Phylogenetic ANCOVA: Estimating Changes in Evolutionary Rates as Well as Relationships between Traits

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ABSTRACT: We present a new phylogenetic comparative method—phylogenetic analysis of covariance (PANCOVA)—that uses interspecific data and a phylogeny to estimate the effects of major events on both the rate of phenotypic evolution and the association between traits. It could be used, for example, to model the impact of a key innovation, colonization of a new habitat, or environmental change. The approach is optimized with maximum likelihood and is formulated under the familiar phylogenetic generalized least squares framework, which is flexible and easily extended to incorporate other factors and parameters. As an example, we explore the relationship between parental investment and relative telencephalon size in birds and contrast the results of PANCOVA with those from other phylogenetic comparative methods.

Keywords: analysis of covariance, birds, evolutionary rates, maximum likelihood, phylogenetic comparative methods, phylogenetic generalized least squares.

Introduction

Major evolutionary forces that lead to bursts of phenotypic diversification (e.g., key innovation, colonization of a new habitat, environmental change) may also lead to changes in the relationships between diversifying traits. Many factors including colonization of coral reefs (Price et al. 2011), evolution of piscivory (Collar et al. 2009), and changes in pollinators (Roalson and Roberts 2016) have recently been proposed as forces driving major changes in the rate of phenotypic diversification. We might expect such forces to also lead to major changes in the relationships between diversifying traits such as limb proportions (Polly 2010), brain-to-body-size ratios (Finarelli and Flynn 2009), or relative metabolic rates (Santos and Cannatella 2011). We can apply phylogenetic comparative methods to data from extant species to infer major shifts in diversification rate (O'Meara et al. 2006; Thomas et al. 2006) or in the relationships between traits (Felsenstein 1985; Martins and Hansen 1997). Here, we propose a phylogenetic comparative method that infers the impact of a single factor on both diversification rates and phenotypic relationships simultaneously.

Our method could be applied, for example, to the question of brain size evolution. The evolution of sociality and the high cognitive demands of social living have often been suggested as drivers of the evolution of large vertebrate brains (Dunbar 1992; Shultz and Dunbar 2006; Pérez-Barbería et al. 2007). Although group size is not always a good predictor of relative brain size (Beauchamp and Fernández-Juricic 2004), more precise measures of social cohesion, such as the amount of biparental care, appear to be associated with the relative size of specific brain regions, at least in birds (Shultz and Dunbar 2010). We may also want to ask more specifically whether the gain of sociality was a type of key innovation, leading to an overall increase in brain size disparity. In addition, evolutionary changes in sociality may lead to shifts in the relative size or allometry of particular brain regions, especially in regions involved in cognitive processing such as the telencephalon. Ideally, we would ask both questions simultaneously, thereby also determining which pattern was a better descriptor of the relationship between sociality and brain size across long periods of evolutionary time.

Several phylogenetic comparative methods have been developed to address the first question about phenotypic diversification rates in a single, continuous, trait. Early approaches focused on estimating the phenotypic variance among species across an entire clade (e.g., Lynch 1991; Foote 1993; Martins 1994), and more recent methods offer ways to compare that variance in different parts of a phylogeny (e.g., O'Meara et al. 2006; Thomas et al. 2006; Eastman et al. 2011). The most recent approaches provide estimates using different assumptions about the underlying evolutionary process (Beaulieu et al. 2012) and with uncertain phylogenetic information (Eastman

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et al. 2011; Revell et al. 2012). Other methods are needed, however, when the question involves relative sizes or the relationship between two or more continuous traits. For example, Revell and Collar (2009) propose a method that describes the relationship between two traits as an evolutionary rate matrix and then tests whether that matrix shifts in different parts of a phylogeny.

Early attempts to study relative sizes in a phylogenetic context started by calculating simple ratios (e.g., brain size divided by body size; Van Valkenburgh and Ruff 1987; Lindenfors 2006; Shultz and Dunbar 2006). However, ratios can lead to precision and accuracy problems (Packard and Boardman 1988, 1999; Jasieński and Bazzaz 1999). One popular alternative is to regress one trait on the other (e.g., brain size [y] on body size [x]) in a phylogenetic regression to extract residuals and then to use those residuals as the new response variable in a phylogenetic ANOVA (e.g., Garland et al. 1992; Revell 2009; Mahler et al. 2010). Expanding this approach to an analysis of covariance (ANCOVA) or multiple regression, a researcher can also test for changes in the relationship between the two traits over evolutionary time (García-Berthou 2001; Freckleton 2002). Phylogenetic ANCOVAs have been applied to interspecific data using Monte Carlo simulation (Garland et al. 1993), phylogenetic generalized least squares (Butler et al. 2000; Lavin et al. 2008), and adaptation-inertia models (Escudero et al. 2012), but all of these applications assume a single diversification rate across the entire phylogeny.

Here, we develop a comprehensive procedure in which both phenotypic diversification and trait relationships can be estimated simultaneously. Our phylogenetic ANCOVA (PANCOVA) combines a phylogenetic generalized least squares (PGLS) model of the relationships between two or more traits with maximum likelihood estimates of unequal evolutionary rates in different parts of the phylogeny. This combination of PGLS and likelihood approaches is familiar enough to encourage complex model fitting and flexible enough to open the door to future extensions incorporating a variety of microevolutionary assumptions and selective perspectives (e.g., Hansen 1997; Martins and Hansen 1997; Hansen et al. 2008). Below, we illustrate the PANCOVA approach (and several alternative approaches) with an example based on the above question of whether evolutionary changes in sociality drive evolutionary changes in brain size.

The PANCOVA Model

We begin with a PGLS framework that places the phylogeny and assumptions about the rates of evolutionary change in a complex error term (Hansen and Martins 1996; Martins and Hansen 1997):

$$y = \beta_0 + \beta_1 x + \beta_2 z + \beta_3 xz + \varepsilon,$$
$$\varepsilon \sim \mathrm{MVN}(\mathbf{0}, \mathbf{V}). \tag{1}$$

The primary trait of interest is given by y, a vector containing species means for the continuous response variable (e.g., telencephalon size). The ANCOVA is specified in the mean structure of the model (i.e., $y = \beta_0 + \beta_1 x + \beta_2 z + \beta_3 xz$) describing how the continuous and categorical variables are associated with each other. As in any ANCOVA, variation in the response variable is predicted by at least two explanatory vectors: the covariate, z (e.g., body size), and a categorical factor that codes for the critical historical event, x (e.g., sociality). The interaction term, xz, estimates a possible difference in the relationship between the response and covariate terms (relative telencephalon size) in different parts of the phylogeny indicated by the categorical factor (sociality). We estimate the regression coefficients: β_0 and β_2 for the global intercept and slope, as well as β_1 and β_3 for a possible change in the intercept or slope (respectively) for taxa in one of the two states identified by the categorical factor. Finally, ε is a vector of multivariate normally distributed error terms with a mean of 0 and variance $\mathbf{V}$, a matrix that describes the expected similarities due to shared evolution along a phylogeny.

Besides the phylogeny, the $\mathbf{V}$ matrix incorporates a model of evolutionary change (Hansen and Martins 1996). Under Felsenstein's (1985) classic Brownian motion model of phenotypic evolution under neutral or simple directional selection, the variance between any two species (i and j) is $\mathbf{V}_{ij} = \gamma\, t_a$, where γ is the amount of new variation added each generation and t_a is the phylogenetic distance from the root to the most recent common ancestor of taxa i and j (Martins and Hansen 1997). When phylogeny has little or no effect on phenotypic variation, a nonphylogenetic approach (Martins and Garland 1991) specifies $\mathbf{V}_{ij} = \gamma\, \mathbf{I}$, where $\mathbf{I}$ is the identity matrix . There are many other possibilities. For example, when using ultrametric trees scaled to a height of one, we can also specify $\mathbf{V}_{ij} = \lambda \gamma\, t_a + (1 - \lambda)\mathbf{I}$, where λ is a measure of phylogenetic signal (Pagel 1999*a*; Freckleton et al. 2002; de Villemereuil et al. 2012) and is equivalent to the phylogenetic heritability of the phylogenetic mixed model (Housworth et al. 2004; Hansen and Orzack 2005; Hadfield and Nakagawa 2010; Ho and Ané 2014). When $\lambda = 0$, change only happens at terminal branches, and the model reduces to a nonphylogenetic approach; when $\lambda = 1$, evolutionary change happens at a constant rate through the tree, and the model reduces to Brownian motion.

To also allow a possible shift in the rate of phenotypic diversification, we apply different values of the rate constant (γ) to different parts of the phylogeny when constructing the $\mathbf{V}$ matrix. First, we specify ancestral states of the categorical factor, describing the trait along each branch of the phylogeny as being in one or the other state of that categorical factor (e.g., social or nonsocial). This is comparable to the noncensored approach of O'Meara et al. (2006). We then add an optional parameter (g) corresponding to the proportion of

each transitional branch that is evolving in each regime. Specifically, $g = 1$ indicates that the shift to the new state of the categorical factor happened immediately after the branching event, whereas $g = 0$ indicates that the shift happens only at the end of the branch, just before it splits again.

In summary, parameters for detecting unequal evolutionary rates under different models are embedded in the error term (ε), whereas parameters for estimating a shift in trait covariation are embedded in the mean structure (βs). A full model considering the predictive value of a categorical factor with two states would include six parameters. There are two β parameters describing the intercept (β_0) and slope (β_1) of a global regression line and one γ parameter (γ_0) describing the global diversification rate. Two additional β parameters (β_1, β_3) estimate the additional amounts needed if the relationship between y and z differs in the two parts of the phylogeny described by the categorical factor (x). Also, a second γ parameter (γ_1) estimates the additional variance needed to describe a change in diversification rate with the categorical factor. In addition, we include two parameters in forming the matrix, $\mathbf{V}$, from distances extracted from the phylogeny: g (indicating at which time point along each branch the historical shift occurs) and λ (on models accounting for phylogenetic signal).

We can estimate the likelihood of a model containing any subset of these parameters using the standard PGLS equation

$$\log(L) = \log\left[\frac{\exp\{-1/2[y - X\beta]'(V)^{-1}[y - X\beta]\}}{[(2\pi)^N \times \det(V)]^{1/2}}\right], \quad (2)$$

where $\mathbf{X}$ is the design matrix with explanatory variables (for eq. [1], the first column would be a vector of ones, followed by x, z, and xz), β is a vector of regression coefficients (for eq. [1], $\beta_0, \beta_1, \beta_2$, and β_3), and N is the number of extant taxa. To estimate parameters, we optimize equation (2) using standard procedures, such as Powell's algorithm, Markov chain Monte Carlo, grid search, or sensitivity simulations.

A Worked Example

To illustrate the PANCOVA, we used the example questions and data developed by Shultz and Dunbar (2010) to ask whether evolutionary shifts in sociality are linked to changes in relative brain size and phenotypic diversification in birds. Shultz and Dunbar (2010) acknowledged that the tree they used for analyses was controversial, so for our example, we worked with a pruned tree (fig. 1) from Hedges et al. (2015) instead. The tree results from a synthesis of studies in molecular evolution and phylogenetics reporting time of divergence among species (Hedges et al. 2006; Hedges and Kumar 2009) that was used to estimate a unique bird tree calibrated to time as described in Hedges et al. (2015). Following Shultz and Dunbar (2010), we choose a measure of sociality (i.e., biparental care) informative of bondness and
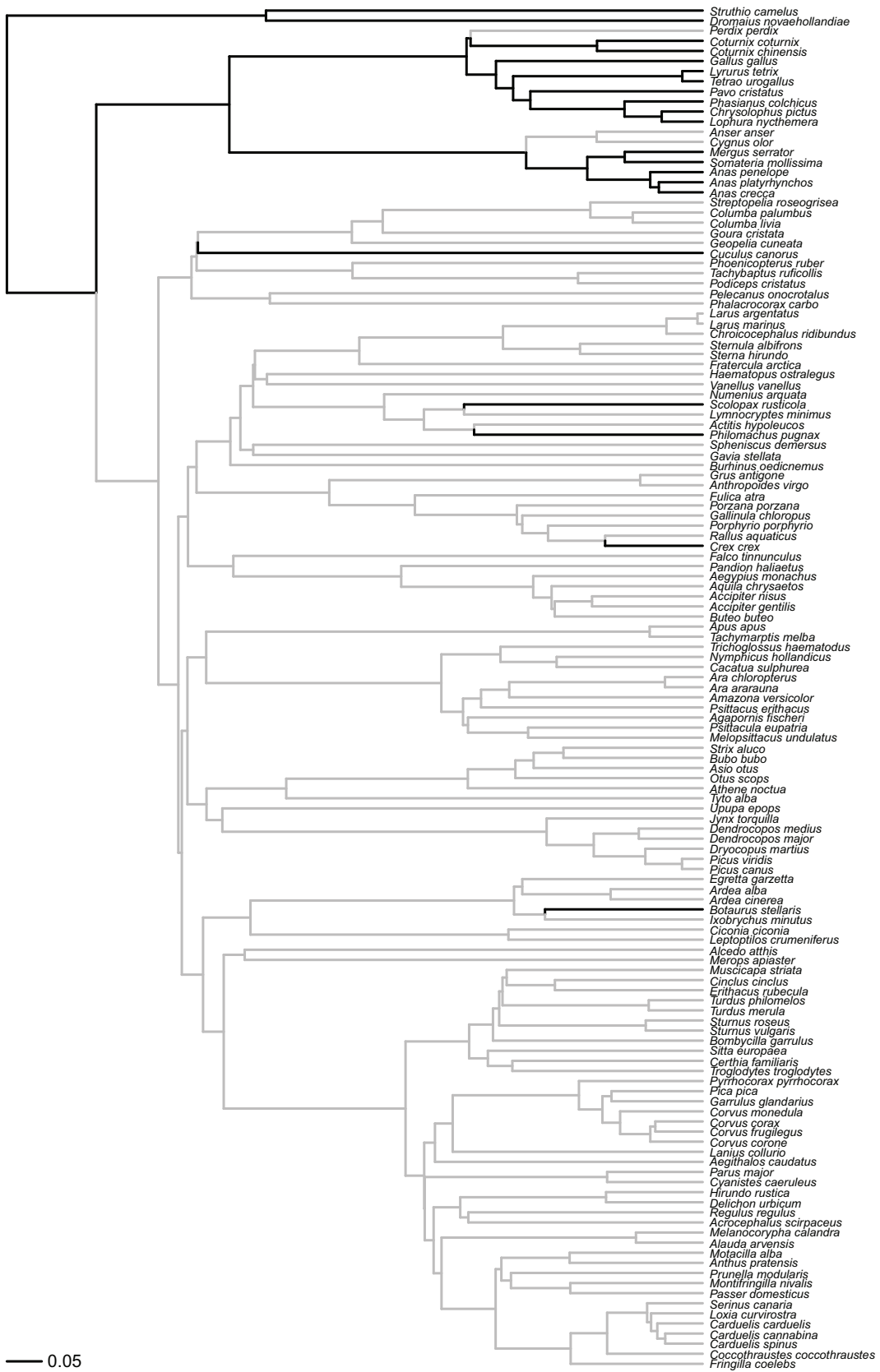
stable relationships, instead of the traditional flock size (e.g., Beauchamp and Fernández-Juricic 2004). Although most birds exhibit biparental care, uniparental care is the ancestral state, and there have been several reversals across all birds (fig. 1). Our question is whether these evolutionary changes in social behavior are associated with changes in relative brain size. We modeled telencephalon mass as the primary response (y), with body mass as a covariate (z) and biparental care as a categorical indicator of sociality (x). Note that both telencephalon mass and body mass were extracted from Shultz and Dunbar (2010), where they had been log transformed. Both data and phylogeny are deposited in the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.2r504 (Fuentes-G. et al. 2016).

We estimated parameters by using Powell's (1964) method to optimize the likelihood (eq. [2]) for three models: (1) the full PANCOVA model (two intercepts, two slopes, two diversification rates), (2) a simple ANCOVA with a single diversification rate (two intercepts, two slopes, one diversification rate), and (3) a simple linear regression (one intercept, one slope, one diversification rate). For each of these models, we estimated parameters assuming Brownian motion, no phylogeny, and phylogenetic signal (λ) models and compared results using the second-order bias correction version of Akaike information criterion (AICc; Akaike 1974, 1992). Following Burnham and Anderson (2002), we considered models with lower AICc values to have better fits and interpreted differences in AICc values (ΔAICc) >2 as supporting real differences between models. We performed all analyses in R (R Development Core Team 2014) and provide the scripts as an electronic enhancement (zip file, available online).[1] For some tasks, the scripts use existing functions available in the supporting packages ape (Paradis et al. 2004), geiger (Harmon et al. 2008), phangorn (Schliep 2011), nlme (Pinheiro et al. 2013), and ctv (Zeileis 2005).

For comparison, we also analyzed the data set under other commonly used approaches. The first set of approaches does not explicitly model the relationship between telencephalon and body mass (like PANCOVA does) but starts by computing a composite variable informative of relative telencephalon size. For these approaches, we obtained composite variables in two ways: (1) by computing ratios dividing telencephalon mass by body mass and (2) by estimating size-corrected residuals of a phylogenetic regression of telencephalon mass on body mass (Revell 2009), using the R package phytools (Revell 2012). We then compared the results of the below methods on these two measures of relative telencephalon size.

To test for differences in evolutionary rates of relative telencephalon size, we applied O'Meara et al.'s (2006) noncensored approach (using phytools; Revell 2012). With this

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1. Code that appears in *The American Naturalist* is provided as a convenience to the readers. It has not necessarily been tested as part of the peer review.

Struthio camelus
Dromaius novaehollandiae
Perdix perdix
Coturnix coturnix
Coturnix chinensis
Gallus gallus
Lyrurus tetrix
Tetrao urogallus
Pavo cristatus
Phasianus colchicus
Chrysolophus pictus
Lophura nycthemera
Anser anser
Cygnus olor
Mergus serrator
Somateria mollissima
Anas penelope
Anas platyrhynchos
Anas crecca
Streptopelia roseogrisea
Columba palumbus
Columba livia
Goura cristata
Geopelia cuneata
Cuculus canorus
Phoenicopterus ruber
Tachybaptus ruficollis
Podiceps cristatus
Pelecanus onocrotalus
Phalacrocorax carbo
Larus argentatus
Larus marinus
Chroicocephalus ridibundus
Sternula albifrons
Sterna hirundo
Fratercula arctica
Haematopus ostralegus
Vanellus vanellus
Numenius arquata
Scolopax rusticola
Lymnocryptes minimus
Actitis hypoleucos
Philomachus pugnax
Spheniscus demersus
Gavia stellata
Burhinus oedicnemus
Grus antigone
Anthropoides virgo
Fulica atra
Porzana porzana
Gallinula chloropus
Porphyrio porphyrio
Rallus aquaticus
Crex crex
Falco tinnunculus
Pandion haliaetus
Aegypius monachus
Aquila chrysaetos
Accipiter nisus
Accipiter gentilis
Buteo buteo
Apus apus
Tachymarptis melba
Trichoglossus haematodus
Nymphicus hollandicus
Cacatua sulphurea
Ara chloropterus
Ara ararauna
Amazona versicolor
Psittacus erithacus
Agapornis fischeri
Psittacula eupatria
Melopsittacus undulatus
Strix aluco
Bubo bubo
Asio otus
Otus scops
Athene noctua
Tyto alba
Upupa epops
Jynx torquilla
Dendrocopos medius
Dendrocopos major
Dryocopus martius
Picus viridis
Picus canus
Egretta garzetta
Ardea alba
Ardea cinerea
Botaurus stellaris
Ixobrychus minutus
Ciconia ciconia
Leptoptilos crumeniferus
Alcedo atthis
Merops apiaster
Muscicapa striata
Cinclus cinclus
Erithacus rubecula
Turdus philomelos
Turdus merula
Sturnus roseus
Sturnus vulgaris
Bombycilla garrulus
Sitta europaea
Certhia familiaris
Troglodytes troglodytes
Pyrrhocorax pyrrhocorax
Pica pica
Garrulus glandarius
Corvus monedula
Corvus corax
Corvus frugilegus
Corvus corone
Lanius collurio
Aegithalos caudatus
Parus major
Cyanistes caeruleus
Hirundo rustica
Delichon urbicum
Regulus regulus
Acrocephalus scirpaceus
Melanocorypha calandra
Alauda arvensis
Motacilla alba
Anthus pratensis
Prunella modularis
Montifringilla nivalis
Passer domesticus
Serinus canaria
Loxia curvirostra
Carduelis carduelis
Carduelis cannabina
Carduelis spinus
Coccothraustes coccothraustes
Fringilla coelebs

0.05

method, we compared the fit of a model with a single global rate across the bird phylogeny with a model that estimates different rates for regions of the phylogeny in which the birds are social (biparental care) and nonsocial (uniparental). Second, we applied Thomas et al.'s (2006) approach, which asks a similar question but allows for a shift also in the mean relative telencephalon size between social versus nonsocial birds. We applied this approach using the code provided by Thomas et al. (2009). Note that both of these approaches estimate only differences in rates of evolutionary diversification and do not consider changes in the relationships between traits such as telencephalon and body size. Thus, we applied both to our ratios and residual measures of relative telencephalon size.

Other methods can be used to explore changes in mean trait values or the relationships between traits but cannot estimate changes in diversification rates. First, we applied a simple, nonphylogenetic ANOVA to test the predictive effect of sociality (x) on relative telencephalon size (y; both ratios and residuals). Second, we applied a nonphylogenetic ANCOVA with telencephalon mass as the response variable (y), body mass as a covariate (z), and sociality (parental investment) as the factor (x). For hypothesis tests of the parameters estimated using these ANOVA and ANCOVA models, we used Monte Carlo simulation procedures, generating 1,000 sets of data using the same phylogeny and a Brownian motion model of phenotypic evolution and creating a null distribution by analyzing each simulated data set with the same ANOVA and ANCOVA procedures (Martins and Garland 1991). We conducted all calculations in R (R Development Core Team 2014), with the ANOVA component as implemented in geiger (Harmon et al. 2008).

Third, we applied an ANCOVA version of the adaptation-inertia method (Hansen 1997; Hansen et al. 2008). Under the adaptation-inertia model, we envision species traits as evolving in a world of multiple, complex, and sometimes conflicting selective pressures and use an Ornstein-Uhlenbeck (OU) model to infer the speed at which a trait responds to a single, identified selective pressure. Here, we regressed telencephalon mass (y) on the total amount of time each species evolved in a social or nonsocial selective context (mapping parental investment as a dichotomous trait onto the phylogeny). We included body mass (z) in two ways: first, as a fixed explanatory variable (as in PANCOVA) and, second, as a trait evolving via Brownian motion process influenced by large unknown stochastic factors (Hansen et al. 2008). Although

use of the Brownian motion version in this case is difficult to justify or to interpret, we include the results for the sake of pragmatic comparison. We applied both options using the R package SLOUCH (Pienaar 2011).

Finally, we applied Revell and Collar's (2009) method to estimate changes in the evolutionary rate matrix describing evolution of telencephalon size and body size between parts of the tree characterized by the presence or absence of biparental care, using the R package phytools (Revell 2012). This approach is the most similar to the PANCOVA in that it also simultaneously estimates evolutionary rates and relationships, albeit in a fundamentally different way.

We also conducted limited computer simulations to explore the statistical performance of the PANCOVA method. Specifically, we chose an arbitrary topology of 33 taxa, generated data using a Brownian motion model of phenotypic evolution for two continuous traits, and allowed changes in both the diversification rate of each trait and the covariance between the two traits at specific points on the phylogeny identified by predetermined shifts in a third, categorical trait. We generated 1,000 data sets for each of several combinations of phenotypic variances and covariances and then analyzed each using an ordinary least squares regression (nonphylogenetic), a correlation of Felsenstein's (1985) contrasts, PGLS-OU regression (Martins and Hansen 1997), and PANCOVA. Because the relationship between the three variables, and their variances and covariances, is complex, we report here only limited results confirming that our implementation of the PANCOVA code could recover evolutionary parameters.

Results

Using PANCOVA, we found that evolutionary gains of avian sociality (as indicated by shifts to biparental care) are associated with shifts in both relative telencephalon size and the rate of evolutionary diversification of telencephalon size (fig. 2). The best-fitting model overall (table 1) was a full PANCOVA showing differences in both the relationship between telencephalon and body size (both intercepts and slopes) and in the evolutionary rates of relative telencephalon size for social versus nonsocial birds. This model also included an estimate of λ (estimating strong phylogenetic signal, closely approaching that of Brownian motion) but not g (the optional parameter that estimates the point along each branch at which the changes occur did not improve

Figure 1: Pruned bird phylogeny from Hedges et al. (2015), with the most parsimonious reconstruction of biparental care (presence = gray; absence = black), which is informative of social cohesion. In species with biparental care, both parents regularly contribute to provisioning and/or incubation. Ancestral state estimates under maximum likelihood using a continuous-time Markov model (Pagel 1999*b*) are consistent with the most parsimonious reconstruction, so only the latter is shown. Both parsimony and maximum likelihood reconstructions were conducted in Mesquite (Maddison and Maddison 2011). Branch lengths are in units of expected phenotypic change (scale provided at bottom left).
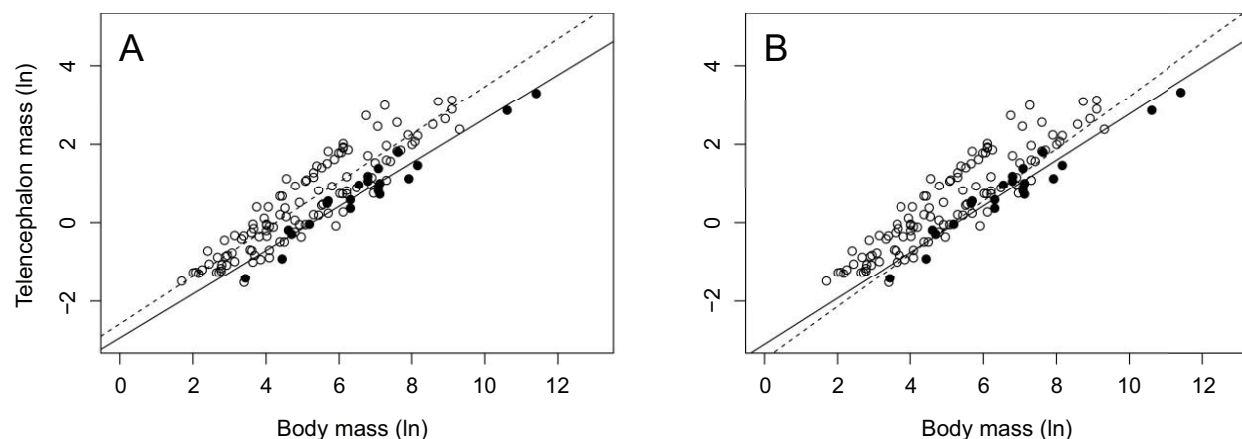
Figure 2: Telencephalon mass is well predicted by body mass for the two categories of sociality (social = empty circles; nonsocial = filled circles). Lines are as estimated according to the phylogenetic analysis of covariance (with two rates) models of table 1 for social (dashed line) and nonsocial (continuous line) birds, under different evolutionary models (*A*, nonphylogenetic; *B*, Brownian motion or phylogenetic signal).

model fit). Note that because estimates of phylogenetic signal (λ) were close to one, the parameter estimates under Brownian motion and phylogenetic signal models were nearly identical.

With any of the three sets of assumptions (no phylogeny, Brownian motion, and estimated phylogenetic signal), bird species that have gained sociality (as indicated by biparental care) exhibit a larger range of relative telencephalon sizes (table 1), and the relationship between telencephalon and body mass (β_2) is positive and allometric (fig. 2). For all three sets of microevolutionary assumptions, the fit of the full PANCOVA model (with two intercepts, two

slopes, and two rates) was better (lower AICc) than that of the simpler ANCOVA (with two intercepts and two slopes but only one evolutionary rate) or the simple linear regression (with one intercept, one slope, and one rate; table 1). This difference was substantial (ΔAICc > 2) for all three models (nonphylogenetic, Brownian motion, and estimated phylogenetic signal).

With no phylogenetic signal (fig. 2*A*), the PANCOVA approach detected two distinct lines with a higher intercept (relatively larger telencephalons) for bird species that have gained sociality (β_1). Under Brownian motion and phylogenetic signal models (fig. 2*B*), the pattern was more complex

Table 1: Parameter estimates for phylogenetic ANCOVA (PANCOVA)

Assumption, model	Intercepts		Slopes		Rates			
	β_0	β_1	β_2	β_3	γ_0	γ_1	λ	AICc
No phylogeny:								
Simple	−2.4 (.13)	...	.6 (.02)	...	.28	...	...	215.3
ANCOVA	−2.9 (.39)	.4 (.41)	.6 (.06)	.1 (.06)	.22	...	...	190.9
PANCOVA	−2.9 (.22)	.4 (.26)	.6 (.03)	.1 (.04)	.07	.18	...	182.8
Brownian motion:								
Simple	−3.5 (.25)	...	.7 (.02)	...	.24	...	...	54.5
ANCOVA	−3.0 (.38)	−.5 (.31)	.6 (.04)	.1 (.05)	.23	...	...	54.2
PANCOVA	−3.1 (.25)	−.4 (.22)	.6 (.03)	.1 (.03)	.08	.18	...	48.6
Phylogenetic signal:								
Simple	−3.4 (.23)	...	.7 (.02)	...	.19	...	.97	42.9
ANCOVA	−3.0 (.36)	−.4 (.29)	.6 (.04)	.1 (.05)	.19	...	.97	43.0
PANCOVA	−3.1 (.25)	−.4 (.22)	.6 (.03)	.1 (.03)	.07	.15	.93	40.0

Note: Shown are phylogenetic analysis of covariance (PANCOVA) parameter estimates for the bird example assuming no phylogeny, Brownian motion, or an estimated phylogenetic signal. For each set of assumptions, a full PANCOVA with two intercepts, two slopes, and two rates fit better (lower corrected Akaike information criterion [AICc]) than did simpler models including an ANCOVA (with two intercepts, two slopes, and one rate) or a simple linear regression (one intercept, one slope, one rate). Standard errors for the regression coefficients are shown in parentheses; in all three sets of assumptions, PANCOVA produces lower standard errors than the simpler ANCOVA. See "A Worked Example" for other details about the estimation and interpretation of model parameters.

since the lines crossed, indicating an interaction effect (β_3). Accounting for phylogeny, we found that only larger-bodied bird species exhibited larger telencephalon sizes with the evolutionary gain of sociality, whereas smaller-bodied bird species that have gained sociality actually have relatively small telencephalons (fig. 2B). In all three cases, the full PANCOVA models also detected an increase in the rate of evolution of telencephalon size with the gain of sociality (the change in evolutionary rate, γ_1, is more than two times the evolutionary rate across all birds, γ_0).

With other methods, we found only limited evidence that relative telencephalon size diversified more quickly in social than in nonsocial birds and completely missed the complex interaction between relative telencephalon size and sociality. We found a doubling of relative telencephalon diversification rates when using methods that estimate evolutionary rates alone (O'Meara et al. 2006; Thomas et al. 2006), however, this difference in rates was statistically significant only when we calculated evolutionary rates using ratios (not phylogenetic residuals; table 2). We found no suggestion that evolutionary shifts in sociality predict relative telencephalon size using phylogenetic ANOVAs with ratios ($P = .7$) but a significant relationship between sociality and relative telencephalon size when using phylogenetic ANOVAs with residuals ($P = .01$). There was again no relationship when tested with an ANCOVA with hypothesis tests conducted in comparison to null distributions generated using Monte Carlo simulation (β_1 within 95% confidence interval; table 3). The ANCOVA found a significant positive and allometric relationship between telencephalon and body mass (β_2 outside 95% confidence interval; table 3) but no difference between slopes for social versus nonsocial species (β_3 within 95% confidence interval; table 3).

From a completely different perspective, the adaptation-inertia method conveyed a similar message (table 4). Regardless of how body mass was modeled (fixed or Brownian motion), the estimated optimum for telencephalon mass was higher for birds that have gained sociality (biparental care).

Although there is some difference between optima for the randomly evolving covariate model (table 4), the difference for the best-fitting model (fixed covariate) is rather small. In fact, there was little difference in support (ΔAICc < 2) between the fixed-covariate model and an optimal regression excluding a factor distinguishing social and nonsocial birds (AICc = 56.9). The large phylogenetic half-life estimate for the best-fitting model (fixed covariate) suggests that there is phylogenetic inertia in telencephalon mass and (much like the large estimate of λ in the PANCOVA) indicates that telencephalon size is tracking the phylogeny.

Using Revell and Collar's (2009) evolutionary rate matrices, we found evidence that phenotypic diversification in telencephalon mass increased with the evolutionary gain of sociality (biparental care; table 5). The full model with different variances and covariances for social and nonsocial birds had a lower AICc score (490.1) than did a model that did not distinguish on the basis of sociality (AICc = 497.5). This difference appeared to be due primarily to a difference in rate estimates, because an intermediate model with different rates but a common correlation (AICc = 492.1) fit almost as well as did the full model, whereas an intermediate model with common rates but different correlations (AICc = 498.9) did not.

Simulations

Our analyses of computer-simulated data confirmed that, as expected, the PANCOVA yielded the same parameter estimates as did PGLS-OU (Martins and Hansen 1997) or Felsenstein's (1985) contrasts method when applied to data generated under a Brownian motion model with no historical shifts in the mean or variance (appendix). In addition, we found that PANCOVA successfully detected shifts in the rate of phenotypic diversification that these other methods were not designed to infer. For example, when we generated data with no relationship ($r = 0$) between the primary response trait and the covariate and an increase in diversifica-

Table 2: Estimates of evolutionary rates under composite variables

Approach, estimate	Single rate	Social	Nonsocial	Likelihood ratio	P
O'Meara et al. 2006 (σ^2):					
Ratios	.10 (.010)	.11 (.014)	.05 (.014)	4.72	.03
Residuals	.24 (.028)	.26 (.035)	.13 (.045)	2.91	.09
Thomas et al. 2006 approach (θ):					
Ratios	. . .	2.3 (1.14, 4.42)	1.0 (.56, 2.16)	4.71	.03
Residuals	. . .	2.1 (.95, 4.13)	1.0 (.49, 2.16)	3.55	.06

Note: Shown are estimates of the rate of diversification of relative telencephalon size using O'Meara et al.'s (2006) and Thomas et al.'s (2006) approaches with the bird example. Using the O'Meara et al. (2006) method, we estimated a single evolutionary rate for the entire phylogeny (σ^2; in parentheses are 1 standard error) and separate evolutionary rates for birds with and without sociality (biparental care). Using the Thomas et al. (2006) method, parameter estimates (θ; with approximate confidence intervals in parentheses) are for birds with and without sociality. For both methods, we compared the fit of single- and two-rate models with likelihood ratio tests (df = 1). We present results for interspecific measures of relative telencephalon size in terms of ratios and phylogenetic residuals in different rows.

Table 3: Parameter estimates for ANCOVA with Monte Carlo null distribution

Statistic	Estimate	Critical values
F	238.2[*]	9.61
β_0	−2.9[*]	−1.36, 2.37
β_1	.4	−1.84, 1.91
β_2	.6[*]	−.23, .22
β_3	.1	−.29, .32

Note: Shown are the F ratio and estimates of intercepts (β_0 and β_1) and slopes (β_2 and β_3) describing the relationship between telencephalon (y) and body (z) size for birds with and without sociality (x) using standard ANCOVA. We conducted hypothesis tests by comparing these estimates with critical values obtained from phylogenetically simulated null distributions.

[*] $P = .05$.

tion rate following evolutionary changes in a specified categorical trait, all of the tested methods correctly estimated the lack of relationship between trait of interest and covariate, but only PANCOVA correctly inferred an increase in diversification rate.

Intriguingly, when we added a larger positive relationship between response and covariate ($r = 0.5$ or 0.9) while retaining the increase in diversification rate, the other methods incorrectly estimated a lower relationship between response and covariate ($r = 0$), while PANCOVA correctly detected both the larger slope and the increase in diversification rate (appendix). The only case involving a serious compromise in parameter estimation was when the variances of the continuous variables were largely unequal. In particular, a high variance in the covariate (z) diluted any relationship between the continuous variables, and therefore, slopes were underestimated even under data simulated with high covariance. This was a general problem, however, that affected all of the tested methods.

Discussion

We present PANCOVA, a phylogenetic ANCOVA model that offers a comprehensive approach to exploring the consequences of major historical shifts (e.g., key innovations, changes in behavior, colonization of new habitats, splitting

of lineages, substantial environmental change). It does so by estimating changes in the rate of phenotypic diversification as well as changes in directional trends or relationships to other phenotypic traits. The method is flexible, easy to implement, and offers evolutionary insights that are not available using other methods.

We illustrated PANCOVA using an example from Shultz and Dunbar (2010), who suggested that sociality in birds would be associated with the evolution of larger brains, if we focused on aspects of sociality such as biparental care. Biparental care requires complex social negotiations, can impose significant cognitive costs, and along with sociality has been associated with evolutionary changes in brain morphology (Barton 1996; Goodson et al. 2012). In brief, biparental care in birds is thought to extend the altricial period, allowing more time for brain development and, consequently, larger brains (Pérez-Barbería et al. 2007; Shultz and Dunbar 2010). Our PANCOVA results support this conclusion by finding that the evolutionary gain of biparental care was associated with an increase in relative telencephalon size, but only in larger-bodied bird species. In smaller-bodied bird species, the inferred relationship was weaker or reversed. In addition, our PANCOVA found that social birds had higher rates of relative telencephalon size diversification. Results from other methods were superficially consistent, but most did not detect significant differences between the relative telencephalons of social (biparental) and nonsocial birds, and none detected the complex interaction between brain size, body size, and sociality. This interaction was also missed in the study of Shultz and Dunbar (2010), who focused on ratios and phylogenetically controlled residuals as measures of relative brain size. Using these composite variables, they found that larger relative brain sizes are associated with biparental care, as well as other life-history traits and behavioral characteristics (e.g., altricial development, pair bonding). Our results are consistent with these in regard to telencephalon size and biparental care, but our PANCOVA did not miss the aforementioned interaction because the continuous variables were modeled rather than synthesized in a single composite variable. In this way, differences in allometric trends according to particular states of parental investment can be explored more thoroughly.

Table 4: Parameter estimates for ANCOVA using the adaptation-inertia method

| Model | Primary optima | | | Phylogenetic Correction | | | |
	Social	Nonsocial	Slope	$t_{1/2}$	Factor	v_y	AICc
Fixed covariate	−3.5 (.43)	−3.6 (.44)	.7 (.03)	19.80	.02	3.37	56.0
Brownian motion covariate	−3.2 (.27)	−3.6 (.28)	.9 (.04)	.21	.71	1×10^{-5}	115.5

Note: Shown are results from regression of bird telencephalon mass (y) on times spent in social and nonsocial contexts (x), with body mass modeled as either a fixed or a randomly evolving (Brownian motion) covariate. Standard errors for the primary optima are shown in parentheses. The optimal regression (slope) is the slope of body mass corrected by the phylogenetic correction factor, which together with the phylogenetic half-life ($t_{1/2}$) can be used as a measure of phylogenetic inertia. The stationary variance (v_y) measures the amount of noise in telencephalon mass as it evolves toward the primary optima. AICc = corrected Akaike information criterion.

Table 5: Evolutionary rate matrices

Bird type, measurement	Telencephalon size	Body size
Nonsocial (correlation = 0.98):		
Telencephalon size	2.0	3.3
Body size	3.3	5.7
Social (correlation = 0.94):		
Telencephalon size	2.2	2.8
Body size	2.8	4.0

Note: Shown are estimates of evolutionary variances (rates) and covariances for telencephalon and body sizes using Revell and Collar's (2009) method. This approach found support for a full matrix with different variances and covariances describing the relationship between telencephalon and body mass in social and nonsocial birds, but the change in the relationship between telencephalon and body size with the evolution of sociality was quite small.

In addition, PANCOVA expands the relevance of the pattern by adding the element of phenotypic diversification, which was not explored by Shultz and Dunbar (2010).

PANCOVA begins with a powerful and flexible PGLS regression model that allows researchers to add and remove likely parameters within the familiar context of regression model fitting. ANCOVA approaches that explicitly model the relationships between variables can be much more useful than ratios or residuals for studying allometry (García-Berthou 2001; Freckleton 2002). In the bird example explored here, ratios reported a difference in rates between social and nonsocial birds but failed to detect a difference in mean relative telencephalon sizes. On the contrary, a difference in mean relative telencephalon sizes, but not rates, was detected under phylogenetic residuals. Therefore, conclusions about the resulting evolutionary trend would have depended on the chosen composite variable (i.e., ratios or phylogenetic residuals), even when both means and evolutionary rates were actually relevant for describing the pattern (as shown by PANCOVA). Besides allometry, ANCOVAs can also be useful in studying the evolution of sexual dimorphism, interspecies coevolution, and other complex phenotypes. Here, we applied three forms of ANCOVAs (with Monte Carlo simulations, the adaptation-inertia model, and the PANCOVA), all of which detected an allometric relationship between telencephalon and body mass. Further exploration of parameters is more difficult with the Monte Carlo simulations (which emphasize hypothesis testing rather than parameter estimation) or with the adaptation-inertia model, which offers a rich evolutionary interpretation but for which there have only been a few limited ANCOVA-type models implemented (Pienaar 2011; Bartoszek et al. 2012).

PANCOVA offers the added benefit of simultaneously estimating rates of diversification. This is an important advance from simpler ANCOVA methods (e.g., Garland et al. 1993; Butler et al. 2000; Lavin et al. 2008) that consider shifts in mean traits and trait covariation after a major historic event but do not allow for the possibility of a change in evolutionary rate or variance. It is also an extension of likelihood procedures that estimate rates but do not allow a shift in mean traits or trait relationships (O'Meara et al. 2006; Thomas et al. 2006, 2009). In our worked example, PANCOVA detected an increase in the rate of relative telencephalon diversification in bird species that have gained sociality (biparental care), a novel finding that raises questions about whether other factors are constraining both parental investment and brain size evolution in these species. For example, baby birds raised by single parents may have slower growth rates (due to inadequate nutrition) or fewer social learning opportunities, both of which may impose constraints on adult brain size.

Our model is most similar in concept to Revell and Collar's (2009) rate matrix approach, although the two approaches differ considerably in both implementation and interpretation. PANCOVA is computationally simpler and thus may be particularly useful when exploring different combinations of mean structures and error terms as well as evolutionary scenarios other than Brownian motion (which was not the best model for the bird data set explored here). More importantly, although PANCOVA's regression slopes are conceptually similar to the covariances estimated by Revell and Collar's (2009) approach, the rate parameters estimated by the two methods are quite different. Whereas Revell and Collar's (2009) rate estimates refer to the two traits independently of each other (e.g., rate of telencephalon mass diversification and rate of body mass diversification, separately), the PANCOVA estimates refer to relative telencephalon size (residuals of the relationship between telencephalon and body mass) and are calculated as functions of the entire ANCOVA model. Thus, PANCOVA rate estimates may be most useful when the covariate is a scaling or other correction factor rather than a trait of independent interest. Note also that because both rate parameters and slopes are jointly estimated, even the slope estimates resulting from application of these two approaches could differ dramatically.

PANCOVA also estimates differences in intercepts that are not provided in Revell and Collar's (2009) evolutionary rate matrix approach. For example, PANCOVA could be used to detect an evolutionary shift from isometry (intercept = 0) to linear allometry (intercept ≠ 0), even when the slopes of the lines (or, equivalently, the covariances of the evolutionary rate matrices) are the same (Albrecht et al. 1993; Packard and Boardman 1999). Different PANCOVA intercepts could be also interpreted as shifts in directional trends or optimal traits, yielding results that are superficially similar to those produced by application of the adaptation-inertia model (Hansen 1997; Hansen et al. 2008). The PANCOVA may be most useful, as in the bird example, when the covariate is a correlated trait (e.g., a scaling factor), and the cate-

gorical variable is an indicator trait (e.g., biparental care) that is linked to a number of important factors (e.g., social behavior and development) rather than an explicitly causal selective regime. Hansen's adaptation-inertia model would usually be a better choice when more is known about the relevant selective forces, and it is important to avoid the problem of inherited maladaptation (Hansen and Orzack 2005), which can result from ignoring the effects of past values of the explanatory variable on the current values of the response. Development of an adaptation-inertia model that also estimates evolutionary rates (or a PANCOVA that relies on an adaptation-inertia framework) could be an important future advance. By accounting for unequal phenotypic rates, PANCOVA showed improved statistical properties such as higher precision (lower standard errors in regression coefficients when compared with ANCOVA), better model fitting (lower AICc values in table 1), and more accurate descriptions of trait relationships, as simulation results showed that assuming homogeneity of phenotypic rates, when rate shifts were present, compromised the sensitivity of other methods to detect positive relationships between continuous variables (PANCOVA correctly estimated both positive relationships and rate shifts). It is possible that similar statistical properties will benefit the adaptation-inertia framework by accounting for unequal stationary variances.

An adaptation-inertia version of PANCOVA would also be a valuable step toward developing a comprehensive comparative method for studying adaptive radiations. Adaptive radiation is a complex evolutionary phenomenon that often includes cladogenesis and rapid speciation as well as phenotypic diversification in response to selection (Gavrilets and Losos 2009; Glor 2010). By considering different evolutionary rates and optima, the extension we suggest here would cover the diversification and selection components of an adaptive radiation when combining continuous and discrete variables. Another useful extension would be to also add estimates of species richness, using existing comparative methods that infer changes in speciation and extinction rates (e.g., Alfaro et al. 2009; Stadler 2011; Hunt 2013). As comparative methods considering phenotypic diversification, adaptation, and speciation become available in common frameworks, it becomes more possible to combine them into single approaches that address some of the most important questions in evolutionary biology. We hope that our approach will inspire additional theoretical development around this important theme.

Acknowledgments

APPENDIX

Supplemental Table

Table A1: Selected simulation results

Simulation, method	Intercepts		Slopes		Rates	
	β_0	β_1	β_2	β_3	γ_0	γ_1
Zero XY correlation, diversification increases:						
No phylogeny	.1 (.74)	…	.0 (.18)	…	…	…
Contrasts	…	…	.0 (.18)	…	…	…
PGLS-OU	…	…	.0 (.18)	…	…	…
PANCOVA	.1 (1.55)	.1 (1.20)	.0 (.69)	.0 (.66)	16.1	6.2
Positive XY correlation, diversification increases:						
No phylogeny	.1 (.33)	…	.9 (.08)	…	…	…
Contrasts	…	…	.9 (.08)	…	…	…
PGLS-OU	…	…	.9 (.08)	…	…	…
PANCOVA	.1 (1.50)	.0 (1.19)	.9 (.54)	.0 (.56)	3.0	1.2

Table A1 (*Continued*)

Simulation, method	Intercepts		Slopes		Rates	
	β_0	β_1	β_2	β_3	γ_0	γ_1
Positive XY correlation, high X variance, diversification increases:						
No phylogeny	.2 (1.97)	…	.1 (.18)	…	…	…
Contrasts	…	…	.1 (.18)	…	…	…
PGLS-OU	…	…	.1 (.18)	…	…	…
PANCOVA	.2 (2.65)	.3 (2.09)	.3 (.95)	—.2 (.84)	56.8	353.6

Note: Parameter estimates resulting from analysis of data simulated along a 33-taxon phylogeny using a nonphylogenetic regression, a regression based on the Felsenstein independent contrasts method, phylogenetic generalized least squares–Ornstein-Uhlenbeck (PGLS-OU) regression, and phylogenetic analysis of covariance (PANCOVA), assuming Brownian motion. Numbers in parentheses are 1 standard error. See "A Worked Example" for other details about the simulation process and phylogenetic comparative methods.

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A female southern lapwing (*Vanellus chilensis*) takes a walk with her chicks in the Aburrá Valley (Antioquia, Colombia). Photo credit: Jorge I. Atehortúa, http://jatehortua.wixsite.com/atehophoto.