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RESEARCH ARTICLE

Measuring Morphological Integration Using Eigenvalue Variance

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Abstract The concept of morphological integration describes the pattern and the amount of correlation between morphological traits. Integration is relevant in evolutionary biology as it imposes constraint on the variation that is exposed to selection, and is at the same time often based on heritable genetic correlations. Several measures have been proposed to assess the amount of integration, many using the distribution of eigenvalues of the correlation matrix. In this paper, we analyze the properties of eigenvalue variance as a much applied measure. We show that eigenvalue variance scales linearly with the square of the mean correlation and propose the standard deviation of the eigenvalues as a suitable alternative that scales linearly with the correlation. We furthermore develop a relative measure that is independent of the number of traits and can thus be readily compared across datasets. We apply this measure to examples of phenotypic correlation matrices and compare our measure to several other methods. The relative standard deviation of the eigenvalues gives similar results as the mean absolute correlation (W.P. Cane, Evol Int J Org Evol 47:844–854, 1993) but is only identical to this measure if the correlation matrix is homogenous. For heterogeneous correlation matrices the mean absolute correlation is consistently smaller than the relative standard deviation of eigenvalues and may thus underestimate integration. Unequal allocation of variance due to variation among correlation coefficients is captured by the relative standard deviation of eigenvalues. We thus suggest that this measure is a better reflection of the overall morphological integration than the average correlation.

Keywords Morphological integration · Evolutionary constraint · Phenotypic correlation · Eigenvalue distribution

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Introduction

> I mean by [the correlation of growth] that the whole organization is so tied together during its growth and development, that when slight variations in any one part occur, and are accumulated through natural selection, other parts become modified.
>
> *Charles Darwin, On the Origin of Species, 1859*

The seminal work of Olson and Miller (1958) on the concept of morphological integration brought the evolution of correlated characters (Darwin 1895: correlation of growth; Chetverikov 1929 in: Chetverikov 1961: correlative variability; Terentjev 1931, Berg 1960: correlation pleiades) to the attention of evolutionary biologists. Olson and Miller (1958) proposed that traits that develop or function in concert tend to be phenotypically correlated and will evolve together. Later, quantitative genetic models (Lande 1980; Lande and Arnold 1983; Cheverud 1982, 1984; Jones et al. 2007; Revell 2007) placed these ideas on solid theoretical ground by showing that if functionally and developmentally integrated phenotypic traits experience correlative stabilizing

selection they will become genetically correlated. These genetic correlations then will result in correlated responses to selection for functionally and developmentally related traits (Pearson 1903; Lande and Arnold 1983). The features of an organ or organism do not represent stochastically independent dimensions but are correlated with one another. Intertrait correlations reduce the amount of independent variation in their respective dimensions. The new methods to characterize the multivariate phenotypic structure are still being developed, reflecting the interest in this field and the need for tools (e.g., Magwene 2008).

Our focus here is on overall level of inter-correlation among traits, defined by Olson and Miller (1958) as the magnitude of morphological integration. For the purpose of this note, we name the overall correlation of traits correlatedness, to distinguish it from pair wise correlation. There is often discussion in evolutionary studies as to whether morphological or other forms of integration should be considered in terms of covariance or correlation matrices. While the two matrices are related by a simple matrix operation ($R = W^{-1}CW^{-1}$ where W is a diagonal matrix of the inverses of trait standard deviations) and a correlation is a variance-standardized covariance, it is the genetic covariance matrix that appears in the multivariate response to selection equation (Lande 1979). Therefore the covariance matrix is often considered more relevant for evolutionary studies (Hansen and Houle 2008; Kirkpatrick 2008). However, a covariance matrix combines two separate, fundamental aspects of quantitative variation; the level and pattern of variation, as described by a vector of trait variances or coefficients of variation, and the strength and pattern of relationship between traits. Olson and Miller (1958) were specifically concerned with this second aspect, i.e., with the pattern of intertrait relationships. With a focus on intertrait relationships, it is most appropriate to analyze correlation, rather than covariance, matrices in studies of morphological integration. Relative levels of variation can and have been considered separately from strength of intertrait relationships (Cheverud 1996).

We thus focus on the traditional measure of integration, namely the correlation and specifically explore the relationship between the attribute measured and the measurement scale, i.e., the representational aspect of the measurement. Integration of a complex phenotypic unit will be assessed by the measurement of the correlatedness of its traits. One measure of the correlatedness is the dispersion of the eigenvalues of the correlation matrix among phenotypic dimensions (Wagner 1984; Cheverud et al. 1989). Each eigenvalue is equal to the amount of variance distributed along its corresponding eigenvector. Morphological integration leads to the concentration of variation in a few dimensions and thus leads to increased differences among eigenvalues. The more different the eigenvalues, the more biased the variation and thus the higher the integration. For example, when traits are highly correlated, most of the independent variance is concentrated in the first few eigenvalues which results in a high eigenvalue variance. Contrastingly, when the traits are uncorrelated, the eigenvalues will tend to be similar and thus have low variance. Eigenvalue variance has thus been proposed to assess the shape of the eigenvalue distribution (Wagner 1984; Cheverud et al. 1989). High eigenvalue variance is characteristic of the highly integrated phenotypic units, whereas low eigenvalue variance is typical of phenotypes with low integration. Because the eigenvalue variance also depends on the number of eigenvalues, it needs to be corrected for the number of traits (i.e., number of eigenvalues) included in the matrix. This enables comparison across matrices of different size.

Eigenvalue variance is frequently applied to measure phenotypic integration in wide range of fields (e.g., Herrera et al. 2002; Peres-Neto and Magnan 2004; Hallgrimsson et al. 2004; Young and Hallgrimsson 2005; Hallgrimsson et al. 2006; Parsons and Robinson 2006; Young 2006), and also indirectly to assess the effective number of independent variables in a correlation matrix on a continuous scale (Wagner et al. 2008). The latter purpose was originally developed to determine the number of independent tests to calculate the significance threshold in QTL mapping based on the Bonferroni adjustment (Cheverud 2001; Nyholt 2004).

In the present contribution, we provide a simple derivation showing how the dispersion of eigenvalues relates to overall correlatedness represented in a correlation matrix. Eigenvalue variance scales linearly with the square of mean correlation while the standard deviation of the eigenvalues scales with average level of correlation.

Eigenvalue Variance Depends on Number of Traits

Eigenvalue variance is the average squared deviation of the eigenvalues from the mean eigenvalue. The sum of the eigenvalues of a correlation matrix equals the number of variables (N) because the trace of a matrix (i.e., the sum of the diagonal elements) is invariant under rotation of the coordinate system. Thus the sum of the eigenvalues is equal to the trace of the correlation matrix, which is of course equal to the number of rows and columns. For that reason the mean eigenvalue of a correlation matrix always equals 1.0. The eigenvalue variance is

$$\mathrm{Var}(\lambda) = \frac{\sum_{i=1}^{N} (\lambda_i - 1)^2}{N}$$

Note that we use here the number of traits, N, not $N - 1$ for normalization, because this is a definition and not a statistical estimate of a population variance. The range of

eigenvalue variance is from zero (all eigenvalues are equal), to a rank-specific maximal eigenvalue variance. The maximal eigenvalue variance is reached when only one eigenvalue is larger than zero. In this case the first eigenvalue is equal to N, and all the others equal to zero, because of the constraint $\sum_{i=1}^{N} \lambda_i = N$. This is the case if all variables are fully correlated with each other. The maximal eigenvalue variance thus is:

$$\mathrm{Var}_{\max}(\lambda) = \frac{(N-1)^2 + \sum_{i=2}^{N} (0-1)^2}{N} = \frac{N(N-1)}{N} = N - 1$$

The range of eigenvalue variance is therefore zero to $(N - 1)$.

To account for the dependency on the size of the matrix when eigenvalue variance is compared among matrices, we can define the *relative eigenvalue variance* by dividing the observed eigenvalue variance by the maximum eigenvalue variance for the particular number of traits:

$$\mathrm{Var}_{\mathrm{rel}}(\lambda) = \frac{\mathrm{Var}(\lambda)}{\mathrm{Var}_{\max}(\lambda)} = \frac{\mathrm{Var}(\lambda)}{N-1}$$

The relative eigenvalue variance is independent of the number of traits and can thus be used to compare morphological integration across different matrices. The range of this measure is from zero to one.

The relationship between correlatedness and eigenvalue variance will in the following be explored both in homogeneous and heterogeneous matrices. Whereas for the homogeneous matrices, where all off-diagonal elements are equal, an analytical path is straightforward, we utilize both empirical and analytical approaches for the heterogeneous matrices.

Homogeneous Correlation Matrices

Here we consider homogeneous matrices, characterized by all off-diagonal elements being equal (no variance among correlations; see also Morrison 1976, pp. 289). The eigenvalues of this set of matrices can easily be described algebraically. Consider the (uniform) correlation to be r and consider building the matrix by adding one trait at a time. The trivial matrix with a single trait has eigenvalue 1. Adding a trait leads to the simple 2×2 matrix,

$$\mathbf{R}_{N=2} = \begin{bmatrix} 1 & r \\ r & 1 \end{bmatrix}$$

whose eigenvalues can simply be calculated by solving the eigenvalue equation

$$\det(\mathbf{R}_{N=2} - \lambda \mathbf{I}) = 0$$
$$(1-\lambda)^2 - r^2 = 0$$

For easier handling, we substitute x for $(1 - \lambda)$ and solve by factorization $(x - r)(x + r) = 0$, which has the solutions $x_1 = (1 - \lambda_1) = r$ and $x_2 = (1 - \lambda_1) = (-r)$ and hence $\lambda_{1,2} = 1 \pm r$.

For homogenous matrices of size $N = 3$ the eigenvalue equation becomes

$$\det(\mathbf{R}_{\mathbf{N=3}} - \lambda \mathbf{I}) = (1-\lambda)\det(\mathbf{R}_{N=2} - \lambda \mathbf{I}) - r\det\begin{bmatrix} r & r \\ r & 1-\lambda \end{bmatrix} + r\det\begin{bmatrix} r & 1-\lambda \\ r & r \end{bmatrix}$$

$$\det(\mathbf{R}_{\mathbf{N=3}} - \lambda \mathbf{I}) = (1-\lambda)^3 - 3r^2(1-\lambda) + 2r^3$$

Again, we substitute x for $(1 - \lambda)$ and obtain the equation $x^3 - 3r^2x + 2r^3 = 0$ and solve with solutions $x_1 = (1 - \lambda_1) = (-2r)$ and $x_{2,3} = (1 - \lambda_1) = r$ and hence $\lambda_1 = 1 + 2r$, $\lambda_{2,3} = 1 - r$.

The first eigenvalue seems to follow the rule $1 + (N - 1)r$, where N is the number of traits, and the remaining eigenvalues are $(1 - r)$. It is easy to show that these are in fact solutions for homogenous correlation matrices of any size N (see Appendix 1). The number of eigenvalues will increase by one to match the number of traits, the eigenvalues following the first eigenvalue are all equal to $(1 - r)$. Thus the sum of the eigenvalues remains equal to the number of traits $\sum \lambda_i = (1 + (N-1)r) + (N-1)(1-r) = N$.

The eigenvalue variance can be obtained by substituting the $(1 + (N–1)r)$ for λ_1, and $(1 - r)$ for all remaining eigenvalues:

$$\mathrm{Var}(\lambda) = r^2(N-1)$$

and

$$\mathrm{Var}_{\mathrm{rel}}(\lambda) = \frac{r^2(N-1)}{N-1} = r^2$$

For homogenous correlation matrices, the relative eigenvalue variance is equal to the squared correlation coefficient.

These considerations suggest an alternative to the eigenvalue variance as a measure of morphological integration, namely the relative standard deviation of eigenvalues, which then is equal to the correlation for homogenous correlation matrices:

$$\mathrm{SD}_{\mathrm{re1}}(\lambda) = \frac{\sqrt{\mathrm{Var}(\lambda)}}{\sqrt{N-1}} = r$$

Note that the above equation is valid for the matrices involving either only positive, only negative, as well as mixed-sign correlations. While the absolute eigenvalues

of the matrices change, their variance and standard deviation is independent of the sign of correlations. As the eigenvalue variance scales with r^2, the sign of the correlations makes no difference.

Generating Random Correlation Matrices

To build the heterogeneous correlation matrices, the off-diagonal elements of the matrix were randomly drawn from the normal distribution with various variance levels (see Fig. 1). The correlations were constrained to the values between -1 and $+1$. When the variance of correlation values in the matrix is equal to zero this yields homogeneous matrices with each correlation being the same, while increasing the variance of the distribution from which the pair wise correlations are drawn increases the heterogeneity of the correlations within matrix. If necessary, the matrices were subsequently bent to assure they were nonnegative (Jorjani et al. 2003). Singular matrices are commonly obtained in quantitative genetic analyses (Searle 1961; Cheverud 1988). Matrix bending shrinks the eigenvalues around the mean eigenvalue until the smallest eigenvalue is greater than or equal to 0. New matrices were produced in such cases by multiplication of the shrunken eigenvalues with the original eigenvectors. These matrices were then studied instead of the original ones, if the originally generated matrices were not non-negative definite. For all generated matrices, the relative variance and the relative standard deviation of eigenvalues were calculated. We also calculated the mean absolute correlation. The mean correlation substitutes for the uniform correlation coefficient r in the above homogeneous matrices.

Heterogeneous Correlation Matrices

To explore the relationship between the correlatedness and the eigenvalue variance in heterogeneous matrices empirically, we generated 1000 random matrices, with sizes of 4–30 traits, and randomly drawn pair wise correlations. We recorded the mean absolute pair wise correlation. As described above, the eigenvalue variance and eigenvalue standard deviation is independent of the sign of correlation. On the contrary, when calculating the mean correlation in matrices with positive and negative pair wise correlations, these would partially cancel out instead of giving a measure of correlatedness. For this reason, the absolute correlation values are used (Cane 1993). In Fig. 1, we plot the two measures of eigenvalue distribution against the mean absolute correlation in the matrix. Plots A and B show this relationship for two levels of matrix heterogeneity, as measured by the coefficient of variation. Plot A shows cases with CV < 0.6 and plot B with CV > 0.6. The plots demonstrate that the general relationship between the mean correlation of the correlation matrix and the eigenvalue variance, or eigenvalue standard deviation is maintained in heterogeneous correlation matrices. However, the observed values lay consistently above the lines for the corresponding homogeneous matrices with r equal to the mean absolute correlation. Hence the two measures, r^2 (or r) and relative eigenvalue variance (or relative standard deviation) differ in heterogeneous matrices.

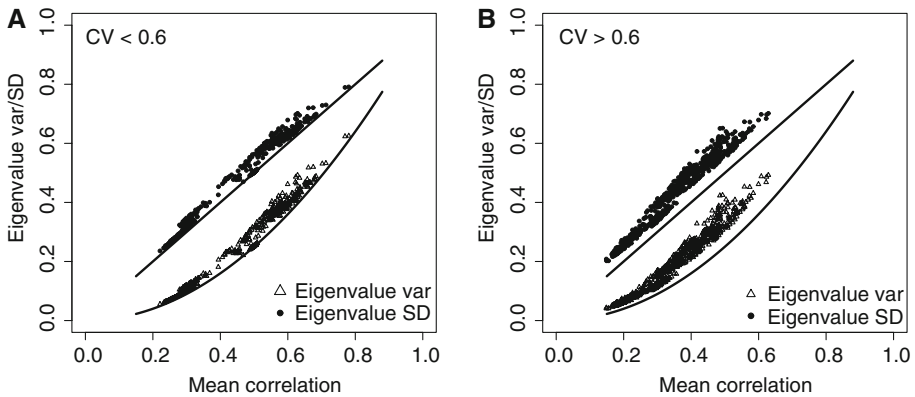

Fig. 1 The relationship between relative (i.e., matrix size invariant) eigenvalue variance, respectively relative standard deviation of eigenvalues, and the mean correlation in heterogeneous matrices; for different degrees of matrix heterogeneity: CV < 0.6 (**a**) and CV > 0.6 (**b**). Note that the general squared and linear tendencies are maintained, however the results (i.e., empirical rel SD (λ) and rel Var(λ)) are consistently above the lines predicted for the homogeneous matrices in which r corresponds to $\bar{r}$ of the heterogeneous matrices. *Straight line* predicts the relative eigenvalue standard deviation, while quadratic line predicts the relative eigenvalue variance of the corresponding homogeneous matrix. Absolute value of correlations was used to calculate $\bar{r}$. Note that due to the absolute correlation being limited to 1.0, the high mean absolute correlation necessarily means that the matrices will be less heterogeneous

To explore the effect of heterogeneity of correlation on eigenvalue variance analytically, we consider a 3×3 matrix with the following structure:

$$\mathbf{R}_{N=3} = \begin{bmatrix} 1 & r-\varepsilon & r \\ r-\varepsilon & 1 & r+\varepsilon \\ r & r+\varepsilon & 1 \end{bmatrix}$$

Note that the mean correlation of this matrix is r, while the standard deviation is $\frac{2}{3}\varepsilon$.

The eigenvalue equation of this matrix is

$$\begin{aligned}\det(\mathbf{R}_{N=3} - \lambda\mathbf{I}) = (1-\lambda)\left[(1-\lambda)^2 - (r+\varepsilon)^2\right] \\ - (r-\varepsilon)[(r-\varepsilon)(1-\lambda) - r(r+\varepsilon)] \\ + r[(r-\varepsilon)(r+\varepsilon) - r(1-\lambda)]\end{aligned}$$

after substituting x for $(1-\lambda)$ and multiplying out

$$\det(\mathbf{R}_{N=3} - \lambda\mathbf{I}) = x^3 - x(3r^2 + 2\varepsilon^2) + 2r^3 - 2r\varepsilon^2$$

We do not present a formal solution of this polynomial as it is rather complex. The numerical examples of this equation are plotted in Fig. 2 (mean correlations $r = 0.3$ and 0.7, and deviations $\varepsilon = 0.05$, 0.10, 0.15, 0.20, 0.25, and 0.30 for each of the mean r values), and reveal that the three solutions can be approximated. Compared to the solutions for homogeneous matrices the first solution ($x_1 = -2r$) varies only marginally with increasing ε, and the remaining two solutions vary equidistantly around r, with a deviation ($\pm\delta$) from r increasing with increasing ε.

We therefore approximate the three solutions with $x_1 \approx -2r$, $x_2 \approx r - \delta_\varepsilon$, $x_3 \approx r + \delta_\varepsilon$

The variance of eigenvalues is then:

$$\mathrm{Var}(\lambda) \approx \frac{4r^2 + (r+\delta)^2 + (r-\delta)^2}{3} = 2r^2 + \frac{2}{3}\delta^2$$

Or, in general

$$\lambda_1 \approx 1 + (N-1)r$$
$$\lambda_{i>1} \approx 1 - (r + \delta_i)$$

Note that in the above model, correlations are symmetrically distributed. Then, $\sum_{i=1}^{N} \delta_i = 0$ and $\frac{1}{N}\sum_{i=1}^{N} \delta_i^2 = \sigma^2 > 0$. This model will respect the constraint that $\sum_{j=1}^{N} \lambda_j = N$. It is then easy to see that

$$\mathrm{Var}(\lambda|\varepsilon \neq 0) > \mathrm{Var}(\lambda|\varepsilon = 0) \text{ because}$$
$$\mathrm{Var}(\lambda|\varepsilon \neq 0) \approx (N-1)r^2 + \sigma^2$$
$$\mathrm{Var}(\lambda|\varepsilon \neq 0) \approx \mathrm{Var}(\lambda|\varepsilon = 0) + \sigma^2$$

The eigenvalue variance in random heterogeneous matrix is therefore strictly greater than the eigenvalue variance in the corresponding homogeneous matrix with r corresponding to the mean correlation of heterogeneous matrix. Heterogeneity of correlation therefore increases eigenvalue variance as well as eigenvalue standard deviation of the correlation matrix. This explains the pattern we see in the Fig. 1, where the scatter invariably lays above the line of SD(λ) and Var(λ) for the corresponding homogeneous matrices.

The formulation above decomposes the eigenvalue variance into the effects of mean correlation and the dispersion around mean in heterogeneous correlation matrix. The consequence is that equivalent eigenvalue variance can be achieved by different combinations of both

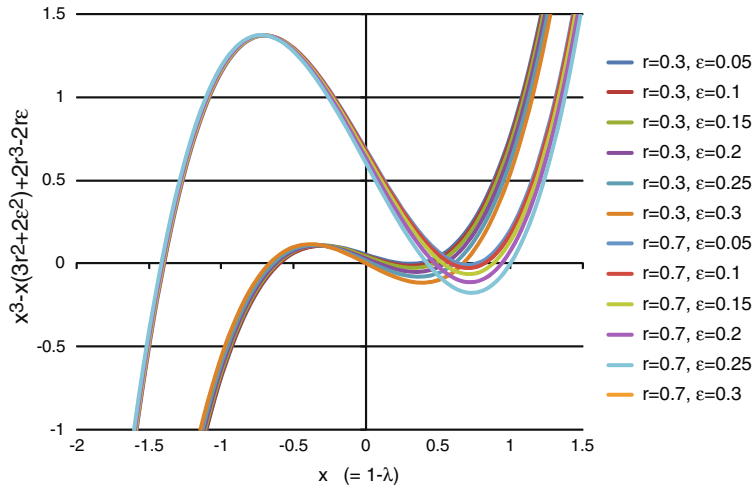

Fig. 2 The plot of the polynomial with solutions equal to the three $(1-\lambda)$ of the heterogeneous correlation matrix with $N = 3$. The three solutions of the polynomial are the intersections of the curve with x-axis. The two groups of curves show the cases with mean correlations of $r = 0.3$ and 0.7. The within-group variances are due to deviations from the mean correlation ($\varepsilon = 0.05$, 0.10, 0.15, 0.20, 0.25, and 0.30). Note that the solution to the left of 0 is almost invariant with the varying ε, and hence dependent solely on r, whereas the two solutions >0 vary dependent on ε. We use this fact to approximate the eigenvalues of heterogeneous matrices (see text)

contributions. For example, the same integration level can be reached by a relatively low mean correlation with strong dispersion, or by a high mean correlation with less dispersion. A simple consideration is sufficient to see when this is the case for two matrices, using the above results, divided by $(N-1)$ to calculate relative eigenvalue variance:

$$r_1^2 + \frac{\sigma_1^2}{N_1 - 1} = r_2^2 + \frac{\sigma_2^2}{N_2 - 1}$$

Thus two matrices have the same relative eigenvalue variance score if the above equation is satisfied. However note also that the model assumes symmetrically distributed correlations. Correlation coefficient r is restricted to $\leq \max|r| = 1$. It follows that the maximal symmetrical deviations possible for a particular $\bar{r}$ diminish as the absolute correlation increases. Maximal correlation variance corresponding to each $\bar{r}$ at symmetrical distribution can be easily derived (Appendix 2) and amounts to $\sigma^2_{\max} = (1 - |\bar{r}|)^2$. As a consequence, possible pairs of $(\bar{r}, \sigma^2)$ that overlap in their integration scores (measured as relative eigenvalue variance) are restricted.

Non-Random Heterogeneous Matrix: Independent Modules

An especially relevant case of heterogeneous matrices are modular matrices, in which clusters of traits are more strongly correlated within than between such clusters. Here we consider a simple case of non-hierarchical modular matrices, where the within-module correlations are different from zero and homogeneous for the module, and between-module correlations are zero, i.e., the modules are independent from each other. The distribution of correlations corresponding to such modular matrix is a k-modal distribution, where k is the number of modules. Each of the modules in this case can be considered as a separate homogeneous submatrix. An example of such correlation matrix is:

$$\mathbf{R}_{N=7} = \begin{bmatrix} 1 & r_{w1} & r_{w1} & 0 & 0 & 0 & 0 \\ & 1 & r_{w1} & 0 & 0 & 0 & 0 \\ & & 1 & 0 & 0 & 0 & 0 \\ & & & 1 & r_{w2} & r_{w2} & r_{w2} \\ & & & & 1 & r_{w2} & r_{w2} \\ & & & & & 1 & r_{w2} \\ & & & & & & 1 \end{bmatrix}$$

where r_{w1}, r_{w2} are within-module correlations. There are two modules in the above case ($k = 2$) with sizes $N_1 = 3$ and $N_2 = 4$. The clusters are non-overlapping and together account for the whole matrix: $N = N_1 + N_2$.

The eigenvalues in this special case follow the derivations above for homogeneous matrices, but separately for each submatrix. The eigenvalues are:

$$\lambda_1 = 1 + (N_1 - 1)r_{w1} = 1 + 2r_{w1}$$

$$\lambda_2 = 1 + (N_2 - 1)r_{w2} = 1 + 3r_{w2}$$

$$\lambda_3,\ \lambda_4 = 1 - r_{w1}$$

$$\lambda_5,\ \lambda_6,\ \lambda_7 = 1 - r_{w2}$$

Or, in general the indices of modules are $i = \{1, 2, 3, \ldots k\}$ there are k leading eigenvalues λ_i:

$$\lambda_{1,i} = 1 + (N_i - 1)\, r_{wi}$$

where r_{wi} is the within-module correlation of the i-th module and there are $(N_i - 1)$ remaining eigenvalues $\lambda_{j,i}$ per module:

$$\lambda_{j,i} = 1 - r_{wi}$$

where N_i is the size of the i-th module, and j is the index of eigenvalues belonging to the same module $j = \{2, 3, \ldots N_i\}$

Eigenvalue variance is then

$$\mathrm{Var}(\lambda) = \frac{\sum_{i=1}^{k}\left((1-(1+(N_i-1)\,r_{wi}))^2+(N_i-1)\,(1-(1-r_{wi}))^2\right)}{N} = \frac{\sum_{i=1}^{k} N_i r_{wi}^2 (N_i-1)}{N}$$

For some examples of special cases, see Appendix 3. In this simple case with independent homogeneous submodules, eigenvalue variance amounts to the weighted arithmetic mean of the eigenvalue variances of homogeneous submatrices, $r^2(N-1)$, where the weight is equal to the size of each module. It is important for our purpose here to notice that eigenvalue variance is therefore affected by the module size and within-module correlation in the same way these two parameters affect eigenvalue variance in the homogeneous (i.e., uni-modular) matrices.

A more general analysis of heterogeneous matrices to see how different modular structures in particular, and non-randomness in general, affect the measure of integration exceeds the scope of this paper and requires a separate treatment (in prep.; see also Kotz et al. 1984). Here, we restrict ourselves to show a general relationship between correlation coefficients and eigenvalue dispersion on simulated matrices, without restricting the distribution of r to be symmetrical. We will in the following refer to the *r-scale,* when correlation coefficients are measured as the Pearson's product–moment correlations on the scale $-1 \leq r \leq 1$; and we will refer to the *z-scale*, when talking about the transforms of the correlation coefficients, transformed to be normally distributed on a continuous scale $-\infty \leq z \leq \infty$ and related to the r-scale by the Fisher's

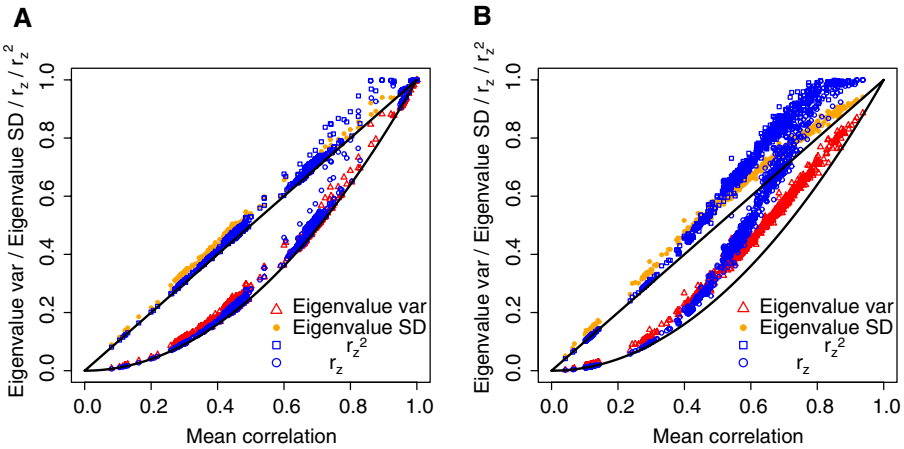

Fig. 3 The relative eigenvalue variance and relative standard deviation of eigenvalues in the correlation matrices with non-symmetrical distribution of correlation. The matrices were produced with z values and consequently transformed by inverse Fisher's z-transformation onto r-scale. The two averages, one calculated from z-scaled data and subsequently transformed onto r-scale ($\bar{r}_z$), and the one calculated on r-scale ($|\bar{r}|$) are compared. The two lines show $|\bar{r}|$ and its squared values. The data are separated by different degrees of heterogeneity as in Fig. 1: CV < 0.6 (**a**) and CV > 0.6 (**b**). The x-axis scales according to the r-scale. Note that eigenvalue variance (*red scatter*) and SD (*orange scatter*) follow the respective $\bar{r}^2$ and $|\bar{r}|$ values, not the values calculated in z-distribution (*blue*)

z-transformation (Fisher 1928). We restrict ourselves to non-singular matrices, i.e., all off-diagonal elements are smaller than $|1|$ on r-scale.

For Fig. 3, the matrices were generated by drawing z-values from normal distribution with various combinations of means and variances. This yielded a more general representation of matrices, including the ones with high mean correlation and hence strongly skewed distribution on the r-scale. Next the average z was calculated across the off-diagonal elements of the matrix, and transformed by the inverse of Fisher's z-transformation (Fisher 1928) onto the r-scale ($\overline{|r|}_z$).The whole matrix was consequently transformed to the r-scale, and the relative eigenvalue variance, relative eigenvalue standard deviation, the average $\overline{|r|}$, and $\overline{|r|}^2$ were calculated. Figure 3 shows the behavior of eigenvalue variance and standard deviation, as well as $\overline{|r|}$, squared $\overline{|r|}$, average inverse-transformed $\overline{|r|}_z$, and squared $\overline{|r|}_z$. The two values $\overline{|r|}$, $\overline{|r|}^2$ are represented by lines. The X-axis is on the r-scale. The relationship between r and z is well known (Fisher 1928). With respect to this relationship the plot simply shows that the average correlation calculated on z-scale and back-transformed exceeds the $\overline{|r|}$ (calculated on the r-scale), increasingly so at higher $\overline{|r|}_z$. This is because the distribution of correlation at high average r is increasingly skewed towards zero. Figure 3 also shows that eigenvalue variance and eigenvalue distribution follow the $\overline{|r|}^2$, and $\overline{|r|}$, respectively, rather than the back-transformed $\overline{|r|}_z$ (the latter was suggested by Van Valen 1965). This is consistent with the result on a subset of heterogeneous matrices with symmetrical distribution of correlations (Fig. 1). Also note, that the scatter lays above the values of $\overline{|r|}^2$, respectively $\overline{|r|}$, as in Fig. 1. However, in this case the deviation of eigenvalue dispersion from the $\overline{|r|}^2$ cannot be expressed as variance of correlations, due to the non-symmetrical distribution of r. See Appendix 4 for a version of the equation for eigenvalue variance when the requirement for symmetry is relaxed.

Sample Size

Note that for the present purpose we paid no attention to sample size, but dealt with ideal matrices, as if built from an infinite population. With finite sample size, r^2 becomes biased upwards and correspondingly, the dispersion of eigenvalues, measured by the standard deviation or variance, changes. Thus, when testing hypotheses of integration as deviation of the observed eigenvalue dispersion from the one expected in a random correlation matrix, the sample size used to derive the empirical correlation matrix needs to be considered as it will influence the expected null distribution. Even for uncorrelated random variables, the estimated correlation matrix will have non-zero off diagonal elements and the eigenvalue variance will be larger than zero. The expected value of the eigenvalue variance for the finite sample correlation matrices for uncorrelated variables is $(N-1)/M$, where N is the number of variables and M is the sample size (Wagner 1984). Hence the standard deviation of eigenvalues should be tested against $\sqrt{(N-1)/M}$, and the relative standard deviation against $\sqrt{1/M}$, the expectations for a random population correlation matrix based on sample size M.

Examples

To demonstrate how the relative integration measures perform on real data, and compare it to other indices, we applied it to several datasets: cranial measurements taken on cotton-top tamarins (*Saguinus oedipus*), measurements on wings of the Northern Goshawk (*Accipiter gentilis*), and mouse skeletal measurements. For the description of datasets, see Appendix 5. In the Tamarin cranial dataset the standard deviation and variance of eigenvalues were calculated for correlation matrices consisting of left and right side measurements separately, as well as for the combined total matrix. The plots of the eigenvalues of the three datasets are shown in Fig. 4. Table 1 lists the level of integration for several phenotypic datasets. The observed standard deviation is listed next to the relative standard deviation that can be compared between partial and total datasets, as well as among datasets with different numbers of variables. In Table 1, we also provide our results compared with ones produced by several other proposed measures of integration (see short descriptions in Appendix 6). A concern with comparing these measures is that they may not be on the same scale and thus be hard to compare, however most (with exception of absolute eigenvalue variance and absolute standard deviation) are on the scale 0–1. In fact, for most of the indices (but see Hansen and Houle 2008) it is not well defined exactly what attribute of the correlation matrix the *integration* measure represents and how it should scale. Therefore the primary question here is whether they correspond on the ordinal scale, i.e., whether the order from the less to more integrated phenotypes is maintained across integration measures. All the measures in Table 1 and Fig. 5 were applied to correlation matrices which already consist of dimensionless quantities.

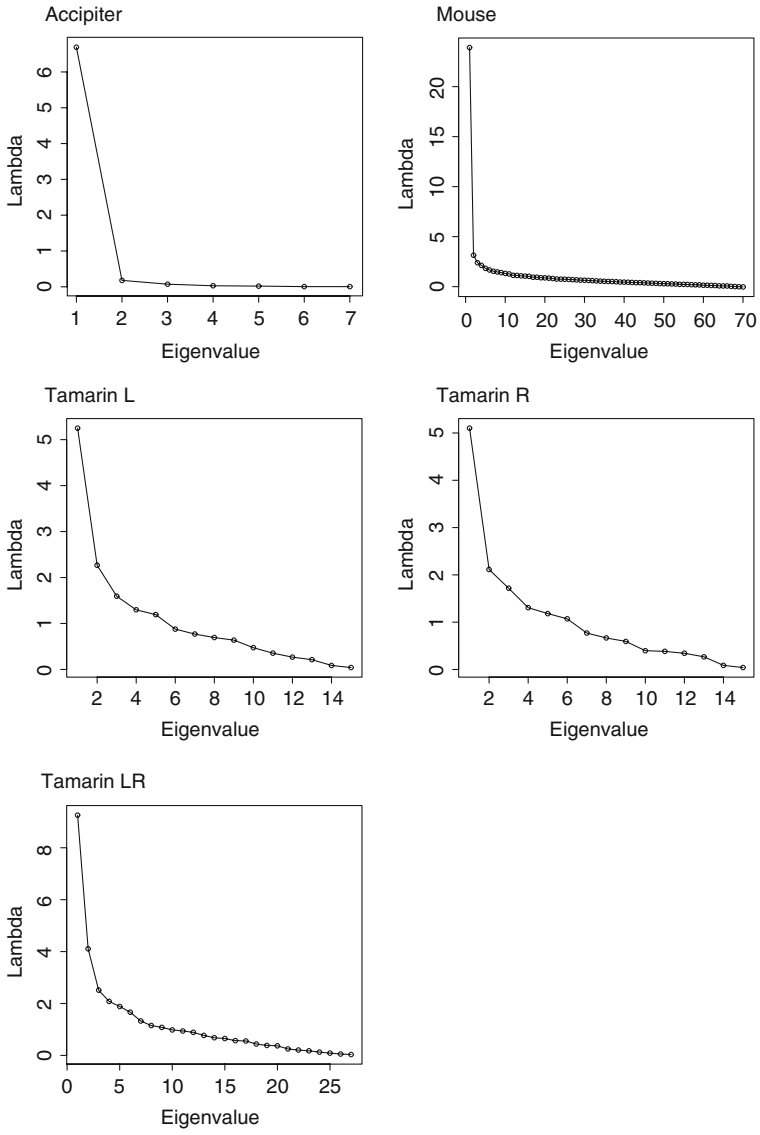

Fig. 4 The eigenvalue distributions of the empirical datasets. The measurement error was assumed to be independent and the error variance has been subtracted from the diagonal of the covariance matrix, prior to building correlation matrix. Negative eigenvalues are not plotted. Note that the integration is higher in the combined (L + R) Tamarin dataset than in the separate lateral datasets. Also note very high integration in *Accipiter* wing, where most variation is concentrated in the leading eigenvalue, while the remaining eigenvalues are almost equal

Table 1 Comparison of integration levels measured by the proposed index to the ones measured by several other commonly used indices on empirical data

dataset	M	N	observed SD (λ)	rel. SD (λ)	$\sqrt{\frac{1}{M}}$	I^{a}	Var(λ)^{b}	rel. Var(λ)	i^{c}	z^{d}	I_r^{e}
Tamarin L	275	16	1.252	0.323	0.06	0.418	1.568	0.104	0.768	0.118	0.256
Tamarin R	275	16	1.214	0.314	0.06	0.401	1.474	0.098	0.684	0.114	0.246
Tamarin RL	275	32	1.750	0.314	0.06	0.401	3.063	0.099	0.752	0.108	0.235
Mouse skeleton	1040	70	2.820	0.339	0.03	0.520	7.952	0.115	0.791	0.138	0.298
Accipiter wing	64	7	2.324	0.949	0.125	0.948	5.401	0.900	0.975	0.688	0.948

[a] Cheverud et al. (1983): (1-geometric mean of eigenvalues)

[b] Wagner (1984): eigenvalue variance

[c] Hansen and Houle (2008): (1-autonomy)

[d] Van Valen (1965): mean of the Fisher's z-transformed correlation, back-transformed onto the 0–1 scale

[e] Cane (1993): mean absolute r

The measure is originally based on covariance matrix, however here we used correlation matrix (variance-standardized data). M: the sample size; N: the number of traits; SD(λ): standard deviation of eigenvalues; rel. SD(λ): relative standard deviation of eigenvalues; $\sqrt{1/M}$: the value of rel. SD(λ) expected in random correlation matrix with no integration, based on the sample size; the indices are described in Appendix 6. The results are compared graphically in Fig. 5

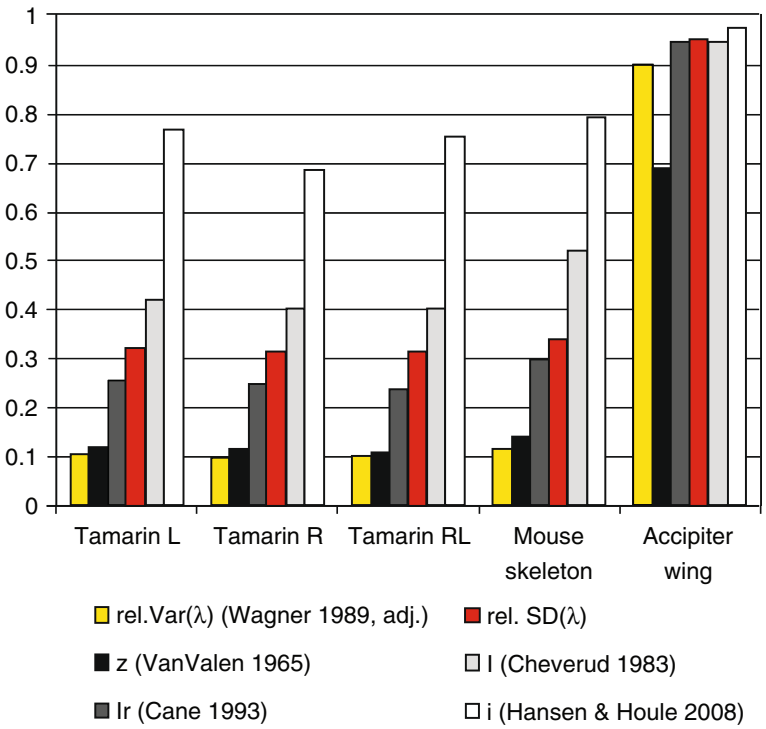

Fig. 5 The values of different integration indices for the exemplary empirical data in comparison. Note that the Hansen and Houle's (2008) average integration (i), originally based on covariance matrix, is calculated here on correlation matrix (i.e., variance-standardized matrix). Note that the particular exemplary datasets maintain their relative ranking under different measures, but the absolute magnitudes of integration measurement vary

Even though the measures yield considerably different values, the rank of integration is maintained across the different measures in these examples. Van Valen's (1965) z-measure gives similar values as the relative eigenvalue variance for weakly integrated data, while giving much lower values in the strongly integrated data from the bird wing. As expected the relative standard deviation of eigenvalues gives similar values as the mean absolute correlation. The relative standard deviation of eigenvalues, however, is always larger than the mean absolute correlation because of the effect of variation among the correlation coefficients. Only for homogenous correlation matrices these two measures the same (see above), but the mean absolute correlation ignores the effect of deviations from homogeneity on morphological integration, i.e., on variance allocation among eigenvectors (see above for the analytical proof). Hence we argue that the mean absolute correlation consistently underestimates the degree of morphological integration. We also calculated the new integration measure by Hansen and Houle (2008) using

correlation matrices, although their measure is originally based on covariance matrices and has a different theoretical basis than the other more intuitive measures. While the absolute values differ relative to other measures, the results are the same on an ordinal scale.

In conclusion, we show that the relative standard deviation of eigenvalues of correlation matrices scales linearly with the mean absolute correlation (Cane 1993) but is only identical to this measure if the correlation matrix is homogeneous. For random heterogeneous correlation matrices the mean absolute correlation consistently underestimates integration, since it is invariant with respect to the differences among correlation coefficients. Hence unequal allocation of variance due to variation among correlation coefficients is not reflected in the mean absolute correlation coefficient, but is captured by the relative standard deviation of eigenvalues. We thus suggest that this measure, or its squared version, is a better reflection of overall morphological integration. We furthermore show that this relationship is not limited to the random matrices with symmetrical distribution of correlation coefficients, but that the eigenvalue variance and standard deviation also follow the pattern in more general, asymmetrical distributions of correlations.

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Appendix 1

Let $\mathbf{R}$ be a homogenous correlation matrix of size N with $\mathbf{R}_{ij} = r$ for all $i \neq j$. If $1 = (1,\ldots,1)$ is an eigenvector of $\mathbf{R}$ and $\lambda_1 = 1 + (N-1)r$ the corresponding eigenvalue, then $\mathbf{R1} = \lambda_1 \mathbf{1}$. Considering this equation component wise:

$$(\mathbf{R1})_i = (\lambda_1 \mathbf{1})_i$$

$$(\mathbf{R1})_i = \sum_{j=1}^{N} \mathbf{R}_{ij}\mathbf{1}_j$$

we see that the right hand side of the latter equation is simply the row sum of the i-th row. Because of the symmetry of $\mathbf{R}$ this sum is the same for all rows in $\mathbf{R}$, and which has $(N-1)$ terms of size r and one equal to 1:

$$\sum_{j=1}^{N} \mathbf{R}_{ij}1_j = 1 + (N-1)r = \lambda_1 1_i$$

The other eigenvalues of $\mathbf{R}$ can be obtained by considering the constraint $\sum_{i=1}^{N} \lambda_i = \mathrm{tr}\mathbf{R} = N$, from which we see that

$$\sum_{i=1}^{N} \lambda_i = \lambda_1 + \sum_{i=2}^{N} \lambda_i = N$$

$$\sum_{i=2}^{N} \lambda_i = N - \lambda_1$$

$$\sum_{i=2}^{N} \lambda_i = N - 1 - (N-1)r$$

$$\sum_{i=2}^{N} \lambda_i = (N-1)(1-r)$$

Due to the symmetry of the $\mathbf{R}$ matrix the $N-1$ non-leading eigenvalues are all the same and $\lambda_{i>1} = 1 - r$.

Appendix 2

To derive the maximum variance corresponding to a particular $\bar{r}$, we consider maximal symmetrical dispersal of r. Maximum dispersal is reached when half of the sample size is located at outermost values, in equal distances to both sides from the $\bar{r}$. This can be expressed as

$$\mathrm{Var}_{\max}(r) = \frac{\frac{N^2-N}{4}(r_{i\max} - \bar{r})^2 + \frac{N^2-N}{4}(r_{i\min} - \bar{r})^2}{\frac{N^2-N}{2}} = \frac{1}{2}\left[(r_{i\max} - \bar{r})^2 + (r_{i\min} - \bar{r})^2\right]$$

where $r_{i\max}$, $r_{i\min}$ are the maximal and minimal values of r_i, given the symmetrical distribution. For example, if $\bar{r} = 0.2$, the $r_{\max} = 1$ and $r_{\min} = 0.2 - (1 - 0.2) = -0.6$. In general:

$$r_{\max} = \bar{r} + (1 - |\bar{r}|)$$

$$r_{\min} = \bar{r} - (1 - |\bar{r}|)$$

And therefore, the correlation variance of the maximal symmetrical dispersion for a given average r is:

$$\mathrm{Var}_{\max}(r) = (1 - |\bar{r}|)^2 = \bar{r}^2 - 2|\bar{r}| + 1$$

Appendix 3

Here we investigate the eigenvalue variance in two special cases of non-hierarchical modular matrices. General equation for eigenvalue variance in matrices with k homogeneous submodules, each of size N_i, and of no correlation between modules is:

$$\mathrm{Var}(\lambda) = \frac{\sum_{i=1}^{k}\left(((1+(N_i-1)r_{wi})-1)^2+(N_i-1)((1-r_{wi})-1)^2\right)}{N} = \frac{\sum_{i=1}^{k} N_i r_{wi}^2 (N_i-1)}{N}$$

It is helpful to consider a case where all modules are of the same size, $N_i = \frac{N}{k}$; and $\sum_{i=1}^{k} N_i = N$, and equal within-module correlation r_w. In this case the equation simplifies to

$$\mathrm{Var}(\lambda) = r_w^2\left(\frac{N}{k}-1\right)$$

$N > k$, thus eigenvalue variance (i.e., integration) is lower than in a homogeneous (unimodular) matrix by a factor

$$\frac{N-k}{k(N-1)}$$

To compare it with integration estimated from the overall mean correlation, we calculate overall mean correlation:

$$\bar{r} = \frac{r_w k(N_i(N_i-1))}{N(N-1)} = r_w\frac{N_i-1}{N-1}$$

And therefore

$$\mathrm{Var}(\lambda) \geq \bar{r}^2 = r_w^2\left(\frac{N_i-1}{N-1}\right)^2$$

Here, the two integration levels differ by a factor

$$\frac{N-k}{k(N-1)^2}$$

the factor is smaller than 1, indicating that the estimate by the mean squared correlation is lower than the true integration of modular matrix. In fact we can see that the smaller the module size (and the greater thus the k), the smaller this factor will be.

The second case considered here is a case with different-sized modules of *equal* within-module correlation.

$$\mathrm{Var}(\lambda) = r_w^2\frac{\sum_{i=1}^{k} N_i^2 - N_i}{N} = r_w^2\left(\frac{\sum_{i=1}^{k} N_i^2}{N}-1\right)$$

In which case only the term of the eigenvalue variance describing the effect of submodule size is averaged.

Appendix 4

Eigenvalue Variance for the Three-Trait Case with Unsymmetrical Distribution of Correlation

The correlation coefficients in matrices underlying this consideration are normally distributed on a Fisher's z-transformed scale (Fisher 1928), but not on the Pearson's correlation scale $-1 < r < 1$. Note that in the following we refer to the result of the normalizing Fisher's z-transformation as to the *z-scale*. This is not to be confused with z-standardization of normal distribution. The Pearson's product–moment correlations (r) are referred to as being on the *r-scale*.

Consider a given mean correlation on r-scale ($\bar{r}$) and given maximum deviation from the mean (d) on z-scale. The $\bar{r}$ has a corresponding value when transformed to z-scale, and the deviation d is added and subtracted to gain the lowest and the highest values of the correlation coefficients on the z-scale. The two values were back-transformed onto the r-scale and the deviations ε_1, ε_2 from $\bar{r}$ were calculated as differences of the extreme values from the mean. The third correlation coefficient is $e_3 = 0 - \varepsilon_1 - \varepsilon_2$ due to the constraint of $\bar{r}$, i.e., $\sum \varepsilon_i = 0$. The three off-diagonal elements of the correlation matrix are now $(\bar{r}+\varepsilon_1), (\bar{r}-\varepsilon_2), (\bar{r}+\varepsilon_3)$. The corresponding eigenvalue polynomial (as in previous symmetrical case, see text), substituting x for $(1-\lambda)$ and multiplying out reduces to

$$\begin{aligned}\det(\mathbf{R}_{N=3} - \lambda\mathbf{I}) &= x^3 - x\left(3r^2 + 2r(\varepsilon_1 - \varepsilon_2 + \varepsilon_3) + \varepsilon_1^2 + \varepsilon_2^2 + \varepsilon_3^2\right) \\ &\quad + 2r\left(r^2 + r(\varepsilon_1 - \varepsilon_2 + \varepsilon_3) + \varepsilon_1\varepsilon_3 - \varepsilon_1\varepsilon_2 - \varepsilon_2\varepsilon_3\right) \\ &\quad - 2\varepsilon_1\varepsilon_2\varepsilon_3\end{aligned}$$

Figure 6 shows plot of a family of polynomials with different combinations of parameters $\bar{r}$ and d; the three solutions representing the three $x_i(=1-\lambda_i)$. Bundles of polynomials share the same mean correlation of matrix ($\bar{r}$), but differ in dispersion of correlations (d).

Analogous to the matrices with symmetrical distribution of correlation coefficients (main part of the text), we do not present the exact solutions, but rather approximate the three solutions here. Note that the three solutions follow the same pattern as above in symmetrical matrices. The following can be concluded from the plot: (i) when allowing the asymmetrical distribution of correlation, the heterogeneity affects all three eigenvalues; (ii) the effect of heterogeneity of matrix on first eigenvalue is smaller at higher $|\overline{r}|$; (iii) the first eigenvalue increases with increased heterogeneity (i.e., x_1 decreases) while the remaining two diverge.

Approximating the three solutions in a way similar to symmetrical situation, but noting that all three eigenvalues vary depending on the heterogeneity, rather than only the second and the third, we consider the following solutions:

$$x_1 \approx -2\bar{r} - \delta_{\varepsilon 1}$$

$$x_2 \approx \bar{r} - \delta_{\varepsilon 2}$$

$$x_3 \approx \bar{r} + \delta_{\varepsilon 3}$$

and the resulting eigenvalues are

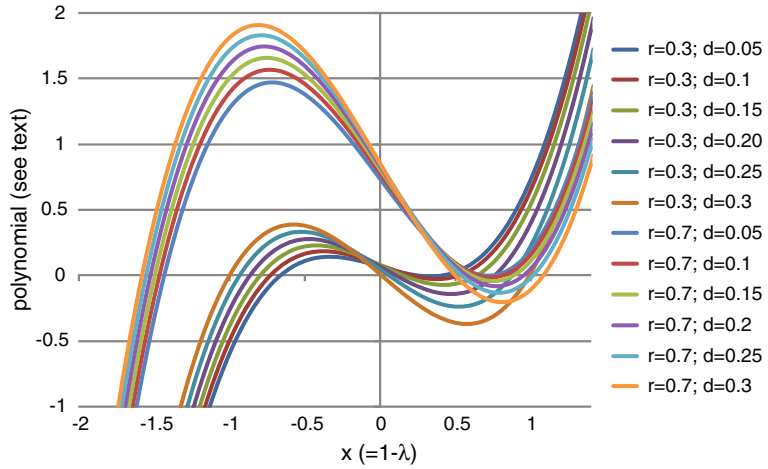

Fig. 6 The plot of the polynomial with solutions equal to the three $(1 - \lambda)$ of the heterogeneous correlation matrix with $N = 3$, but with relaxed constraint on symmetry of the correlation distribution. The three solutions of the polynomial are the intersections of the curve with x-axis. The two groups of curves show the mean correlations of $r = 0.3$ and 0.7. The within-group variability is due to variable deviations from the mean correlation ($\varepsilon = 0.05, 0.10, 0.15, 0.20, 0.25$, and 0.30). Note that, in contrast to the analogous plot in Fig. 3 where the correlation distribution in underlying matrices was constrained to be symmetrical, in this plot the solution to the left of 0 is not invariant with the varying ε. Rather, similar to the two solutions >0, it varies dependent on ε

$$\lambda_1 \approx 1 + 2\bar{r} + \delta_{\varepsilon 1}$$
$$\lambda_2 \approx 1 - (\bar{r} - \delta_{\varepsilon 2})$$
$$\lambda_3 \approx 1 - (\bar{r} + \delta_{\varepsilon 3})$$

If we substitute these into the equation for eigenvalue variance, it yields for three traits:

$$\mathrm{Var}(\lambda) = \frac{1}{N}\left((N-1)^2\bar{r}^2 + 2\bar{r}^2 + 2\bar{r}((N-1)\delta_1 - \delta_2 - \delta_3) + \sum_{i=1}^{N}\delta_i^2\right)$$

And after some rearrangement for a general case:

$$\begin{aligned}\mathrm{Var}(\lambda) &= (N-1)\,\bar{r}^2 \\ &\quad + \frac{1}{N}\left[\sum_{i=1}^{N}\delta_i^2 + 2\bar{r}\left((N-1)\delta_1 - \sum_{i=2}^{N}\delta_i\right)\right] \\ &= (N-1)\,\bar{r}^2 + \frac{1}{N}\left[\sum_{i=1}^{N}\delta_i^2 + 2\bar{r}\sum_{i=2}^{N}(\delta_1 - \delta_i)\right]\end{aligned}$$

This version corresponds to the eigenvalue variance equation for the heterogeneous matrices with symmetrical distribution of correlations, but involves an additional term (that yields zero if the distribution is symmetrical). This equation also shows that the eigenvalue variance in heterogeneous matrices will be greater than the corresponding eigenvalue variance of the homogeneous matrix of the same average correlation.

Appendix 5

Phenotypic Datasets

Tamarin Cranial Dataset

This dataset is comprised of 16 linear lateral cranial measurements on the skull of 275 individuals of the pure bred cotton-top tamarin (*Saguinus oedipus*). Detailed description of the population, measurement method and the list of measurements can be found in Hutchison and Cheverud (1995). The linear measurements were derived from 3D coordinate data for 16 landmarks per each side of the skull. All landmarks were taken twice, to estimate the measurement error and improve repeatability of short measurements by using their average. The average repeatability per trait of these data was 0.788 on the right and 0.776 on the left side.

Bird Wing Dataset

The lengths of seven wing feathers have been measured on 64 museum specimens of Eurasian Goshawk (*Accipiter gentilis gentilis,* data are available in online supplementary material, Table S1). This dataset comprises the first five primary—('hand') as well as the third and the fifth secondary ("forearm") flight feathers, counted from distal end of the wing towards the body. The feathers were measured as a distance from bend of the wing (i.e., wrist joint) to the tip of the feather along the shaft, measured over the folded wing. The sample size consists of individuals covering

most of the subspecies range. All measurements were taken three-fold. The average repeatability of single measurements in these data is 0.978.

Mouse Skeletal Dataset

Phenotypic traits comprise 70 skeletal measurements representing the cranium, axial and appendicular skeleton, as well as body weight at 10 weeks and at necropsy. The experimental population results from an intercross of inbred mouse strains LG/J and SM/J, selected for large and small body weight at 60 days of age, respectively. The data stems from two consecutive analogous intercrosses, comprising a total of 1040 F_2 mice. For the list of measurements and details on measurement techniques see Kenney-Hunt et al. (2008). Extreme outliers were identified by being more than 2.7 standard deviations from the sample mean (SYSTAT 10.2) and were eliminated to avoid biasing the data. The data were corrected for the effects of dam, litter size, experimental block, sex, age at necropsy, and intercross. The correction was done by regressing each of the phenotypic trait measurements on all of the respective predictor variables (dam, litter size, sex, etc.) and taking the residuals as corrected scores for the trait. The repeatability was assessed for each trait by repeated measurements on 30–50 individuals and amounts on average 0.93, with the range from 0.82 to 0.992 for single traits.

Appendix 6

Several Other Indices of Integration

Some of the listed measures require the matrices to be non-negative, or even positive definite. Errorless phenotypic matrices are always at least non-negative definite, however due to error, either sampling or measurement error, empirical matrices are often negative. To apply the methods 2 and 5 to the empirical datasets in the paper, we used only dimensions with eigenvalues greater than zero, in case the matrices were not positive definite.

1. Van Valen (1965) suggested calculating the average Fisher's z-transformed correlation, and transforming it back to the scale 0–1.
2. Cheverud et al. (1983) defined the index of integration as one minus the geometric mean of eigenvalues of a correlation matrix:

$$I = 1 - \sqrt[N]{\prod_{i=1}^{N} \lambda_i}$$

3. Wagner (1984) suggested the variance of eigenvalues of a correlation matrix. We provide also the relative eigenvalue variance, as described in the main body of this paper:

$$\mathrm{Var}_{\mathrm{rel}}(\lambda) = \frac{\mathrm{Var}(\lambda)}{\max \mathrm{Var}(\lambda)} = \frac{\mathrm{Var}(\lambda)}{N-1}$$

4. Cane (1993) used an average absolute pair wise correlation
5. Hansen and Houle (2008) suggested a measure that is based on covariance matrix and applies the harmonic mean of eigenvalues ($H(\lambda)$):

$$i = 1 - \left[\frac{H(\lambda)}{\bar{\lambda}}\left[1 + \frac{1 + I\left(\frac{1}{\lambda}\right) + I(\lambda) - \frac{H(\lambda)}{\bar{\lambda}}}{k+1}\right]\right]$$

where

$$I\left(\frac{1}{\lambda}\right) = \frac{\mathrm{Var}\left(\frac{1}{\lambda_i}\right)}{\left(\frac{1}{\bar{\lambda}_i}\right)^2} \qquad I(\lambda) = \frac{\mathrm{Var}(\lambda)}{(\bar{\lambda})^2}$$

The data for this procedure need to be on similar scale, which can be accomplished by variance or mean standardization. We used variance standardization in the examples, which corresponds to correlation.

References

Berg, R. L. (1960). The ecological significance of correlation pleiades. *Evolution; International Journal of Organic Evolution,14*, 171–180. doi:10.2307/2405824.

Cane, W. P. (1993). The ontogeny of post-cranial integration in the common tern, *Sterna hirundo*. *Evolution; International Journal of Organic Evolution,47*, 844–854. doi:10.2307/2409980.

Chetverikov, S. S., Barker, M., & Lerner, I. M. (1961). On certain aspects of the evolutionary process from the standpoint of modern genetics. *Proceedings of the American Philosophical Society, 105*(2), 167–195.

Cheverud, J. M. (1982). Relationships among ontogenetic, static, and evolutionary allometry. *American Journal of Physical Anthropology,59*(2), 139–149. doi:10.1002/ajpa.1330590204.

Cheverud, J. M. (1984). Quantitative genetics and developmental constraints on evolution by selection. *Journal of Theoretical Biology,110*(2), 155–171. doi:10.1016/S0022-5193(84)80050-8.

Cheverud, J. M. (1988). A comparison of genetic and phenotypic correlations. *Evolution; International Journal of Organic Evolution,42*, 958–968. doi:10.2307/2408911.

Cheverud, J. M. (1996). Developmental integration and the evolution of pleiotropy. *American Zoologist, 36*, 44–50.

Cheverud, J. M. (2001). A simple correction for multiple comparisons in interval mapping genome scans. *Heredity,87*(1), 52–58. doi:10.1046/j.1365-2540.2001.00901.x.

Cheverud, J. M., Rutledge, J. J., & Atchley, W. R. (1983). Quantitative genetics of development: Genetic correlations among age-specific trait values and the evolution of ontogeny. *Evolution; International Journal of Organic Evolution,37*(5), 895–905. doi:10.2307/2408405.

Cheverud, J. M., Wagner, G. P., & Dow, M. M. (1989). Methods for the comparative analysis of variation patterns. *Systematic Zoology,38*(3), 201–213. doi:10.2307/2992282.

Darwin, C. (1895). *On the origin of species*. London: John Murray.

Fisher, R. A. (1928). The general sampling distribution of the multiple correlation coefficient. *Proceedings of the Royal Society of London. Series A, Containing Papers of a Mathematical and Physical Character,121,* 654–673. doi:10.1098/rspa.1928.0224.

Hallgrimsson, B., Brown, J. J., Ford-Hutchinson, A. F., Sheets, H. D., Zelditch, M. L., & Jirik, F. R. (2006). The brachymorph mouse and the developmental-genetic basis for canalization and morphological integration. *Evolution & Development,8*(1), 61–73. doi:10.1111/j.1525-142X.2006.05075.x.

Hallgrimsson, B., Dorval, C. J., Zelditch, M. L., & German, R. Z. (2004). Craniofacial variability and morphological integration in mice susceptible to cleft lip and palate. *Journal of Anatomy,205*(6), 501–517. doi:10.1111/j.0021-8782.2004.00356.x.

Hansen, T. F., & Houle, D. (2008). Measuring and comparing evolvability and constraint in multivariate characters. *Journal of Evolutionary Biology,21*(5), 1201–1219. doi:10.1111/j.1420-9101.2008.01573.x.

Herrera, C. M., Cerda, X., Garcia, M. B., Guitan, J., Medrano, M., Rey, P. J., et al. (2002). Floral integration, phenotypic covariance structure and pollinator variation in bumblebee-pollinated *Helleborus foetidus*. *Journal of Evolutionary Biology,15*, 108–121. doi:10.1046/j.1420-9101.2002.00365.x.

Hutchison, D. W., & Cheverud, J. M. (1995). Fluctuating asymmetry in tamarin (*Saguinus*) cranial morphology: Intra- and interspecific comparisons between taxa with varying levels of genetic heterozygosity. *The Journal of Heredity,86*(4), 280–288.

Jones, A. G., Arnold, S. J., & Burger, R. (2007). The mutation matrix and the evolution of evolvability. *Evolution; International Journal of Organic Evolution,61*(4), 727–745. doi:10.1111/j.1558-5646.2007.00071.x.

Jorjani, H., Klei, L., & Emanuelson, U. (2003). A simple method for weighted bending of genetic (co)variance matrices. *Journal of Dairy Science,86*(2), 677–679.

Kenney-Hunt, J. P., Wang, B., Norgard, E. A., Fawcett, G., Falk, D., Pletscher, S. L., et al. (2008). Pleiotropic patterns of quantitative trait loci for 70 murine skeletal traits. *Genetics,178*(4), 2275–2288. doi:10.1534/genetics.107.084434.

Kirkpatrick, M. (2008). Patterns of quantitative genetic variation in multiple dimensions. *Genetica*, in press.

Kotz, S., Pearn, W. L., & Wichern, D. W. (1984). Eigenvalue-eigenvector analysis for a class of patterned correlation matrices with an application. *Statistics & Probability Letters,2*, 119–125. doi:10.1016/0167-7152(84)90001-4.

Lande, R. (1979). Quantitative genetic analysis of multivariate evolution, applied to brain: Body size allometry. *Evolution, 33*, 402–416.

Lande, R. (1980). The genetic covariance between characters maintained by pleiotropic mutations. *Genetics, 94*, 203–215.

Lande, R., & Arnold, S. J. (1983). The measurement of selection on correlated characters. *Evolution; International Journal of Organic Evolution,37*(6), 1210–1226. doi:10.2307/2408842.

Magwene, P. M. (2008). Using correlation proximity graphs to study phenotypic integration. *Evolutionary Biology,35*, 191–198. doi:10.1007/s11692-008-9030-y.

Morrison, D. F. (1976). *Multivariate Statistical Methods* (2nd ed.). New York: McGraw-Hill, Inc.

Nyholt, D. R. (2004). A simple correction for multiple testing for single-nucleotide polymorphisms in linkage disequilibrium with each other. *American Journal of Human Genetics,74*(4), 765–769. doi:10.1086/383251.

Olson, E. C., & Miller, R. L. (1958). *Morphological integration*. Chicago: University of Chicago Press.

Parsons, K. J., & Robinson, B. W. (2006). Replicated evolution of integrated plastic responses during early adaptive divergence. *Evolution; International Journal of Organic Evolution,60*(4), 801–813.

Pearson, K. (1903). Mathematical contributions to the theory of evolution. XI. On the influence of natural selection on the variability and correlation of organs. *Philosophical Transactions of the Royal Society of London. Series A: Mathematical and Physical Sciences,200*, 1–66. doi:10.1098/rsta.1903.0001.

Peres-Neto, P. R., & Magnan, P. (2004). The influence of swimming demand on phenotypic plasticity and morphological integration: A comparison of two polymorphic charr species. *Oecologia,140*(1), 36–45. doi:10.1007/s00442-004-1562-y.

Revell, L. J. (2007). The G matrix under fluctuating correlational mutation and selection. *Evolution; International Journal of Organic Evolution,61*(8), 1857–1872. doi:10.1111/j.1558-5646.2007.00161.x.

Searle, S. R. (1961). Phenotypic, genetic and environmental correlations. *Biometrics, 17*, 474–480.

Terentjev, P. V. (1931). Biometrische Untersuchungen ueber die morphologischen Merkmale von *Rana ridibunda* Pall. (Amphibia, Salientia). *Biometrika,23*, 23–51.

Van Valen, L. (1965). The study of morphological integration. *Evolution; International Journal of Organic Evolution,19*(3), 347–349. doi:10.2307/2406444.

Wagner, G. P. (1984). On the eigenvalue distribution of genetic and phenotypic dispersion matrices: Evidence for a nonrandom organization for quantitative character variation. *Journal of Mathematical Biology,21*, 77–95.

Wagner, G. P., Kenney-Hunt, J. P., Pavlicev, M., Peck, J. R., Waxman, D., & Cheverud, J. M. (2008). Pleiotropic scaling of gene effects and the 'cost of complexity'. *Nature,452*(7186), 470–472. doi:10.1038/nature06756.

Young, N. M. (2006). Function, ontogeny and canalization of shape variance in the primate scapula. *Journal of Anatomy,209*(5), 623–636. doi:10.1111/j.1469-7580.2006.00639.x.

Young, N. M., & Hallgrimsson, B. (2005). Serial homology and the evolution of mammalian limb covariation structure. *Evolution; International Journal of Organic Evolution,59*(12), 2691–2704.