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On the selection of phylogenetic eigenvectors for ecological analyses

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Among the statistical methods available to control for phylogenetic autocorrelation in ecological data, those based on eigenfunction analysis of the phylogenetic distance matrix among the species are becoming increasingly important tools. Here, we evaluate a range of criteria to select eigenvectors extracted from a phylogenetic distance matrix (using phylogenetic eigenvector regression, PVR) that can be used to measure the level of phylogenetic signal in ecological data and to study correlated evolution. We used a principal coordinate analysis to represent the phylogenetic relationships among 209 species of Carnivora by a series of eigenvectors, which were then used to model log-transformed body size. We first conducted a series of PVRs in which we increased the number of eigenvectors from 1 to 70, following the sequence of their associated eigenvalues. Second, we also investigated three non-sequential approaches based on the selection of 1) eigenvectors significantly correlated with body size, 2) eigenvectors selected by a standard stepwise algorithm, and 3) the combination of eigenvectors that minimizes the residual phylogenetic autocorrelation. We mapped the mean specific component of body size to evaluate how these selection criteria affect the interpretation of non-phylogenetic signal in Bergmann's rule. For comparison, the same patterns were analyzed using autoregressive model (ARM) and phylogenetic generalized least-squares (PGLS). Despite the robustness of PVR to the specific approaches used to select eigenvectors, using a relatively small number of eigenvectors may be insufficient to control phylogenetic autocorrelation, leading to flawed conclusions about patterns and processes. The method that minimizes residual autocorrelation seems to be the best choice according to different criteria. Thus, our analyses show that, when the best criterion is used to control phylogenetic structure, PVR can be a valuable tool for testing hypotheses related to heritability at the species level, phylogenetic niche conservatism and correlated evolution between ecological traits.

It is well known that the presence of spatial, temporal or phylogenetic autocorrelation poses both a challenge and an opportunity for the analysis of ecological and macroecological datasets (Legendre 1993, Peres-Neto 2006). The challenge arises because standard statistical methods assume independence among observations, so the presence of autocorrelation tends to inflate type I error rates (but see Revell 2010). However, the use of the new statistical methods developed to account for autocorrelation also provides opportunities for gaining much deeper insights into ecological/evolutionary patterns and processes. The gain will come from the fact that autocorrelation should not be considered only as a nuisance affecting significance tests, but also as an interesting pattern resulting from ecological (e.g. population dispersal and neutral dynamics) and evolutionary (e.g. niche conservatism and phylogenetic inertia) processes (Martins and Hansen 1996, Wiens and Graham 2005, Dray et al. 2006, Bellier et al. 2007).

Among the statistical methods available to tackle autocorrelated data, those based on the extraction of eigenvectors from a matrix containing the levels of geographic, temporal or phylogenetic similarity among the units of analysis are becoming increasingly important tools in ecology (Thioulouse et al. 1995, Diniz-Filho et al. 1998, Borcard and Legendre 2002, Desdevises et al. 2003, Borcard et al. 2004, Diniz-Filho and Bini 2005, Dray et al. 2006, Dormann et al. 2007, Griffith and Peres-Neto 2006, Peres-Neto et al. 2006, Blanchet et al. 2008, Siqueira et al. 2008, Angeler et al. 2009, Bini et al. 2009, Legendre et al. 2010). In these cases, eigenvectors represent, in a vector form, the relationship among units of analysis (e.g. species or sampling sites), and thus can be easily added as covariates in linear and non-linear models, being a flexible and general tool useful for minimizing the undesirable effects of autocorrelation on significance tests and, at the same time, allowing evaluation of the relative importance of processes generating spatial, temporal or phylogenetic autocorrelation.

In the phylogenetic context, Diniz-Filho et al. (1998) proposed the phylogenetic eigenvector regression (PVR) as a simpler and more flexible alternative to Cheverud et al.'s

(1985) autoregressive model (ARM). The main goals of PRV and ARM are to estimate the magnitude of phylogenetic signal in data and estimate the evolutionary correlation between traits and its associated probability. The logic underlying both variance partitioning methods, derived from quantitative evolutionary genetics, is that the total variation in a given trait (T) measured for n species can be partitioned into a phylogenetic (P) and a specific (S) component, so that T = P + S. The P-component expresses the part of the variation in T that is phylogenetically autocorrelated (usually expressed in a pairwise distance matrix **D** derived from a phylogeny), whereas the S-component is the unique variation in each species that arises after the divergence from the most recent common ancestor. The coefficients of determination (R^2) of these models (i.e. PVR or ARM) measure the ratio P/T (or 1 – S/T), which is an estimate of the amount of phylogenetic signal in the trait, so that a high R^2 indicates that more phylogenetically-related species tend to be more similar for the trait (Cheverud et al. 1985, Gittleman and Kot 1990; but see Rohlf 2001). Moreover, patterns in the S-component can be interpreted as the result of independent recent adaptive responses of species with respect to variation in another traits or to environmental variation (but see Harvey et al. 1995, Westoby et al. 1995a, b, Desdevises et al. 2003 for discussion and more complex interpretations of this partition).

PVR starts by extracting eigenvectors from a double-centered phylogenetic distance matrix (using a principal coordinate analysis – PCoA – based on phylogenetic distances among species – Legendre and Legendre 1998), which are used as explanatory variables in a standard OLS multiple regression. Initial applications of PVR were focused on estimating the phylogenetic signal in data using the model R^2 (Morales 2000, see Sakamoto et al. 2010 for a recent application), but it is also straightforward to use PVR to estimate the correlation between traits (i.e. by correlating S-components, as in ARM). Indeed, simulation studies have shown that PVR has acceptable type I and II errors rates when estimating these correlations, showing similar results to other methods, such as phylogenetic independent contrasts (PIC) and phylogenetic generalized least-squares (PGLS) under distinct evolutionary models (Diniz-Filho and Torres 2002, Martins et al. 2002). Desdevises et al. (2003) expanded the method using a partial regression approach to decouple the effects of ecological and phylogenetic (plus their overlap, interpreted as phylogenetic niche conservatism) components in trait variation.

Eigenvectors from a phylogenetic distance matrix reflect the phylogenetic relationship among species in a vector form, and the first eigenvectors tend to represent (or map) larger distances among species, thus expressing divergences closer to the root of the phylogeny. Rohlf (2001) criticized PVR stating that all eigenvectors would be needed to capture all phylogenetic information. Consequently, if all eigenvectors are used as predictors in a multiple regression model then all variability in a trait Y is explained (i.e. $R^2 = 1$), leaving no residual variation (so the S-component is zero). Although the reasoning is correct and PVR's R^2 should be interpreted with caution, we emphasize that not all of them are actually necessary to model trait variation among species. Some carefully selected eigenvectors suffice for modeling purposes, because rarely, if ever, traits are free from measurement error and will not evolve under very simple models throughout the phylogeny. Moreover, the advantage of PVR is that eigenvectors can deal with complex evolutionary patterns in a trait (e.g. allowing for non-stationarity and stasis, Diniz-Filho et al. 2010), and can also be incorporated in a range of statistical models to represent and quantify phylogenetic structure (Desdevises et al. 2003), as well as to model variation in discrete or categorical traits (Griffith and Peres-Neto 2006). We agree, however, that Rohlf's (2001) criticism highlights the need for a better evaluation of which and how many eigenvectors must be used in a PVR analysis, which is the main theme of this paper.

The problem of selecting eigenvectors generated by eigenfunction spatial analysis (i.e. principal coordinates of neighbor matrices PCNM, Borcard and Legendre 2002, Borcard et al. 2004; Moran's eigenvector maps – MEM, Dray et al. 2006; spatial filtering, Griffith 2003) has been recently addressed by several authors (Diniz-Filho and Bini 2005, Tiefelsdorf and Griffith 2007, Blanchet et al. 2008, Jombart et al. 2009). However, there is no consensus on how to select phylogenetic eigenvectors, despite the fact that a well-defined set of eigenvectors is of paramount importance for all the above mentioned techniques (Peres-Neto et al. 2006, Griffith and Peres-Neto 2006). For instance, when unnecessary eigenvectors are included in PVR, the patterns of trait variation due to phylogenetic structure might be slightly overestimated, decreasing our ability to detect non-phylogenetic patterns in trait variation (but see Peres-Neto et al. 2006 for distinct results in variance partition using spatial eigenfunction analyses). On the other hand, using too few eigenvectors in PVR will leave residual autocorrelation so phylogenetic and non-phylogenetic patterns will be confused in the S-component.

There is an increasing interest in the application of phylogenetic methods to test hypotheses related to niche conservatism (Cooper et al. 2010, Hof et al. 2010), ecogeographical rules (Diniz-Filho et al. 2007, 2009), spatially-structured phylogenetic patterns in trait variation (Freckleton and Jetz 2009, Safi and Pettorelli 2010) and phylogenetic diversity in general (Kuhn et al. 2009, Diniz-Filho et al. 2010, Pillar and Duarte 2010). PVR, as well as other phylogenetic comparative methods, can be used to analyze patterns and processes that require estimating the magnitude of phylogenetic signal, as well as to modeling species' trait responses to environmental drivers independent of the phylogenetic structure.

However, we believe that one of the most interesting potential applications of PVR is the evaluation of ecogeographical rules by combining interspecific and assemblage approaches (Diniz-Filho et al. 2007, 2009, Ramirez et al. 2008, Terribile et al. 2009, Olalla-Tárraga et al. 2010). Rather than using PVR, for example, to evaluate Bergmann's rule by correlating species body size with mean temperature (i.e. a general estimate of temperature within species' ranges, in a typical 'cross-species' analysis), the evolutionary and ecological components of assemblage patterns can be investigated using PVR by partitioning body size variation into phylogenetic and specific components and then obtaining mean values for these components within assemblages. Partitioning trait variation using PVR allows not only studying the spatial variation in mean body size across species assemblages (as in a classical assemblage-based approach), but also the mean S-component (PVR residuals). For instance, Diniz-Filho et al. (2009) showed that total mean body size in Carnivora decreases towards higher latitudes (see also Rodriguez et al. 2008), probably related to macroevolutionary events such as higher species turnover in these regions. On the other hand, the mean S-component, expressing deviations from expected body size due to phylogenetic effects, shows a clearer Bergmannian gradient, with large bodied species (or deviations from phylogenetic expectation) in colder parts of the world, reinforcing adaptive explanations linked with climatic variation.

However, and, admittedly, the estimation of the S-component is a key issue to accurately detect these ecogeographical patterns. Thus, our goal here is to examine the core of the PVR method, by comparing different approaches for eigenvector selection using several criteria. We also evaluate here the implications of these eigenvector selection approaches for detecting patterns (i.e. ecogeographical rules) by comparing the S-component derived from different PVR models with analogous results from two other methods, i.e. the autoregressive model (ARM) (Cheverud et al. 1985) and the phylogenetic generalized least-squares (PGLS) (Martins and Hansen 1997, Garland and Ives 2000, Rohlf 2001). Finally, we used simulations to evaluate the ability of PVR to quantify accurately the phylogenetic signal with different sets of eigenvectors and under different evolutionary scenarios (i.e. assuming traits evolving 1) independently among species, 2) under a neutral process and 3) under stabilizing selection processes).

Methods

Data and the basic PVR model

We use a global Carnivora database with log-transformed body size data for 209 species (see Diniz-Filho et al. 2009 for details) to illustrate the application of PVR and to evaluate the approaches used to select eigenvectors (see below). We used different sets of eigenvectors in an OLS multiple regression model of the form

$$\mathbf{Y} = \mathbf{X}\beta + \boldsymbol{\varepsilon}$$

where **X** contains a set of *k* eigenvectors from a phylogenetic distance matrix **D** derived from Bininda-Emonds' et al. (1999, 2007) supertree, obtained using a standard principal coordinate analysis (PCORD) (Legendre and Legendre 1998). The vector β comprises the partial regression coefficients, whereas $\boldsymbol{\varepsilon}$ represents the model residuals that can be equated with S-component, as in Cheverud's et al. (1985) ARM. The phylogenetic signal is given by the R^2 of the OLS model, and its statistical significance can be tested by F-statistics from ANOVA of the regression. However, all estimates in PVR depend on which combination of eigenvectors are used to define **X** and, thus, the critical issue in PVR consists in defining an approach to select the eigenvectors to be retained for further analyses.

According to recent applications of PVR in studying Bergmann's rule (Diniz-Filho et al. 2007, 2009), we also analyzed geographic patterns in the mean S-component by mapping them using assemblages from a total of 4031 cells (1° latitude × 1° longitude) covering the New World (see Diniz-Filho et al. 2009 for details). The idea is that Bergmann's rule can be interpreted as a recent non-phylogenetic (referred to here as 'adaptive') response in the mean S-component within cells, indicating independent responses in the species found in a region, that correlates positively with climate (see also Diniz-Filho et al. 2007, 2009, Ramirez et al. 2008, Terribile et al. 2009, Olalla-Tárraga et al. 2010). On the other hand, patterns in the P-component or total mean body size could go in the opposite direction, with large-bodied species being found, on average, at lower latitudes (see also Rodriguez et al. 2008). This geographic pattern might arise due to higher extinction rates and taxonomic turnover in the glaciated part of the world (Diniz-Filho et al. 2009). Clearly, using an approach that does not estimate correctly the phylogenetic and specific components in data could make it difficult to distinguish between adaptive responses and geographic patterns in extinction rates and taxonomic turnover.

Eigenvector selection approaches

We initially ran different PVR analyses (both in terms of calculating the S-components and mapping them), sequentially increasing the number of eigenvectors from $k = 1$ to 70, following the sequence of their associated eigenvalues. Although it is possible to extract 208 eigenvectors from the data set of 209 species, the initial set comprising the first 70 eigenvector explained 99% of the phylogenetic structure in **D** (i.e. the percentage of variation explained by the principal coordinate analysis applied to the squared phylogenetic distance matrix). Thus, this set was used as an initial cut-off for the comparative analyses. Beyond analyzing how models with increased number of eigenvectors perform according to different criteria (R^2, AIC, residual autocorrelation, type I error rate and so on – see below), the sequential addition of eigenvectors allow us to test two commonly used approaches to select eigenvectors or axes in multivariate analyses (see Peres-Neto et al. 2005 for an evaluation in the case of principal component analysis): 1) comparison of observed and a broken-stick distribution of eigenvalues and; 2) using a pre-established percentage of the trace of the distance matrix, e.g. 95% of the variation in the phylogenetic distances.

The purpose of PVR is to explain variation in a trait due to the structure of the phylogeny and to control for autocorrelation, thus the approaches described above tend to represent as best as possible the phylogenetic structure in respect to trait variation. However, traits frequently show complex patterns of variation among species, not evolving at a constant rate or under the same process along the entire phylogeny. Moreover, because there are also measurement errors both in the traits and in the branch lengths of the phylogeny, the use of all eigenvectors is seldom necessary to account for the phylogenetic patterns in trait variation. Thus, it is sensible to directly search for the eigenvectors (representing parts of the phylogenetic relationships) related to the trait(s) under study. Here we compare three non-sequential approaches under this framework. The first consists of using the eigenvectors that are significantly correlated ($p < 0.05$) with the trait (Diniz-Filho et al. 2009). The second uses an automatic stepwise procedure (because eigenvectors are orthogonal, forward and backward analyses provide identical results). Finally, we used an iterative search for the eigenvector that reduces the largest amount of autocorrelation in the residuals, as previously proposed in the spatial context (Griffith and Peres-Neto 2006). As new eigenvectors are added to the model, residuals are updated and autocorrelation re-estimated. The search stops when residual autocorrelation is reduced below an arbitrarily defined threshold for Moran's I or its statistical significance (here we used Moran's I smaller than 0.05 as a stopping rule).

Model evaluation and simulations

We evaluated the results of each PVR model using different criteria. First, we used the coefficients of determination (R^2) of the models as measures of goodness of fit. As a second criterion, we used the Akaike information criterion (AIC) to select the best model in terms of minimum adequate model (Diniz-Filho and Nabout 2009), which can be viewed as representing a compromise between the magnitude of phylogenetic signal captured by the regression and the number of eigenvectors used. To measure the amount of phylogenic structure of **D** retained by the eigenvectors, we also computed, for each set of eigenvectors, an Euclidean distance among species based on the selected eigenvectors, and correlated this matrix with the original matrix of phylogenetic distances (this is called cophenetic correlation in multivariate analyses – Sokal and Rohlf 1962, Legendre and Legendre 1998).

Gittleman and Kot (1990) pointed out that the assumption of independence must always be confirmed after model fit, because incorporating the phylogenetic matrix into a model does not ensure that residuals will be independently distributed, which is critical for the correct estimate of the S-component. Therefore, Gittleman and Kot (1990) suggested that Moran's I based correlograms (Legendre and Legendre 1998; see also Diniz-Filho 2001 and Pavoine et al. 2007 for recent interpretations) could be used as a diagnostic tool after fitting Cheverud's et al. (1985) ARM to check the validity of this assumption (i.e. phylogenetically independent S-component). The same reasoning applies to PVR, and thus we retained model residuals for evaluation using phylogenetic correlograms based on Moran's I coefficients calculated for five distance classes, establishing the significance of each coefficient using 500 randomizations.

We also evaluated the performance of each PVR model in estimating the amount of phylogenetic signal (i.e. the PVR's coefficient of determination, R^2) using simulations under three scenarios. The first scenario was generated by randomizing trait values (250 times) along the tips of the phylogeny. For each randomization, we estimated the R^2 of a regression between the resultant trait vector and the set of eigenvectors. By doing so, we derived the null distribution of R^2 expected in the absence of phylogenetic signal in the data. We then counted how many F-values of the OLS multiple regression were significant at the 5% level, which allows calculation of the type I error rate of the PVR in finding a significant phylogenetic signal when it does not exist.

In a second simulation scenario, we simulated a trait evolving under a pure neutral (Brownian motion) model (plus random errors, which would go to zero as the number of species increases). In this case, one could estimate the coefficient of determination estimated by PVR when all trait variation can be explained by phylogenetic relationships. Therefore, we implemented a total of 250 evolutionary realizations of the Brownian motion process in the PDSIMUL routine of PDAP (phenotypic diversity analysis program – Garland et al. 1993), and the distributions of R^2 and associated F-values were evaluated as before.

Changing the parameters of the Brownian motion simulation allows another evaluation of how PVR captures phylogenetic signal. The Brownian motion process can be viewed as a particular case of an Ornstein–Uhlenbeck (O–U) process, in which rather than evolving under a neutral process, a trait is subjected to stabilizing selection (Felsenstein 1988, Hansen and Martins 1996, Martins et al. 2002). This O–U process is expressed as a negative exponential relationship between the interspecific variance and time since divergence, with an alpha parameter that regulates the intensity of selection. Therefore, in a third simulation scenario we evaluated how increasing the intensity of selection in the model by adjusting alpha, ranging from 0 (Brownian motion) to 10 (strong stabilizing selection), affects the R^2 estimates (see Diniz-Filho 2001 for details of simulating O–U parameters).

Comparison with other methods

Finally, we correlated the S-components estimated by the PVR models with others methods, to evaluate how the similarity between these related methods is affected by PVR's eigenvector selection. We initially compared PVR with Cheverud's et al. (1985) autoregressive model (ARM), which also partitions T into P and S by fitting the model

$$\mathbf{Y} = \rho\mathbf{W}\mathbf{Y} + \varepsilon$$

where ρ is the autoregressive coefficient (the slope of **Y** against **Wy**), **W** is a weighting matrix derived from the phylogeny and ε represents the model residuals. The term $\rho\mathbf{WY}$ estimates the P-component as a linear combination of the trait that expresses the expected value of each species as a function of the values in all other species weighted by their phylogenetic distances. The residuals (ε) express the S-component and, again, the ratio P/T is estimated by the ρ^2, or the coefficient of determination (R^2) of this linear model, which is fitted using maximum likelihood estimation (Cheverud et al. 1985). Here we used a modified implementation of ARM by Gittleman and Kot (1990), where the **W**-matrix was given by $W_{ij} = 1/D_{ij}^2$, so that only small residual autocorrelation (estimated by Moran's $I = 0.073$, $p = 0.035$) remains after fitting the model.

Finally, because of discussion of the relationship between phylogenetic generalized least-squares (PGLS) and PVR (Adams and Church 2011, Freckleton et al. 2011), we also compared S-component from PVR with a PGLS transform of log-body size, following Garland and Ives (2000, p. 361). The idea is to calculate a vector **Z**, given by

$$\mathbf{Z} = \mathbf{DY}$$

where **D** comes from a singular value decomposition of the form

$$\mathbf{DCD}^{\mathrm{T}} = \mathbf{I}$$

and **C** is the phylogenetic covariance among species (**I** is an identity matrix). So, the vector **Z** can be interpreted as the values of the trait that would be expected if species were phylogenetically independent and hence can be used in any other statistical analysis (see also Butler et al. 2000). Thus, this **Z**-vector can be roughly comparable to PVR's S-component. Because body size can evolve under more complex models than simple Brownian motion, we fitted the PGLS model by searching for the λ that maximizes the likelihood of the model, and compared it with a null model of absence of signal and with a Brownian model ($\lambda = 1$) (Freckleton et al. 2002).

Results

Sequential approach

The first 25 eigenvalues of the PCoA accounted for 95% of the variation among the distances contained in **D**. Only the first six eigenvectors would be selected for PVR according to the broken-stick approach. However, residuals resulting from regressing body size against these first six eigenvectors were strongly autocorrelated ($I = 0.525$, $p < 0.001$), and the model had a low coefficient of determination ($R^2 = 0.455$). On the other hand, when selecting the 25 eigenvectors that explain 95% of the variance, all criteria suggest that PVR model falls within the interval of k for which all estimates start to be stable and produce similar results. These models possess R^2 values always higher than 0.75, and especially with a non-significant Moran's I for S-component (Table 1).

It is clear that increasing the number of eigenvectors k results in an increase in the coefficient of determination, as pointed out by Rohlf (2001) and expected for any multiple regression (although adjusted-R^2 may be less sensitive to this). However, after adding the first 25–30 eigenvectors, the cophenetic correlations are all very high (i.e. $r \geq 0.99$), implying that these eigenvectors tend to summarize efficiently the main patterns of phylogenetic relationships among the species. Also, AIC indicates that the minimum adequate model must add sequentially 30–40 eigenvectors (the delta AIC between the models with 25 and 30 eigenvectors is relatively large, but not between those models with 30 and 40 eigenvectors), which is similar to the minimum number of eigenvectors needed to remove all autocorrelation from the residuals (i.e. about 25 eigenvectors).

The absence of phylogenetic autocorrelation is critical to ensure that species are independent so that S-component can be used for further statistical analyses. Note that when increasing the number of eigenvectors (e.g. $k \geq 40$) the residual autocorrelation becomes increasingly negative and statistically significant. Although not intuitive, this result indicates that the data provide more information (i.e. there are more degrees of freedom) than would be obtained from uncorrelated residuals, so this can be interpreted as an overcorrection or overfitting problem (Table 1).

The spatial patterns in mean body size and mean S-component possess very low correlations with environmental predictors (Diniz-Filho et al. 2009). However, the maps of the S-components (Fig. 1) clearly show the impacts of an inefficient partition of phylogenetic and specific components resulting from selecting too few eigenvectors (e.g. Fig. 1B). It is possible to see that mean body size does not show strong geographical patterns, although there is a tendency for species to be, on average, larger in the tropics than

Table 1. Coefficients of determination (R^2) and F-statistics evaluating the significance of each phylogenetic eigenvector regression (PVR) between Carnivora body size (log-transformed) and variable numbers of eigenvectors (k) under sequential and non-sequential approaches for eigenvector selection. The columns also contain the Moran's I of the model residuals estimated for the first distance class (IRES, and their associated p-values), the correlation of the S-components estimated by PVR with the S-component from autoregressive model ($r_{PVR,ARM}$) and with the **Z**-transform from PGLS ($r_{PVR,PGLS}$), correlation between the original phylogenetic matrix and the Euclidean matrix calculated with base on the selected eigenvectors (r_{coph}) and Akaike information criterion (AIC) of the phylogenetic regression models. Non-sequential eigenvector selection approaches include eigenvectors significantly related with body size at $p < 0.05$ (ESRBS), eigenvectors selected by a standard stepwise algorithm (STEP), and eigenvectors selected to minimize the phylogenetic autocorrelation of the residuals (MINI).

Approaches	k	R^2	F	IRES	p	$r_{PVR,ARM}$	$r_{PVR,PGLS}$	r_{coph}	AIC
Sequential	1	0.02	3.1	0.701	<0.001	0.73	0.799	0.86	408.50
	5	0.40	26.8	0.434	<0.001	0.80	0.833	0.98	314.90
	10	0.57	26.0	0.405	<0.001	0.77	0.826	0.99	255.79
	15	0.62	21.8	0.122	<0.001	0.79	0.839	0.99	238.71
	20	0.69	20.6	0.080	<0.001	0.81	0.835	0.99	212.44
	25	0.76	23.5	−0.036	0.487	0.75	0.826	0.99	206.28
	30	0.78	20.6	−0.070	0.256	0.73	0.82	1.00	168.78
	40	0.81	17.5	−0.108	0.006	0.70	0.797	1.00	168.29
	50	0.82	14.5	−0.116	0.002	0.69	0.776	1.00	184.86
	60	0.84	13.0	−0.126	<0.001	0.65	0.765	1.00	199.05
	70	0.86	11.6	−0.135	<0.001	0.64	0.753	1.00	223.20
Non-sequential									
ESRBS	27	0.82	29.9	−0.096	0.014	0.70	0.786	0.99	118.56
STEP	36	0.84	24.3	−0.122	0.001	0.69	0.782	1.00	126.43
MINI	14	0.70	31.6	0.046	0.172	0.77	0.818	0.96	191.00

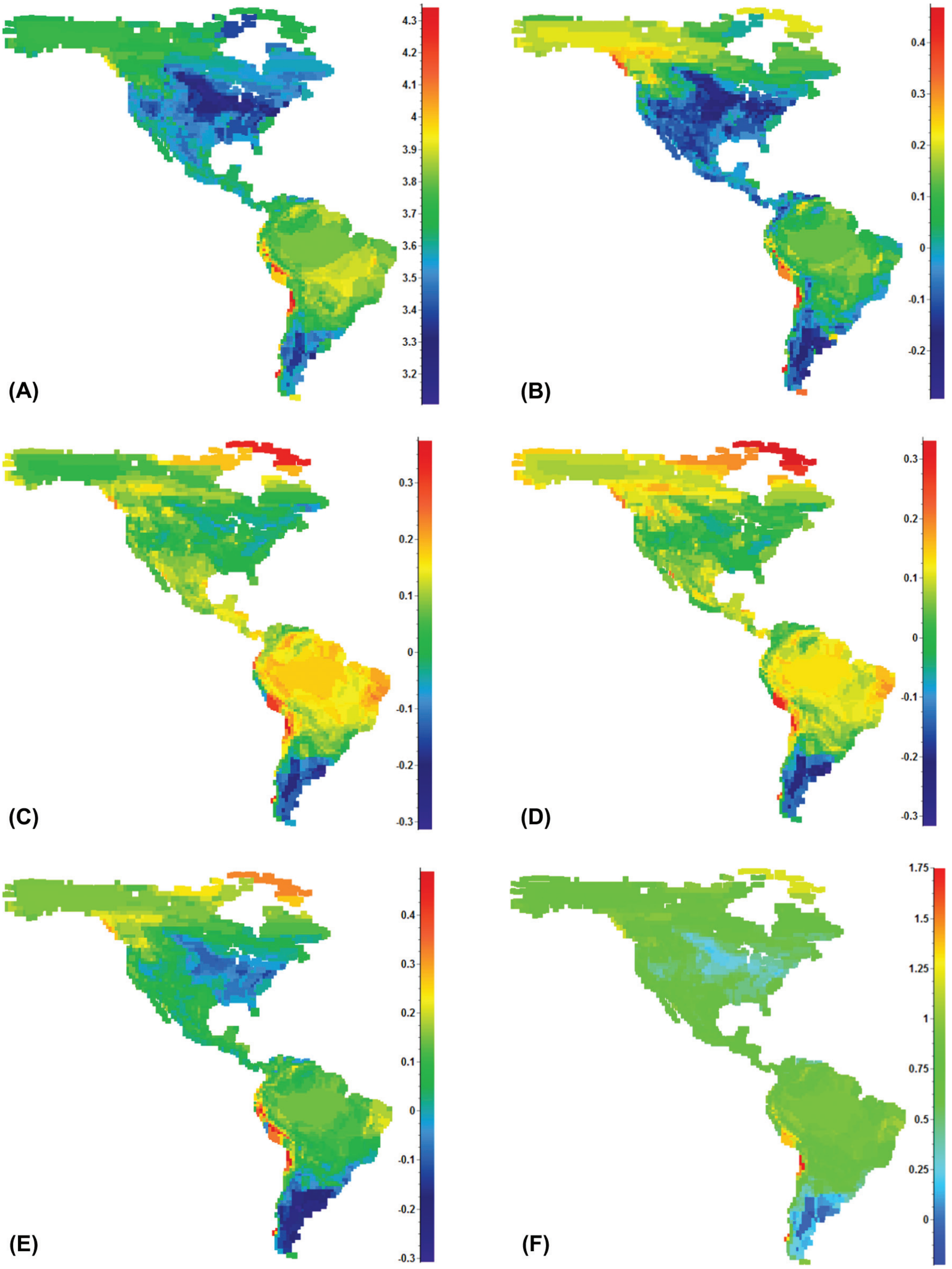

Figure 1. Spatial variation of mean body size of Carnivora assemblages in the New World (A) and examples of mean S-components of PVR estimated using different sequential and non-sequential approaches, including the first 5 eigenvectors (B), the 25 first eigenvectors (C), and the eigenvectors that minimize residuals Moran's I (D). For comparison, (E) contains the mean S-component derived from autoregressive method and (F) contains the mean **Z**-vector from PGLS.

in the North America. When only a few eigenvectors are used, there is also no evidence of Bergmann's rule, because the S-component is not phylogenetically independent and, thus, patterns are still similar to those found for mean total body size. However, when averaging S-components, mainly when $k > 20$, this result tends to invert, and a Bergmann-like pattern is clearer, mainly in northeastern North America (Fig. 1C).

Also, in this range (i.e. $k > 20$), PVR results are similar to those obtained using ARM and PGLS, as expected by the correlation among the S-components with results from the two methods (**Z**-vector in the case of PGLS; Fig. 1F). The best PGLS model for body size was obtained using a λ of 0.936. As, as expected, the PGLS **Z**-vector is not autocorrelated (Moran's $I = -0.055$; $p = 0.108$). In general, correlations between PVR's S-component, estimated with different sets of eigenvectors, and the PGLS's **Z**-vector were around 0.8, slightly higher than with ARM's S-component (Table 1). The correlation between PGLS **Z**-vector and S-component from ARM was even higher, and equal to 0.905. All of these correlations are even higher when an assemblage approach is used, after averaging the phylogenetic-free values estimated by both methods within cells (for example, with $k = 25$, the correlation between these values was equal to 0.826 across species, but increased to 0.882 across space).

Non-sequential approaches

All three non-sequential approaches provided good results according to the model evaluation criteria previously discussed for sequential approaches (Table 1). However, the R^2 values tend to be slightly higher when using a stepwise multiple regression than when utilizing the sequential approaches (with similar *k*).

It is important to note that using the 27 eigenvectors that are significantly correlated with body size produced the smallest AIC value, and therefore the minimum adequate model, among all compared models (Table 1). To achieve a comparable R^2 (≈ 0.8) using the sequential criterion it would be necessary to add about 50 eigenvectors. Despite being similar the minimum adequate model, this PVR model with 50 eigenvectors possess autocorrelated residuals, but with large negative Moran's I value in the first distance class (as expected for over-fitted models). This also occurs when using a stepwise multiple regression, which produced the model with the second smallest AIC value, but also with negative and significant autocorrelation in the residuals.

The criterion that minimizes Moran's I in the residuals yields a model with only 14 eigenvectors, and with small autocorrelation. The R^2 is not as high as in the other non-sequential models (0.70), but still much higher than expected for a sequential model with similar *k* (i.e. under sequential approach approximately 20 eigenvectors would be needed to achieve a similar R^2; Table 1). Mean S-components were also stable and similar to geographic patterns from other PVR models using the sequential approach.

Simulations and evolutionary models

Even under a null model of no phylogenetic signal in the data, there is a clear increase in the median value of R^2 as the number of phylogenetic eigenvectors increases (as expected in any multiple regression). The same pattern emerges when the non-sequential approaches were used. However, in both cases, type I error rates after simulating random values across the phylogeny are rather close to the expected value of 5% and have no relationship with *k* (Table 2). The same stability after $k > 20$ was observed for the R^2 according to the Brownian model of trait evolution, which increases slowly and is around 0.81 when 30 eigenvectors are used in the multiple regression model. There is also a more or less constant ratio between observed and expected (under a Brownian motion model) values of R^2 of around 95%.

Increasing the intensity of stabilizing selection in the O–U model by adjusting the alpha-parameter reduces monotonically the R^2 estimated by PVR independently of the criterion used to select eigenvectors (Fig. 2). Based on those criteria for which no residual autocorrelation appears, the mean R^2 dropped from 0.73 under a pure Brownian motion process (when alpha = 0), to 0.35 when alpha is equal to 10.

Discussion

The issue of eigenvector selection

Because PVR was proposed as an alternative to Cheverud's et al. (1985) autoregressive model to partition trait variation into P- and S-components based on an eigen-analysis of phylogenetic distances, the most important issue in PVR is how to determine which eigenvectors will be used in the analyses. However, this issue has been rarely discussed in the literature, both for spatial and phylogenetic eigenvector methods.

The primary counterintuitive aspect in the phylogenetic context is that, although all eigenvectors are indeed necessary to represent the entire phylogeny (as correctly pointed out by Rohlf 2001), not all of them are necessary to remove phylogenetic autocorrelation in a particular trait. The distribution of eigenvalues and the ability of the first eigenvectors to describe the relationships among species (i.e. to describe what can be called 'the geometry of relationships') will depend on phylogeny properties, such as balance and stemminess. However, the phylogenetic signal will emerge

Table 2. Type I error rates, median coefficients of determination (R^2) and critical values of R^2 (at confidence level of 95%) obtained by phylogenetic eigenvector regressions (PVR) after randomly reshuffling Carnivora body size (log-transformed) along the tips of the phylogeny. Different approaches for selecting eigenvectors were used (Table 1). Also shown are the median coefficients of determination (R^2) obtained under a Brownian motion process.

			R^2		
Approaches	*k*	Type I error	Null	95%	Brownian
Sequential	1	0.040	0.002	0.018	0.184
	5	0.040	0.022	0.049	0.541
	10	0.050	0.043	0.082	0.652
	15	0.030	0.070	0.094	0.750
	20	0.050	0.090	0.147	0.754
	25	0.060	0.119	0.179	0.778
	30	0.030	0.140	0.200	0.811
	40	0.050	0.200	0.239	0.845
	50	0.040	0.259	0.270	0.856
	60	0.035	0.300	0.295	0.866
	70	0.040	0.346	0.312	0.870
Non-sequential					
ESRBS	27	0.055	0.127	0.190	0.769
STEP	36	0.040	0.172	0.243	0.816
MINI	14	0.060	0.065	0.117	0.635

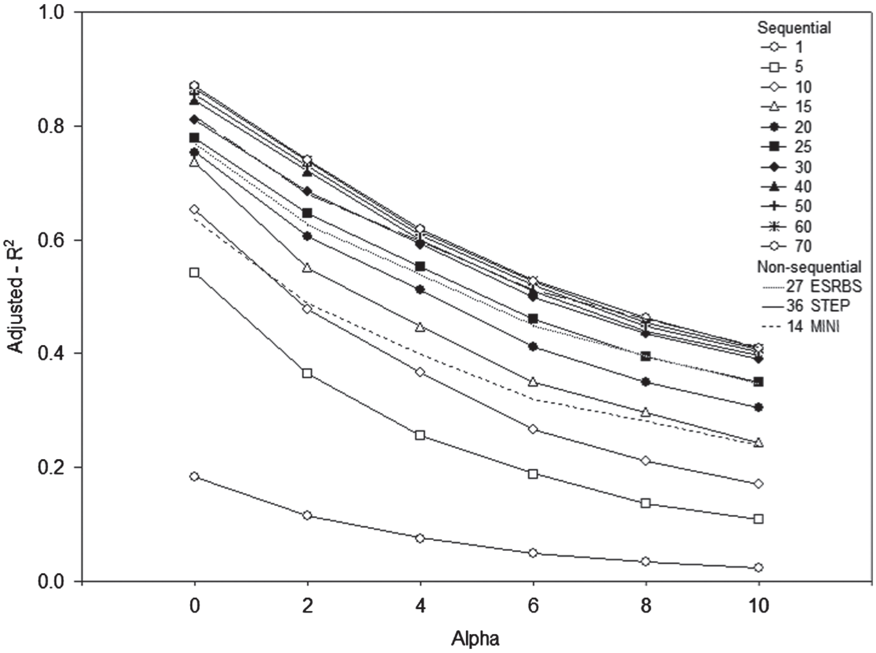

Figure 2. Variation in the coefficient of determination obtained by a phylogenetic eigenvector regression with increasing intensity of selection (as regulated by the alpha parameter) and according to different approaches for selecting phylogenetic eigenvectors. Inserts are the numbers of eigenvectors.

in this phylogeny as a combination of phylogeny shape and evolutionary dynamics of traits (i.e. the evolutionary model), so we may think of at least three reasons why not all eigenvectors are necessary in a PVR analysis: 1) only parts of the phylogeny that are relevant to trait evolution need to be incorporated into the OLS model, especially if traits evolve under complex patterns along the phylogeny, and 2) even if the trait varies continuously with phylogeny from the root to the tips under simple models such as Brownian motion or O–U process, a few eigenvectors can capture most of this interspecific trait variation if the geometry of the relationships creates very strong structures at the base of the phylogeny (e.g. very distinct clades that diverged for the trait). Moreover, 3) measurement errors in both the data and the phylogenies exist, so it is expected that some eigenvectors – especially those representing short branch lengths, which are more difficult to estimate and are more prone to error – will not be useful, also because they will account for small accumulated variation in trait.

Moreover, regarding Rohlf's (2001; see also Freckleton et al. 2011) criticisms, it is important to emphasize that one can consider PVR adequate to model trait variation only when the values in the S-component are independent among species. This can be tested by phylogenetic correlograms, as originally suggested by Gittleman and Kot (1990) for the ARM. PVR will incorrectly estimate the strength of the phylogenetic signal when, for example, few eigenvectors representing the base of the phylogeny are used to describe the patterns and few species differ greatly from this expectation due to very recent adaptations. In this case the R^2 will be an underestimate because eigenvectors describing the part of the phylogeny when recent shifts in species' trait occurred will be missing (Monteiro and Abe 1999). The same will happen under phylogenetic non-stationarity, in which processes change through the phylogeny (Diniz-Filho et al. 2010) and create more complex and unexpected (by the phylogeny) interspecific variation. However, all comparative methods are subject to this problem and, in PVR and other partitioning methods, the problem can be easily detected by a careful analysis of model residuals.

Our analyses reveal that both sequential and non-sequential approaches may be useful to select eigenvectors, and results tend to be robust (at least in terms of the magnitude of phylogenetic signal and estimated S-components; Table 1) for some ranges of *k* values, especially considering selection procedures that yield models with residuals with no autocorrelation. As a consequence, geographical analyses of mean S-component, which have recently used to evaluate multiple processes underlying Bergmann's rule, are also stable after a critical number of eigenvectors is selected. Correlations with ARM and PGLS are also relative high and show qualitatively similar patterns, being all very different from those ignoring phylogeny and mapping mean body size. Therefore, recent criticisms by Adams and Church (2011) and Freckleton et al. (2011) should be viewed with caution, suggesting that particular cases and troubles in the selection of eigenvectors used in the models may lead to a misleading comparison between methods.

Even so, although it is advantageous for practical purposes that results are not very dependent on which eigenvectors are selected, it is important to provide guidelines for eigenvector selection. These guidelines will depend on the statistical comparison of models shown in Table 1, and on the definition of the most important criteria for PVR model.

In a theoretical (and statistical) sense, the first and most important critical diagnosis for most applications (i.e. correlated evolution and phylogenetic signal) is the evaluation of residual autocorrelation, and this should be done whatever approach is adopted. The sequential approach of successively adding eigenvectors until the Moran's I of residuals becomes non-significant or reach values below a defined critical level (Diniz-Filho and Tôrres 2002) is an initial option, and relatively easy to compute, but it is much less effective than other more computationally intense approaches, such as directly minimizing residual Moran's I. Indeed, the sequential method required at least 20 eigenvectors to reduce autocorrelation below 0.05, whereas the iterative search method required only fourteen eigenvectors to achieve similar results.

The AIC criterion also favors the approach of including eigenvectors significantly related with body size and the stepwise approach, when compared to sequential approaches. However, using eigenvectors that are correlated with **Y** and stepwise selection tend to produce over-fitted models (especially stepwise). Thus, AIC tends to favor models with more eigenvectors than necessary to take autocorrelation into account, which can be problematic to perform further analyses with S-component. The iterative search for low residual autocorrelation approach represents a good compromise between model explanation and ensuring independence among species.

The phylogenetic signal

When the S-component is independent among species, by definition the ratio in the variances of P/T expresses the phylogenetic signal in data. Thus, the R^2 of PVR estimates the amount of phylogenetic signal in data, or, more explicitly, the proportion of variation in a trait that is explained by the phylogenetic distances (represented by a few eigenvectors, which implies in some loss of information) among species. However, as pointed out by Rohlf (2001), although the R^2 expresses the part of the trait variation described by the selected phylogenetic eigenvectors, it is clear that this interpretation may be also related to how eigenvectors describe the geometry of the relationships as well. Thus, the amount of signal must be interpreted considering how well the phylogeny is represented by a relatively small number of eigenvectors, which in turn depends on tree topology and stemminess.

Other methods have been proposed to estimate the phylogenetic signal, including ARM (Cheverud et al. 1985, Gittleman and Kot 1990), Moran's I coefficients (Diniz-Filho 2001), Blomberg's et al. (2003) *K*-statistics, and Pagel's (1999) λ, but a comparative evaluation of these estimates has yet to be performed. As a reference, the R^2 estimated by the ARM used here to compare the S-components was 0.585, not very different from the value obtained by PVR using the optimization approach ($R^2 = 0.7$). Other methods to estimate phylogenetic signal are also popular in ecological analyses, especially Pagel's (1999) λ (see also Freckleton et al. 2002), which was used here to fit PGLS and was equal to 0.936, suggesting a strong phylogenetic signal in body size. But this coefficient actually measures the departure from a Brownian motion model, not being strictly comparable to the R^2 of a PVR or ARM (Housworth et al. 2004). Although this has yet to be demonstrated, the λ could be comparable to the ratio between the observed PVR's R^2 and the one expected under Brownian motion. If this is the case, $\lambda = 0.936$ is close from the ratios obtained when k is around 20 (i.e. equal to 0.976).

One of the difficulties in testing the robustness of methods used to estimate phylogenetic signal consists in simulating trait evolution with a predefined value of phylogenetic signal, which in turn must be measured using a given criterion. Diniz-Filho (2001) showed that there is a monotonic relationship between increasing strength of stabilizing selection in O–U models (which decrease the phylogenetic signal) and Moran's I, and the analyses performed here show that the same relationship between strength of O–U and signal can be detected using PVR's R^2 (Fig. 2). This indicates that PVR captures well the magnitude of phylogenetic signal in data, because increasing the adaptive strength in simulation models (α; Martins et al. 2002), which causes in turn a loss of 'deep-time' historical signal, is well expressed in PVR by a reduction of R^2. Of course, it is possible to infer that different sets of eigenvectors would be necessary to show how 'short-time' historical signal (generated by higher alphas in an O–U process) affect trait variation. These processes would be better modeled by eigenvectors associated with low eigenvalues, which are usually not used in the sequential selection procedures (and would be even among those excluded from our initial set of 99% of the trace). However, if this 'short-time' signal is present, the first eigenvectors should not be selected for modeling phylogenetic effects because any 'deep time' historical signal was deleted by selective process under O–U dynamics, thus dropping the final PVR's R^2 anyway. Further investigations about how eigenvector selection captures different and more complex evolutionary models may be then necessary.

Using randomly reshuffled data, we have shown that PVR gives the correct type I error rates, which also tend to be independent of eigenvector selection. Thus, in the analysis of empirical data, a non-significant relationship between a trait and a set of eigenvectors (as tested by the F-statistics) would be sufficient to indicate the absence of phylogenetic signal in those data. In addition, authors should provide the range of R^2 values obtained after randomly shuffling values over the phylogeny. The observed R^2 resulting from regressing a trait against the selected set of eigenvectors can then be compared to that range of values obtained under the null simulations. If the observed value is clearly within this range, then any further interpretation on the level of phylogenetic signal and on the P- and S-components would be misleading.

Our analyses confirm that PVR is a useful technique for comparative analysis, despite criticisms and the lack of an explicit evolutionary model (Rohlf 2001, Adams and Church 2011, Freckleton et al. 2011). However, even if PVR was found to be robust with respect to selection approaches, our analysis suggests that a poor choice of eigenvectors, particularly when only a few of them are used to represent the phylogeny, can have serious impacts on the ecological interpretation of the variance partitioning. We claim that optimization approaches that search for the minimum Moran's I provide the best compromise between the amount of phylogenetic signal, independence of S-component values and number of eigenvectors used. Moreover, our results can also shed some light on how to use other eigenfunction methods that have been used to take phylogenetic, spatial and temporal autocorrelation into account.

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