatrick and Heckman 1989; Kirkpatrick and Meyer 2004), or the shapes of anatomical objects as quantified from geometric morphometric methods (Klingenberg and Gidaszewski 2010; Adams 2014; see also McPeek et al. 2008). As with sets of univariate traits treated simultaneously, evolutionary changes in multidimensional traits correspond to shifts in the position of a species in a high-dimensional trait space whose axes correspond

to trait dimensions (Klingenberg and Gidaszewski 2010; Adams 2014). However, unlike sets of traits, the values that describe multidimensional traits (i.e., trait dimensions) are mathematically interrelated, and cannot be assessed independently of one another (Adams and Rosenberg 1998; Rohlf 1998; Klingenberg and Gidaszewski 2010; Adams 2011). Thus, because each trait dimension does not have independent biological meaning, phenotypic patterns across species must be characterized using all dimensions of the multidimensional trait simultaneously, rather than being estimated from each trait dimension individually. This distinction is important, as conceptually it implies that while a set of phenotypic traits should have multiple estimates of phylogenetic signal (one per trait), a complex multidimensional trait has but one phylogenetic signal. Put simply, a single trait has a single phylogenetic signal, even if that trait is multidimensional.

To estimate phylogenetic signal for multidimensional traits, Klingenberg and Gidaszewski (2010) proposed fitting the phenotypic data to the phylogeny using squared-change parsimony. They then estimated the sum of squared changes (SSCs) of the trait across all branches of the phylogeny to obtain a single estimate of phylogenetic signal in the multidimensional trait (Klingenberg and Gidaszewski 2010). Under this formulation, smaller values of SSC correspond to a better fit of the data to the phylogeny, and thus represent a higher degree of phylogenetic signal. However, while the approach is intuitive and conceptually straightforward, a number of complications limit its utility. First, the method relies on ancestral state estimation; a procedure known to have large standard errors (see Losos [2011] and references therein). Thus, uncertainty in ancestral states can lead to less reliable estimates of phylogenetic signal. Second, the statistical properties of the approach have not been investigated under known (simulation) conditions. Third, SSC has no *a priori* expected value, and as such estimates of SSC cannot be compared among phenotypic traits as there is no relative scale on which those comparisons can be made. Finally, as I demonstrate below, estimates of SSC change in proportion with increasing levels of trait variation among species, and as the number of trait dimensions is increased. These patterns complicate biological interpretations based on SSC. Thus, for high-dimensional phenotypic data like shape, an alternative estimate of phylogenetic signal is desired.

In this article, I describe a multivariate generalization of the K statistic of Blomberg et al. (2003) that is useful for quantifying and evaluating phylogenetic signal in high-dimensional multivariate traits like shape. The method (K_{mult}) is derived from the equivalency between statistical methods based on covariance matrices and those based on distance matrices. Consistent with this equivalency, the distance-based approach (K_{mult}) provides estimates of phylogenetic signal for univariate data that are numerically identical to those obtained using the standard covariance-based equation for K.

When used on multivariate data, I show that under Brownian motion, the expected value of K_{mult} is 1.0 across a range of trait dimensions, and remains constant as trait variation among species increases or decreases. Using computer simulations I then show that hypothesis testing procedures based on K_{mult} display acceptable Type I error and appropriate statistical power for detecting phylogenetic signal in high-dimensional multivariate traits like shape. Finally, a biological example is presented which demonstrates the utility of the approach. Computer code written in R for implementing the procedure is also provided.

ESTIMATING PHYLOGENETIC SIGNAL WITH KAPPA

The K statistic of Blomberg et al. (2003) estimates the strength of phylogenetic signal in a data set relative to what is expected given a Brownian motion model of evolution. Under Brownian motion, evolutionary changes in a single trait along the phylogeny have an expected value of zero and a variance among species (σ^2) that accumulates proportional to time (Edwards and Cavalli-Sforza 1964; Felsenstein 1973, 1981, 1985, 2004). For multivariate data, the Brownian process is described by a covariance matrix (**R**) whose diagonal elements represent the evolutionary rate for each trait dimension (σ^2), and whose off-diagonal elements express the covariation in changes between trait dimensions (see Felsenstein 1988; Revell and Harmon 2008). Analytically, K is obtained as the function of two ratios (see Blomberg et al. 2003; Revell and Harmon 2008). The numerator of K is a ratio of observed variation in the data relative to the observed variation while accounting for phylogenetic covariance. The denominator of K is the ratio of variation expected under Brownian motion relative to the number of taxa in the phylogeny. Thus, the numerator of K is computed from the observed data, the phylogeny, and the evolutionary model (Brownian motion), whereas the denominator is obtained solely as a function of the phylogeny and the evolutionary model.

For a univariate trait, K may be computed as:

$$K = \frac{(\mathbf{Y}-E(\mathbf{Y}))^{\mathbf{t}}(\mathbf{Y}-E(\mathbf{Y}))}{(\mathbf{Y}-E(\mathbf{Y}))^{\mathbf{t}}\mathbf{C}^{-1}(\mathbf{Y}-E(\mathbf{Y}))} \bigg/ \frac{\text{tr}(\mathbf{C})-N(\mathbf{1}^{\mathbf{t}}\mathbf{C}^{-1}\mathbf{1})^{-1}}{N-1},$$

$$(1)$$

where $\mathbf{Y}$ is a $N \times 1$ vector of phenotypic values for N species, $E(\mathbf{Y})$ is a $N \times 1$ vector containing the phylogenetic mean at the root of the phylogeny: $\hat{a} = (\mathbf{1}^{\mathbf{t}}\mathbf{C}^{-1}\mathbf{1})^{\mathbf{t}}(\mathbf{1}^{\mathbf{t}}\mathbf{C}^{-1}\mathbf{Y})$, $\mathbf{1}$ is a $N \times 1$ vector of ones, and $\mathbf{C}^{-1}$ is the inverse of the $N \times N$ phylogenetic covariance matrix. Under Brownian motion, the diagonal elements of the phylogenetic covariance matrix (**C**) contain the phylogenetic distances from each tip to the root of the phylogeny, whereas the off-diagonal elements contain the phylogenetic distances from the root of the tree to the most recent common ancestor for each pair of species (Martins and Hansen 1997; Garland and Ives 2000; Rohlf 2001). In Equation (1), t represents the matrix transpose operation, tr designates

the trace of the matrix, and -1 is the matrix inverse operation. Values of K range from $0 \rightarrow \infty$, with an expected value of 1.0 under Brownian motion. Values of $K < 1.0$ describe data with less phylogenetic signal than expected, and values of $K > 1.0$ describe data with greater phylogenetic signal than expected. Typically, the observed value (K_{obs}) is evaluated statistically via permutation, where data at the tips of the phylogeny are randomized relative to the tree, and values of K_{rand} are obtained for each permutation of the data which are then compared with K_{obs} (described in Blomberg et al. 2003).

A MULTIVARIATE GENERALIZATION OF KAPPA

The K statistic provides a statistical measure of phylogenetic signal relative to expectations under Brownian motion. However, this measure is only suitable for univariate traits (Hardy and Pavoine 2012). To obtain a comparable measure for high-dimensional multivariate data, I propose a distance-based equation for K_{mult} that is found from the statistical equivalency between covariance-based and distance-based approaches for Euclidean data. From a statistical perspective, analytical procedures often summarize data matrices by their variances and covariances. The K statistic is an example of such an approach, as its numerator is a ratio of variances for $\mathbf{Y}$ given the phylogeny (Equation (1)). However, one can also summarize information in data using the matrix of pairwise distances among specimens, and it has been shown that covariance-based and distance-based approaches often produce equivalent results. For example, both principal components analysis (covariance-based) and principal coordinates analysis (distance-based) yield identical ordinations for the same Euclidean data set (Gower 1966; see also Krzanowksi 1993; Legendre and Legendre 1998). Further, sums of squares obtained from covariance-based methods such as analysis of variance (ANOVA) and regression are numerically identical to those obtained from distance-based procedures such as permutational-ANOVA, when applied to a Euclidean data vector (for a mathematical derivation of this property, see Anderson [2001]; McArdle and Anderson [2001]). Thus, in many cases, statistical summaries based on distances are equivalent to those obtained from the more common covariance-based procedures.

Here, I develop a multivariate generalization of K that can be used to quantify phylogenetic signal in multivariate traits. A worked example outlining all of the computations described in this section may be found in Appendix 1. To accomplish this generalization, several elements of the covariance-based equation must be reformulated. Specifically, those components that describe the ratio of observed variation in the data given the phylogeny (i.e., the numerator of K) must be adjusted mathematically to account for the fact that multivariate data are now being considered. First, we require an alternative characterization of the observed multivariate

variation relative to the phylogenetic mean. This may be achieved by re-expressing the difference between species means and the root of the phylogeny in terms of the Euclidean distance between them:

$$\mathbf{D}_{i,\widehat{a}} = \sqrt{\left(\mathbf{Y}_i - E(\mathbf{Y})\right)\left(\mathbf{Y}_i - E(\mathbf{Y})\right)^t}. \quad (2)$$

In Equation (2), $\mathbf{Y}_i$ is a row vector containing the p-phenotypic values for the ith species and $E(\mathbf{Y})$ is a row vector containing the multivariate phylogenetic mean ($\widehat{a}$). Using Equation (2), the Euclidean distances between all species and the root of the phylogeny are obtained. These are then concatenated into a $N \times 1$ column vector ($\mathbf{D}_{Y,\widehat{a}}$), and the observed variation (mean-squared error, $\mathrm{MSE}_{\mathrm{obs}}$) for the N species relative to the phylogenetic mean is found as:

$$\mathrm{MSE}_{\mathrm{obs}} = \mathbf{D}_{Y,\widehat{a}}^t \mathbf{D}_{Y,\widehat{a}}. \quad (3)$$

Note that Equation (3) is similar to that used to obtain sums of squares for linear models when variation is expressed using distances (see Anderson 2001).

Next, the observed variation in the data while accounting for phylogenetic nonindependence must be calculated. This is accomplished using phylogenetic transformation (Blomberg et al. 2003). First, the phylogenetic covariance matrix ($\mathbf{C}$) is re-expressed via an eigen-decomposition (Garland and Ives 2000; Blomberg et al. 2003). Here, the eigenvectors ($\mathbf{U}$) and eigenvalues ($\mathbf{W}$) of $\mathbf{C}$ are obtained from $\mathbf{C} = \mathbf{U}\mathbf{W}\mathbf{U}^{-1}$, and used to construct the matrix $\mathbf{E}$ as:

$$\mathbf{E} = \left(\mathbf{U}\mathbf{W}^{1/2}\mathbf{U}^t\right)^{-1}. \quad (4)$$

Next, the multivariate phenotypic data for all species are transformed by the phylogeny as:

$$\mathbf{U_Y} = \mathbf{E}\left(\mathbf{Y} - E(\mathbf{Y})\right). \quad (5)$$

where $\mathbf{Y}$ is a $N \times p$ matrix of phenotypic trait values (N species by p dimensions), $E(\mathbf{Y})$ is a $N \times p$ matrix containing the multivariate phylogenetic mean, and $\mathbf{E}$ represents the phylogenetic transformation matrix described in Equation (4). Here, $\mathbf{U}_Y$ represent the phenotypic data transformed by the phylogeny such that they no longer contain phylogenetic covariances. Note that Equation (5) differs from Equation (2) in Blomberg et al. 2003 by including the phylogenetic mean ($\widehat{a}$). The reason is that Equations (2) and (3) of Blomberg et al. 2003 contain an error. Specifically, the phylogenetic mean ($\widehat{a}$) should be in the phylogenetic transformation step as shown above, rather than in subsequent calculations as was originally presented (Ives A.R., personal communication).

From the phylogenetically transformed data, the Euclidean distance between each species value (the rows of $\mathbf{U}_Y$) and the origin is calculated, and these are concatenated into a $N \times 1$ vector containing the Euclidean distances from each species mean to the origin ($\mathbf{PD}_{U,0}$). Finally, the variation for the N species given the phylogeny (MSE) is found as:

$$\mathrm{MSE} = \mathbf{PD}_{U,0}^t \mathbf{PD}_{U,0}. \quad (6)$$

The remaining components of the covariance-based equation of K represent the ratio of expected variation given the phylogeny (i.e., the denominator of K). Because these are obtained solely from the phylogeny, no alteration is required for multivariate data. Thus, combining Equations (1), (3), and (6) provides the multivariate generalization of K as:

$$K_{\mathrm{mult}} = \frac{\mathbf{D}_{Y,\widehat{a}}^t \mathbf{D}_{Y,\widehat{a}}}{\mathbf{PD}_{U,0}^t \mathbf{PD}_{U,0}} \Big/ \frac{tr(\mathbf{C}) - N(\mathbf{1^t C^{-1} 1})^{-1}}{N-1}. \quad (7)$$

Finally, like the K statistic of Blomberg et al. (2003), K_{mult} may be evaluated statistically via permutation, where data at the tips of the phylogeny are randomized relative to the tree, and values of K_{rand} are obtained for each permutation of the data which are then compared with K_{mult}.

It is important to realize that for univariate data, estimates of phylogenetic signal obtained using Equation (7) are numerically identical to those obtained from the variance-based methods typically used (Equation (1)). A demonstration of this property is found in Appendix 1. Thus, for univariate data, the distance–covariance equivalency has been preserved for estimates of phylogenetic signal, because values of K and K_{mult} are numerically identical. However, when used on multivariate data, K_{mult} provides a measure of the strength of phylogenetic signal for multivariate traits quantified in high-dimensional phenotypic spaces. Also, like K, values of $K_{\mathrm{mult}} < 1$ imply that taxa resemble each other phenotypically less than expected under Brownian motion whereas values of $K_{\mathrm{mult}} > 1$ imply that close relatives are more similar to one another phenotypically than expected under Brownian motion. Further, because this approach does not rely on the inversion of covariance matrices, it is not restricted to cases where the number of trait dimensions is less than the number of species in the phylogeny (as is Pagel's λ). This is particularly important for traits such as shape, where the number of trait dimensions frequently exceeds the number of species in a phylogeny (e.g., McPeek et al. 2008; Klingenberg and Gidaszewski 2010). Thus, the distance-based approach proposed here provides a means of quantifying phylogenetic signal in phenotypic traits that is not possible with alternative procedures based on likelihood (e.g., Freckleton et al. 2002).

EXPECTED VALUE OF K_{mult} UNDER BROWNIAN MOTION

Under Brownian motion, it has been shown that K has an expected value of 1.0 for univariate data (Blomberg et al. 2003). Here, I show that for multidimensional data, K_{mult} also exhibits this property. To evaluate the expected value of K_{mult}, multivariate data were generated by simulating data on the phylogeny under a Brownian motion model of evolution. For these

simulations, two distinct patterns of input covariance structure were utilized to generate the multivariate data, as described by the Brownian motion rate parameter, σ^2. For the first case, the input variance for each trait dimension was identical (e.g., $\sigma_1^2 = \sigma_2^2$), thereby describing an isotropic model. For the second, variation in each trait dimension was allowed to differ (e.g., $\sigma_1^2 \neq \sigma_2^2$), thus representing a nonisotropic model. Simulations were conducted on a balanced phylogeny containing 32 species. For each simulation, the number of trait dimensions was first selected ($p = 2, 4, 6, 8, 10, 20, 30$). Next, the initial level of input variance was selected ($\sigma^2 = 0.01, 0.05, 0.1, 0.2, 0.4, 0.6, 1.0, 2.0$), and from this a $p \times p$ input covariance matrix was constructed. For simulations assuming isotropic error, σ^2 was identical for all trait dimensions as described above, whereas for simulations assuming nonisotropic error, the input σ^2 for each trait dimension was drawn from a normal distribution following: $\mu = \sigma_{initial}^2; std = 0.2 * \sigma_{initial}^2$. From these initial covariance matrices, 100 phenotypic data sets were obtained by simulating multidimensional traits along the phylogeny following a Brownian motion model of evolution. For each data set, phylogenetic signal was estimated using K_{mult} as described above, and the mean of the 100 values of K_{mult} was treated as the expected value of the statistic under Brownian motion (for a similar procedure see Blomberg et al. [2003]). Additionally, for each data set I obtained estimates of SSC to evaluate its expected value, because the statistical properties of this measure have not previously been determined.

Results

For all simulation conditions, K_{mult} displayed an expected value near 1.0, and remained consistent as the level of trait variation among species increased or decreased. This finding was the case when data were simulated under both isotropic (Fig. 1a) and nonisotropic conditions (Fig. 1b). By contrast, the expected value of SSC increased when input levels of variation among species increased. When the number of trait dimensions was increased, expected values of K_{mult} remained near 1.0 for all conditions examined (Fig. 1c,d). However, the expected value of SSC increased as the number of trait dimensions increased. Overall these simulations reveal that the expected value of SSC changes with both increasing levels of trait variation among species and with increasing numbers of trait dimensions. Thus, there is no consistent value of SSC that is expected under a Brownian motion model of evolution, because values of SSC depend strongly on the degree of input variation across species at the tips of the phylogeny. Therefore, even under Brownian motion, it is not known whether smaller values of SSC correspond to higher levels of phylogenetic signal or lower levels of trait variation. These properties greatly confound biological interpretations based on SSC. By contrast, K_{mult} exhibits an expected value of 1.0 under Brownian motion whether trait variation among species is increased or decreased, and remains consistent as the number of trait dimensions is increased. Indeed, these are desirable properties, as they imply that K_{mult} describes phylogenetic signal on a relative scale that does not change across phenotypic data sets, even if those data sets differ in their levels of variation and number of trait dimensions.

STATISTICAL PERFORMANCE OF TESTS OF K_{mult}

The simulations presented in the previous section demonstrate that the expected value of K_{mult} remains constant across a range of trait variation and trait dimensions. However, for K_{mult} to be a useful measure of phylogenetic signal, statistical tests based on it must also exhibit appropriate power and Type I error rates. To evaluate the statistical performance of hypothesis tests based on K_{mult}, I executed a series of computer simulations, following the procedure outlined in Blomberg et al. (2003). Initial simulations were conducted on a balanced phylogeny containing 32 species. The multivariate data sets used in these simulations were generated using three different models of input covariance: (i) isotropic, where the input variance for each trait dimension was identical (e.g., $\sigma_1^2 = \sigma_2^2$) and there was no input covariation between dimensions; (ii) nonisotropic, where the input variance in each trait dimension was allowed to differ from one another (e.g., $\sigma_1^2 \neq \sigma_2^2$) and there was no input covariation between trait dimensions; and (iii) nonisotropic with trait covariance, where the input variance in each trait dimension was allowed to differ from one another (e.g., $\sigma_1^2 \neq \sigma_2^2$) but where covariation among trait dimensions was also included. For each simulation, the number of trait dimensions was first selected ($p = 2, 4, 6, 8, 50, 100$). Note that the last two values of p (50 and 100) greatly exceed the number of species in the phylogeny ($N = 32$). Next, an initial $p \times p$ input covariance matrix was constructed. For simulations under an isotropic model, $\sigma^2 = 1.0$ was chosen for all trait dimensions. For simulations under a nonisotropic model, the input σ^2 for each trait dimension was drawn from a normal distribution following: $\mu = 1.0; std = 0.2$, and the $p \times p$ covariance matrix was constructed using these values as the diagonal elements. For simulations under a nonisotropic model with among trait covariance, a random $p \times p$ covariance matrix was generated in the following manner. First, a lower-triangular matrix $\mathbf{L}$ was generated by drawing values from the normal distribution ($\mu = 0; std = 1$). Next, the matrix product $\mathbf{LL^t}$ was calculated, which produces a positive semidefinite covariance matrix (following the Cholesky decomposition: $\Sigma = \mathbf{LL^t}$). Thus, $\mathbf{LL^t}$ represents a random covariance matrix that included differing amounts of input variation among trait dimensions and covariation among trait dimensions.
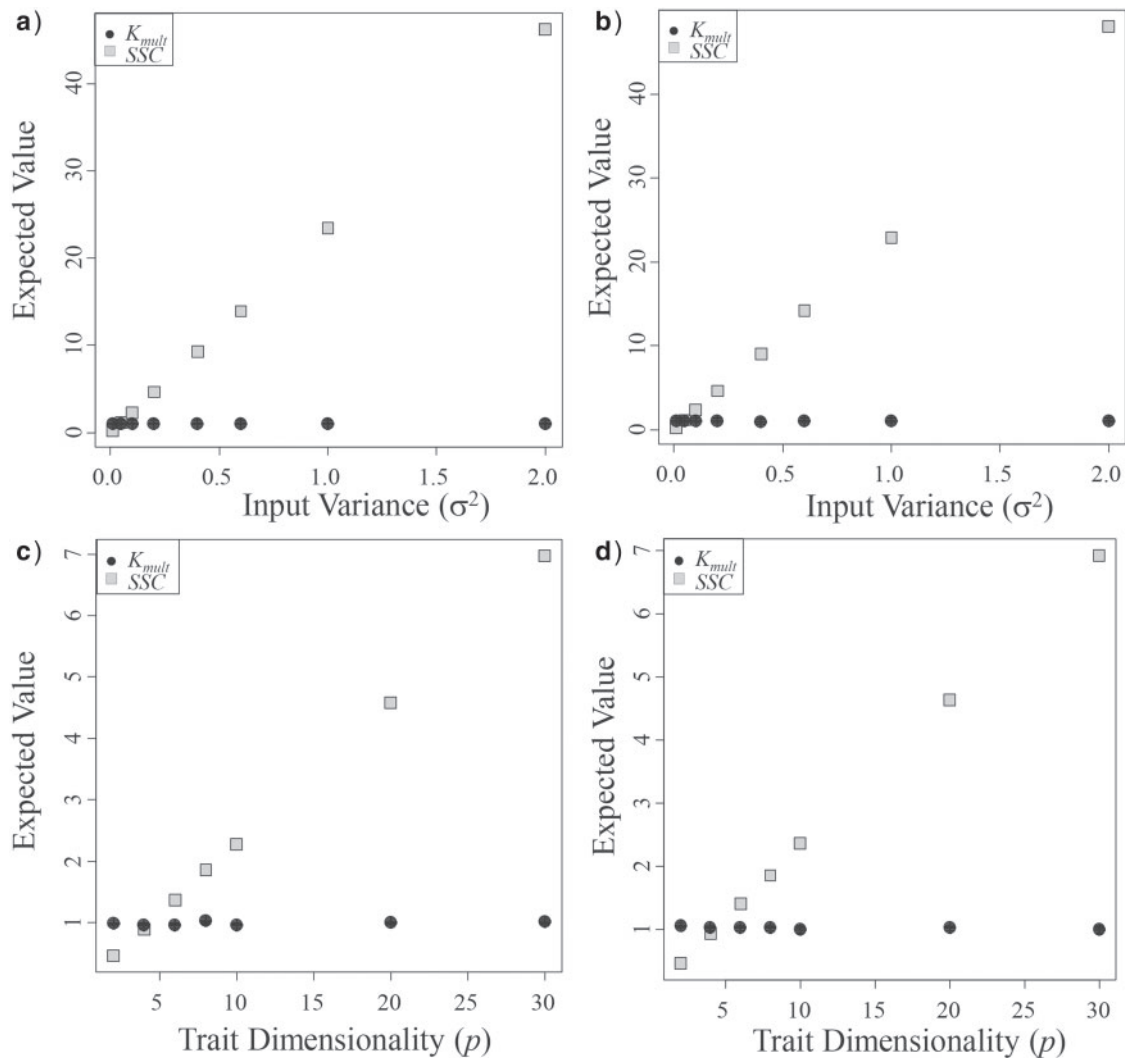
FIGURE 1.   Simulation results evaluating the expected value of K_{mult} and SSC under a Brownian motion model of evolution. Data were simulated on a balanced phylogeny containing 32 species. a) Expected values for K_{mult} and SSC found as the mean across 100 simulations for multidimensional data ($p=10$) generated under an isotropic covariance structure. b) Expected values of K_{mult} and SSC for multidimensional data ($p=10$) simulated under a nonisotropic covariance structure. In both (a) and (b), expected values are plotted as a function of increasing input variation among species. c) Expected values for K_{mult} and SSC found as the mean across 100 simulations for data generated under an isotropic covariance structure, and d) for data simulated under a nonisotropic covariance structure. In both (c) and (d), data were obtained with an initial input variance of $\sigma^2 = 0.1$, and expected values are plotted as a function of the number of trait dimensions (p).

From these initial covariance matrices, 1000 phenotypic data sets were obtained by simulating multidimensional traits along the phylogeny following a Brownian motion model of evolution. Following Blomberg et al. (2003), tests of Type I error were obtained by evolving phenotypic data on a star phylogeny, and testing for phylogenetic signal on a resolved tree. Likewise, statistical power was evaluated by evolving data on a resolved phylogeny and testing for phylogenetic signal on that tree. To obtain a known range of phylogenetic signal, prior to simulating phenotypic data the branch lengths of the phylogeny were transformed by the parameter λ, where λ=0.0 transforms the tree to a star phylogeny whereas λ = 1.0 represents the original fully resolved tree.

Transformation values used in this study were: λ = 0, 0.05, 0.1, 0.2, 0.4, 0.6, 0.8, 1.0 (for a similar procedure see Blomberg et al. [2003]). K_{mult} was then estimated for each data set and statistically evaluated using the permutation described above. The proportion of significant results (out of 1000) was then treated as the Type I error or statistical power of the test, depending upon initial simulation conditions.

Simulations were also performed across a wider set of conditions to evaluate the robustness of the method proposed here. These simulations evaluated the effect of the number of taxa in the phylogeny ($N=16$, 32, 64, 128), as well as the effect of randomly generated phylogenies on statistical performance ($N=$ 16, 32, 64, 128). Results from all simulations are found
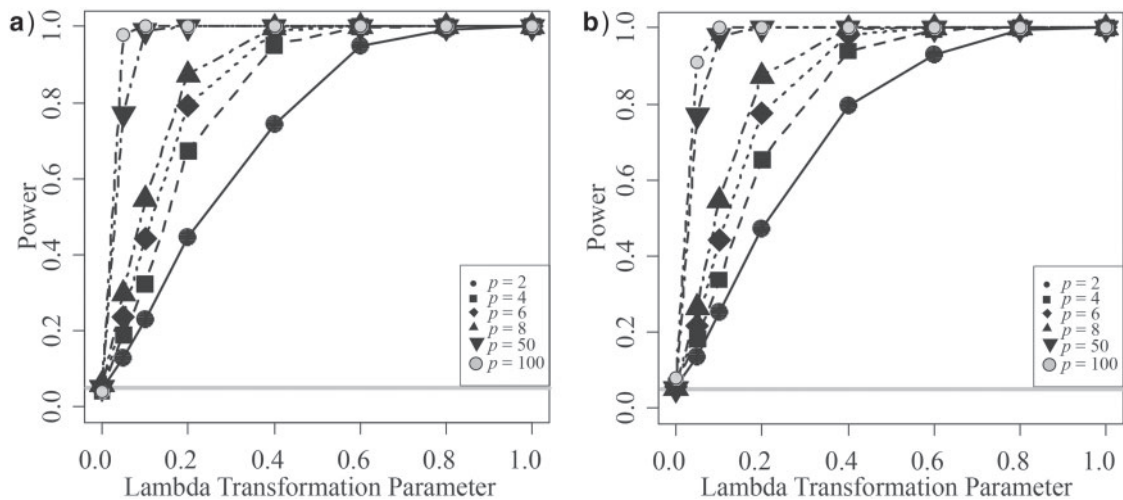
FIGURE 2.   Simulation results evaluating the Type I error and statistical power of hypothesis testing procedures evaluating phylogenetic signal. Data were simulated on a balanced phylogeny containing 32 taxa. a) Statistical power curves for data generated under an isotropic covariance structure. Curves for increasing levels of trait dimensionality are shown. b) Statistical power curves for data generated under a nonisotropic covariance structure. Curves for increasing levels of trait dimensionality are shown.

in the supplementary material available on Dryad (http://dx.doi.org/10.5061/dryad.8fc86).

Results

For all simulations, hypothesis tests of phylogenetic signal displayed appropriate Type I error rates near the nominal value of $\alpha = 0.05$. This pattern remained consistent across the range of trait dimensionality examined in this study, and was consistent when data were generated using an isotropic model (Fig. 2a), a nonisotropic model (Fig. 2b), and a nonisotropic model containing among trait covariance (supplementary material). In addition, the statistical power of tests based on K_{mult} increased rapidly as the degree of phylogenetic signal increased, and this pattern was consistent when data were simulated under both isotropic (Fig. 2a) and nonisotropic conditions (Fig. 2b and supplementary material). Statistical power also increased with increasing trait dimensionality, implying that the ability to detect phylogenetic signal when it was present increased as trait dimensionality increased. For instance, for intermediate levels of input phylogenetic signal on a 32 species phylogeny, statistical power increased from 0.75 when $p = 2$ to 0.95 when $p = 4$ (Fig. 2a). A similar increase in statistical power was observed for tests of phylogenetic signal when several univariate traits were examined simultaneously (Zheng et al. 2009), and when comparing phylogenetic rates of evolution for multidimensional data (Adams 2014). It should also be noted that K_{mult} displayed very high statistical power even in instances when trait dimensionality (p) greatly exceeded the number of species on the phylogeny (N). Similar results were also obtained on phylogenies with different numbers of taxa, and on random phylogenies (supplementary material). Overall these simulations reveal that hypothesis tests based on K_{mult} have appropriate Type I error and

statistical power, and thus provide a useful means of detecting phylogenetic signal in high-dimensional phenotypic traits, regardless of whether those traits display isotropic or nonisotropic covariance structure.

A BIOLOGICAL EXAMPLE

Here, I provide a biological example where I evaluate the strength of phylogenetic signal in a high-dimensional phenotypic trait (head shape) in a lineage of *Plethodon* salamanders. Previous ecological work on *Plethodon* has demonstrated that interspecific competition is widespread in this group (e.g., Jaeger 1971; Hairston 1980; Anthony et al. 1997; Deitloff et al. 2009), and in some instances, interspecific competition has resulted in the evolution of morphological changes in head shape (e.g., Adams 2010). Head shape also displays a strong genetic component (Adams 2011), enabling selection to generate heritable microevolutionary changes. The competitive interactions between species are particularly well studied between members of the monophyletic *Plethodon cinereus* species complex, and this group also displays changes in head shape as a result of these interactions (Adams and Rohlf 2000; Adams et al. 2007; Arif et al. 2007; Adams 2010; Deitloff et al. 2013). Thus, head shape may diversify among species in this lineage and as such it is of interest to determine whether head shape exhibits phylogenetic signal in this group.

To test this, I quantified head shape in all species using geometric morphometric methods (Bookstein 1991; Adams et al. 2013). First, 11 landmarks were digitized from images of the left-lateral side of each head (Fig. 3a) from 478 adult specimens from all species in the *P. cinereus* species complex (data from Adams and Rohlf 2000; Adams et al. 2007; Arif et al. 2007; Myers and Adams 2008; Deitloff et al. 2013). Next, the position of the jaw was standardized relative to the
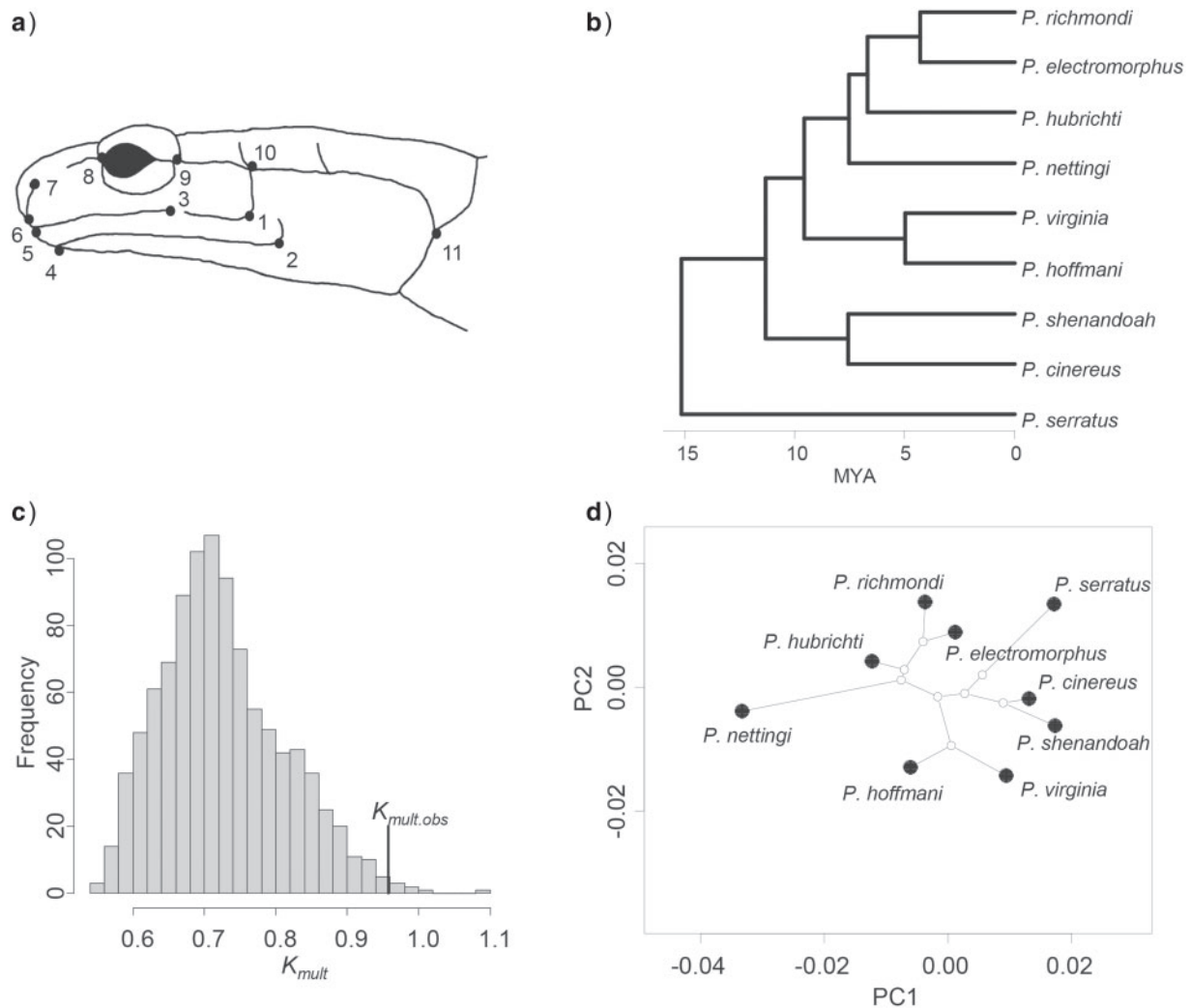
FIGURE 3. a) Positions of 11 anatomical landmarks used to quantify head shape in *Plethodon* salamanders (image from Adams et al. [2007]). b) Fossil-calibrated molecular phylogeny displaying the estimated phylogenetic relationships among species in the *P. cinereus* subclade (from Wiens et al. 2006). c) Histogram of K_{mult} values obtained from 999 permutations of the head shape data on the tips of the phylogeny, with the position of observed value of K_{mult} identified. d) Plot of phylomorphospace viewed as the first two principal component axes of tangent space.

skull by rotating the jaw to a common articulation angle among specimens. Specimens were then aligned using a Generalized Procrustes analysis (Rohlf and Slice 1990), and the set of 22 Procrustes tangent space coordinates treated as shape variables for each specimen. The mean head shape was then calculated for each species. Using these data, the strength of phylogenetic signal in head shape was then evaluated with K_{mult}, calculated on a time-calibrated molecular phylogeny for *Plethodon* containing 9 of the 10 members of this subclade (Wiens et al. 2006; Fig. 3b). Statistical significance of K_{mult} was determined using phylogenetic permutation. In addition, patterns of head shape evolution were visualized in phylomorphospace (Sidlauskas 2008), where the extant taxa and the phylogeny were projected into the morphological trait space, and visualized along the first two axes of this space using principal components analysis. All analyses were performed in R 3.0.1 (R Development Core Team 2013) using routines in

the library geomorph 2.0 (Adams and Otárola-Castillo 2013; Adams 2014), and new routines written by the author (Appendix 2).

Results

Analyses indicated that head shape in *Plethodon* exhibited significant phylogenetic signal ($K_{mult} = 0.957$; $P_{rand} = 0.014$), indicating that closely related species were more similar to one another in head shape than was expected under a Brownian motion model of evolution (Fig. 3c). Thus, for the species examined here, there is a significant degree of phylogenetic structure in patterns of head shape variation among taxa. High levels of phylogenetic signal such as those displayed here are often attributed to processes related to ecological or evolutionary conservatism (e.g., Swenson et al. 2007; Losos 2008). However, the link between levels of phylogenetic signal and evolutionary processes is far

from straightforward, as more than one evolutionary process can produce a particular pattern of phylogenetic signal (see Blomberg et al. 2003; Revell et al. 2008; Ackerly 2009). Nevertheless, the trend of phylogenetic signal in *Plethodon* head shape was quite evident when head shape evolution was viewed in phylomorphospace (Fig. 3d). Here, sister species tended to cluster in similar regions of shape space, and less closely related taxa were more morphologically divergent. Further, head shape variation appeared to diversify and emanate from a central point in shape space, with extant taxa occupying more distant regions when compared with their hypothesized ancestors. Additionally, the lack of overlapping branches of the phylogeny implied that there was little evidence of convergent evolution in head shape in this group. As such it can be inferred that species diversification in the *P. cinereus* species complex is accompanied by similar trends in diversification in head shape among species.

Discussion

A common observation in macroevolution is that closely related species tend to display similar trait values, a pattern called phylogenetic signal. Although quantifying phylogenetic signal in univariate traits is relatively straightforward, methods for high-dimensional multivariate data have remained under-developed. In this article, I described a generalization of the K statistic (Blomberg et al. 2003) that may be used to quantify phylogenetic signal in high-dimensional multivariate traits. The method (K_{mult}) extends existing approaches by quantifying phylogenetic signal for multivariate data such that the observed pattern of phylogenetic signal in high-dimensional traits may be evaluated relative to what is expected under a Brownian motion model of evolution. Using simulations, I demonstrated that K_{mult} exhibits an expected value of 1.0 for traits evolving by Brownian motion. I also showed that tests based on K_{mult} have appropriate Type I error rates and high statistical power, for data simulated under both isotropic and nonisotropic conditions. Further, the approach is capable of detecting phylogenetic signal in multidimensional traits whose dimensionality exceeds the number of species examined. For instance, in the biological example presented here only nine species of *Plethodon* salamanders were examined, yet significant phylogenetic signal was detected in a complex trait (head shape) described by 22-dimensional data. Thus, K_{mult} provides a useful means of evaluating phylogenetic signal in high-dimensional traits like shape, even when the number of trait dimensions greatly exceeds the number of species in the data set.

One important implication of this work is that K_{mult} is the only multivariate measure currently available for evaluating phylogenetic signal that displays the full range of statistical properties that allow both a consistent diagnosis of the presence of phylogenetic signal, and enables evaluations of the strength of phylogenetic signal on a comparative scale relative to expectations under Brownian motion. By contrast, all other approaches have limitations in one or more of these arenas. For instance, both Mantel tests and Pagel's λ describe phylogenetic signal on consistent scales (-1 to 1 and 0 to 1, respectively). So at least in theory, estimates of phylogenetic signal for different data sets may be compared using these measures. However, Mantel tests have low statistical power when used to evaluate phylogenetic signal (Harmon and Glor 2010), which limits their utility. Likewise, Pagel's λ can only be used for multivariate data where the number of species is greater than the number of trait dimensions. For the latter approach this is a serious problem, as high-dimensional multivariate data sets frequently contain more trait dimensions than the number of species in a phylogeny (e.g., McPeek et al. 2008; Klingenberg and Gidaszewski 2010). Alternatively, the SSC measure may be used to evaluate the significance of phylogenetic signal in a multivariate data set. However, values of SSC change in proportion with both the number of trait dimensions and the amount of variation between species. This lack of a consistent expected value means that biological interpretations are challenged, as it is not known whether lower values of SSC correspond to greater phylogenetic signal or simply less phenotypic variation among species. A further implication of this result is that estimates of SSC cannot be compared among phenotypic data sets, as there is no relative scale on which those comparisons can be made.

In contrast to these approaches, values of K_{mult} are represented on a common scale, with an expected value of 1.0 under Brownian motion that remains consistent across levels of trait variation and across numbers of trait dimensions. Thus, reliable comparisons of the strength of phylogenetic signal may be made across data sets when using K_{mult} as a measure of this signal (for an example with univariate data see Blomberg et al. [2003]). Further, because tests based on K_{mult} have appropriate Type I error and high statistical power across a range of trait dimensionality, K_{mult} does not suffer the shortcomings of some of the other approaches mentioned above. When these observations are taken together, only K_{mult} provides a powerful and comparable means of evaluating phylogenetic signal in high-dimensional multivariate data.

It is also important to recognize that the formulation used to derive K_{mult} was obtained by leveraging the distance to covariance equivalency common to many multivariate statistical methods. Beyond enabling a multivariate measure for estimating phylogenetic signal, this advance provides a mathematical merger of two quantitative approaches that are typically not combined: phylogenetic comparative measures of trait evolution (which are frequently described using covariance-based methods), and assessing patterns of phenotypic variation in high-dimensional trait spaces (which are often described in terms of among-specimen distances). Indeed, the insight that distance-based

approaches can be used to estimate phylogenetic signal provides a template for generalizing other phylogenetic comparative approaches for their application to high-dimensional data sets. Thus, current phylogenetic approaches that evaluate macroevolutionary trends using patterns of variation and covariation could be re-expressed using their distance-based counterparts (e.g., Adams 2014). Such analytical advances will be critical to future studies in macroevolution which are increasingly characterizing phenotypes using high-dimensional data. With such a framework, it will thus be possible to evaluate trends in the tempo and mode of evolution in all types of phenotypic traits measured on a continuous scale (univariate, multivariate, and highly dimensional traits).

SUPPLEMENTARY MATERIAL

FUNDING

ACKNOWLEDGMENTS

APPENDIX 1

WORKED EXAMPLE DEMONSTRATING THE EQUIVALENCY OF K AND K_{mult} FOR UNIVARIATE DATA

In this example, five hypothetical species are related by the following phylogeny and have the following phenotypic values ($\mathbf{Y}$):
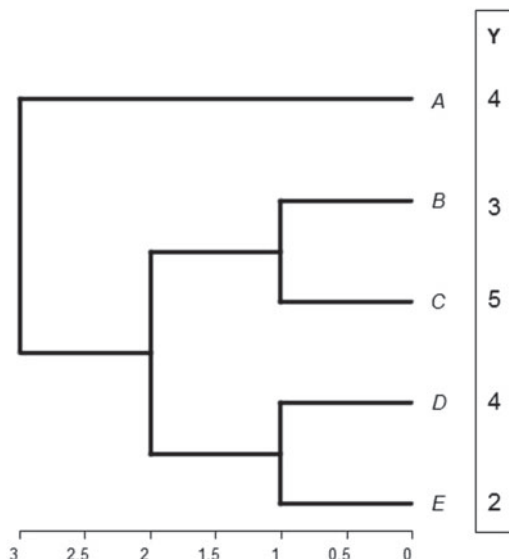
The phylogenetic covariance matrix (C) representing these evolutionary relationships under the hypothesis of Brownian motion is:

$$\mathbf{C} = \begin{array}{c} \\ A \\ B \\ C \\ D \\ E \end{array} \overset{\displaystyle A\ B\ C\ D\ E}{\begin{bmatrix} 3 & 0 & 0 & 0 & 0 \\ 0 & 3 & 2 & 1 & 1 \\ 0 & 2 & 3 & 1 & 1 \\ 0 & 1 & 1 & 3 & 2 \\ 0 & 1 & 1 & 2 & 3 \end{bmatrix}}.$$

Under this model of evolution, the expected value at the root of the phylogeny (or phylogenetic mean) is estimated as: $E(\mathbf{Y}) = \widehat{a} = (\mathbf{1}^t \mathbf{C}^{-1} \mathbf{1})^{-1} (\mathbf{1}^t \mathbf{C}^{-1} \mathbf{Y})$, where $\mathbf{1}$ is a vector of ones, and $\mathbf{C}$ and $\mathbf{Y}$ are as defined above (Rohlf 2001; Revell and Harmon 2008). In this case, $\widehat{a} = 3.684211$. An estimate of phylogenetic signal may then be found using the standard variance-based equation:

$$K = \frac{(\mathbf{Y} - E(\mathbf{Y}))^{\mathbf{t}} (\mathbf{Y} - E(\mathbf{Y}))}{(\mathbf{Y} - E(\mathbf{Y}))^{\mathbf{t}} \mathbf{C}^{-1} (\mathbf{Y} - E(\mathbf{Y}))} \bigg/ \frac{\operatorname{tr}(\mathbf{C}) - N(\mathbf{1}^{\mathbf{t}} \mathbf{C}^{-1} \mathbf{1})^{-1}}{N - 1},$$

which for this example, is: $K = 0.504$.

To obtain the distance-based estimate (K_{mult}), the ratio of observed trait variation relative to the phylogenetic mean is re-expressed in terms of distances. First, the numerator portion is found from the Euclidean distances between species means and the root of the phylogeny: $\mathbf{D}_{i,\widehat{a}} = \sqrt{(\mathbf{Y}_i - E(\mathbf{Y}))(\mathbf{Y}_i - E(\mathbf{Y}))^t}$. These are then concatenated into a $N \times 1$ column vector ($\mathbf{D}_{Y,\widehat{a}}$). For this example, this vector is:

$$\mathbf{D}_{Y,\widehat{a}} = \begin{bmatrix} 0.3157895 \\ 0.6842105 \\ 1.3157895 \\ 0.3157895 \\ 1.6842105 \end{bmatrix}.$$

The observed variation among species relative to the phylogenetic mean is then found as:

$$\mathrm{MSE}_{\mathrm{obs}} = \mathbf{D}_{Y,\widehat{a}}^t \mathbf{D}_{Y,\widehat{a}} = 5.235457.$$

Next, the observed variation while accounting for phylogenetic covariance is estimated. This is calculated by first performing an eigen-decomposition on the phylogenetic covariance matrix: $\mathbf{C} = \mathbf{U}\mathbf{W}\mathbf{U}^{-1}$, and using the eigenvalues and eigenvectors to obtain the phylogenetic transformation matrix: $\mathbf{E} = (\mathbf{U}\mathbf{W}^{1/2}\mathbf{U}^{\mathbf{t}})^{-1}$. For this example, the eigenvalues, eigenvectors, and $\mathbf{E}$ are:

$$\mathbf{U} = \begin{bmatrix} 0.0 & 0.0 & 1 & 0.0000 & 0.0000 \\ -0.5 & 0.5 & 0 & 0.0000 & -0.7071 \\ -0.5 & 0.5 & 0 & 0.0000 & 0.7071 \\ -0.5 & -0.5 & 0 & -0.7071 & 0.0000 \\ -0.5 & -0.5 & 0 & -0.7071 & 0.0000 \end{bmatrix} \quad \mathbf{W} = \begin{bmatrix} 7 & 0 & 0 & 0 & 0 \\ 0 & 3 & 0 & 0 & 0 \\ 0 & 0 & 3 & 0 & 0 \\ 0 & 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 0 & 1 \end{bmatrix}$$

$$\mathbf{E} = \begin{bmatrix} 0.5773 & 0.0000 & 0.0000 & 0.0000 & 0.0000 \\ 0.0000 & 0.7388 & -0.2611 & -0.0498 & -0.0498 \\ 0.0000 & -0.2611 & 0.7388 & -0.0498 & -0.0498 \\ 0.0000 & -0.0498 & -0.0498 & 0.7388 & -0.2611 \\ 0.0000 & -0.0498 & -0.0498 & -0.2611 & 0.7388 \end{bmatrix}$$

With the matrix $\mathbf{E}$, the phenotypic data $\mathbf{Y}$ are transformed by the phylogeny as:

$$\mathbf{U_Y} = \mathbf{E}\big(\mathbf{Y} - E(\mathbf{Y})\big).$$

For this example, this yields the following vector:

$$\mathbf{U_Y} = \begin{bmatrix} 0.1823211 \\ -0.7809499 \\ 1.2190501 \\ 0.6416998 \\ -1.3583002 \end{bmatrix}.$$

The distances between $\mathbf{U}_Y$ and the origin are then obtained, which for this example are:

$$\mathbf{PD}_{U,0} = \begin{bmatrix} 0.1823211 \\ 0.7809499 \\ 1.2190501 \\ 0.6416998 \\ 1.3583002 \end{bmatrix}.$$

From this, the variation while accounting for phylogenetic covariance is found as:

$$\mathrm{MSE} = \mathbf{PD}_{U,0}^t \mathbf{PD}_{U,0} = 4.385965.$$

The expected variation given the phylogeny is then obtained from:

$$\frac{\mathrm{tr}(\mathbf{C}) - N(\mathbf{1^t C^{-1} 1})^{-1}}{N-1} = 2.368421.$$

Finally, K_{mult} is estimated from $\mathrm{MSE}_{\mathrm{obs}}$, MSE, and the denominator of K:

$$K_{\mathrm{mult}} = \frac{\mathbf{D}_{Y,\widehat{a}}^t \mathbf{D}_{Y,\widehat{a}}}{\mathbf{PD}_{U,0}^t \mathbf{PD}_{U,0}} \Big/ \frac{\mathrm{tr}(\mathbf{C}) - N(\mathbf{1^t C^{-1} 1})^{-1}}{N-1}$$

For this example, $K_{\mathrm{mult}} = 0.504$, which is identical to the covariance-based estimate of K as found above.

APPENDIX 2

COMPUTER CODE FOR R

The function below estimates phylogenetic signal (K_{mult}) for multidimensional traits and under a Brownian motion model of evolution. The observed value is statistically assessed using permutation, where data at the tips of the phylogeny are permuted, and an estimate of phylogenetic signal for the permuted data is obtained, and compared with the observed value.

```
Test.Kmult<-function(x,phy,iter=999){
  library(ape)
  Kmult<-function(x,phy){
    x<-as.matrix(x)
    N<-length(phy$tip.label)
    ones<-array(1,N)
    C<-vcv.phylo(phy)
    C<-C[row.names(x),row.names(x)]
    a.obs<-colSums(solve(C))%*%x/sum(solve(C))
      #evol.vcv code
    distmat<-as.matrix(dist(rbind(as.matrix(x),a.obs)))
    MSEobs.d<-sum(distmat[(1:N),(N+1)]^2)
      #sum distances root vs. tips
    eigC <- eigen(C)
    D.mat<-solve(eigC$vectors
      %*% diag(sqrt(eigC$values))
      %*% t(eigC$vectors))
    dist.adj<-as.matrix(dist(rbind((D.mat
      %*%(x-(ones%*%a.obs))),0)))
    MSE.d<-sum(dist.adj[(1:N),(N+1)]^2)
      #sum distances for transformed data)
    K.denom<-(sum(diag(C))-
N*solve(t(ones)%*%solve(C)%*%ones)) / (N-1)
    K.stat<-(MSEobs.d/MSE.d)/K.denom
    return(K.stat)
  }
  K.obs<-Kmult(x,phy)
  P.val <- 1
  K.val <- rep(0, iter)
  for (i in 1:iter){
    x.r<-as.matrix(x[sample(nrow(x)),])
    rownames(x.r)<-rownames(x)
    K.rand<-Kmult(x.r,phy)
    P.val<-ifelse(K.rand>=K.obs, P.val+1,P.val)
    K.val[i] <- K.rand
  }
  P.val <- P.val/(iter + 1)
  K.val[iter + 1] = K.obs
  hist(K.val, 30, freq = TRUE, col = "gray",
    xlab = "Phylogenetic Signal")
  arrows(K.obs, 50, K.obs, 5, length = 0.1, lwd = 2)
  return(list(phy.signal = K.obs, pvalue = P.val))
}
```

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