

# Using the Past to Predict the Present: Confidence Intervals for Regression Equations in Phylogenetic Comparative Methods

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**ABSTRACT:** Two phylogenetic comparative methods, independent contrasts and generalized least squares models, can be used to determine the statistical relationship between two or more traits. We show that the two approaches are functionally identical and that either can be used to make statistical inferences about values at internal nodes of a phylogenetic tree (hypothetical ancestors), to estimate relationships between characters, and to predict values for unmeasured species. Regression equations derived from independent contrasts can be placed back onto the original data space, including computation of both confidence intervals and prediction intervals for new observations. Predictions for unmeasured species (including extinct forms) can be made increasingly accurate and precise as the specificity of their placement on a phylogenetic tree increases, which can greatly increase statistical power to detect, for example, deviation of a single species from an allometric prediction. We reexamine published data for basal metabolic rates (BMR) of birds and show that conventional and phylogenetic allometric equations differ significantly. In new results, we show that, as compared with nonpasserines, passerines exhibit a lower rate of evolution in both body mass and mass-corrected BMR; passerines also have significantly smaller body masses than their sister clade. These differences may justify separate, clade-specific allometric equations for prediction of avian basal metabolic rates.

**Keywords:** allometry, ancestor reconstruction, comparative method, metabolic rate, phylogeny, regression.

Interspecific comparisons have undergone a renaissance in the last decade, and comparative data sets are now analyzed routinely by phylogenetic methods (Eggleson and

Vane-Wright 1994; Losos and Miles 1994; Martins 1996a; Garland et al. 1997). Among these methods are several that use phylogenetic information in an explicitly statistical fashion (Grafen 1989; Harvey and Pagel 1991; Garland et al. 1993; Miles and Dunham 1993; Martins and Hansen 1996, 1997; Reynolds and Lee 1996; Schluter et al. 1997; Pagel 1998; Garland et al. 1999). The rationale for these methods is the theoretical prediction and empirical observation that closely related species are more likely to be similar than are distantly related species. Hence, in comparative studies, species cannot be treated as if they represent independent and identically distributed data points, and ordinary statistical methods, such as standard least squares regression, cannot be used.

The best understood and most prevalent phylogenetically based statistical method is Felsenstein's (1985) independent contrasts (IC). Independent contrasts are calculated as differences in the value of a trait between two sister species (or internal nodes of a phylogenetic tree) divided by the square root of the sum of their branch lengths (branch lengths must be in units of or proportional to expected variance of character evolution). If evolution along the separate branches of the phylogenetic tree occurs independently, then the contrasts will be statistically independent. Felsenstein's (1985) original presentation relied on a Brownian motion model of character evolution, but the procedure can also be justified on first-principles statistical grounds (Grafen 1989; Pagel 1993). In the context of testing for correlated evolution of two characters, simulation studies indicate that independent contrasts are reasonably robust even when character evolution deviates from Brownian motion and when branch lengths used for analyses contain errors, at least if diagnostic tests and transformations of branch lengths are applied (e.g., Type I error rates are not far from what they should be; Grafen 1989; Martins and Garland 1991; Purvis et al. 1994; Díaz-Uriarte and Garland 1996, 1998; Grafen and Ridley 1996; Martins 1996b; Harvey and Rambaut 1998; Garland and Díaz-Uriarte 1999). Although Felsenstein's (1985) original

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presentation considered applications to simple linear correlation and regression, independent contrasts can also be applied to most problems that require such related statistical techniques as principal components analysis, multiple regression, ANOVA, and ANCOVA (e.g., Garland 1992; Garland et al. 1993; McPeck 1995; Díaz et al. 1996; Gray 1996; Martin and Clobert 1996; Clobert et al. 1998; Bonine and Garland 1999; Foufopoulos and Ives 1999). In addition, by rerooting (see below), the method can be used to estimate trait values and standard errors for internal nodes on a phylogenetic tree (Garland et al. 1999), with results equivalent to those estimated by maximum likelihood (Schluter et al. 1997).

An alternative approach relies on generalized least squares (GLS) models. The phylogenetic information required (topology and branch lengths) is identical to that needed for computing independent contrasts. Rather than constructing contrasts, however, GLS involves regression in which error terms are neither independent nor identically distributed. Instead, the expected variances of and correlations between error terms are assumed to be known from the available phylogenetic topology and branch lengths. Under the assumption of Brownian motion character evolution, GLS estimates of regression parameters are also maximum likelihood estimates, with error terms described by a multivariate normal distribution. Grafen (1989), Martins and Hansen (1997), and Pagel (1998) provide discussions of many possible applications of GLS to comparative analyses, including multiple regression, estimating rates of evolution, and reconstructing ancestral traits.

In this article, we address the problem of constructing confidence or prediction intervals for values of a dependent variable regressed against an independent variable. This problem arises frequently in comparative biology, such as in studies of allometry (e.g., Calder 1984; Schmidt-Nielsen 1984; Harvey and Pagel 1991; Garland and Adolph 1994; Garland and Carter 1994; Weathers and Siegel 1995; Reynolds and Lee 1996; Williams 1996; Kozłowski and Weiner 1997; Clobert et al. 1998). As a recent example, Nagy et al. (1999) present a table 2 containing 41 separate allometric equations for predicting field metabolic rates of different clades, habitat, or diet categories. Also in their table can be found the necessary statistics for computing the 95% prediction intervals in the conventional (phylogenetically uninformed) manner. Nagy et al. (1999, p. 259) note that independent contrasts are an alternative, but then state that independent contrasts do not yield "equations that can be used to predict FMR values directly" and do not yield "statistical parameters that allow calculation of confidence intervals for predicted values." Both of these claims were correct when their paper was published but

are now obviated by the new methods presented here and implemented in our PDTREE computer program.

To derive formulae for confidence and prediction intervals, we use both the IC and GLS approaches. We show that the two approaches produce identical results and can estimate all of the same parameters, including the Y-intercept in the original data space (contra Pagel 1998, pp. 337, 341). This demonstration is important because many readers of the comparative literature may have been under the impression that IC and GLS represent fundamentally different ways of analyzing comparative data.

We also present three empirical examples to illustrate the utility and application of these methods. We first show how predictions for unmeasured species can in general be made increasingly accurate and precise as the specificity of their placement on a phylogenetic tree increases. These prediction methods can be used for both extant and extinct (fossil) forms, including putative direct ancestors of extant species (i.e., points along any branch of a phylogenetic tree). Second, we give a specific example in which use of the proposed methods yields greatly increased power to detect whether a particular species deviates from a previously established allometric equation. This example is important not only because it illustrates the increased power that can come from incorporation of phylogenetic information but also because some workers have claimed that independent contrasts could not be used for this purpose (e.g., Smith 1994). Finally, we reexamine the allometry of avian basal metabolic rate using the data compiled by Reynolds and Lee (1996). This analysis highlights the ability of our methods for mapping phylogenetically correct regressions back onto the original data space to reveal new and striking patterns, such as differences in the rate of evolution of passerines as compared with other birds. The example also shows how, contrary to some previous claims (e.g., Weathers and Siegel 1995), allometric equations derived by conventional and phylogenetic methods can differ significantly, even for a data set that encompasses more than four orders of magnitude in body mass, includes hundreds of species that span almost the full range of phylogenetic diversity in a major clade, and shows a high correlation ( $r > 0.95$ ).

### Statistical Approaches to the Problem of Phylogenetic Correlation

Here we set out the general problem posed by phylogenetic correlation (the tendency for related species to resemble each other) in both the IC and GLS formats. Rather than give a detailed account of these approaches, our intention is to compare them conceptually. Appendices A and B provide the formal statistical derivations of the formulae needed to apply the approaches to data. The IC calcula-

tions are implemented in our PDTREE program (available on request from the first author); the GLS approach can be implemented through any commercially available program that manipulates matrices, such as MatLab (MathWorks 1996).

The problem of regression with phylogenetically correlated data can be stated as follows. Let  $x_i$  and  $y_i$  denote the values of an independent and a dependent variable measured for species  $i$  in a group of  $n$  species. Both  $x_i$  and  $y_i$  are assumed to take continuous values. For simplicity, we assume a single independent variable, although our results generalize in a straightforward way to any number of independent variables, including none. With no independent variables, the analysis corresponds to the case of estimating values of a single trait at internal nodes on a phylogenetic tree (Martins and Hansen 1997; Schluter et al. 1997; Cunningham et al. 1998; Martins and Lamont 1998; Garland et al. 1999). The regression problem can be written as

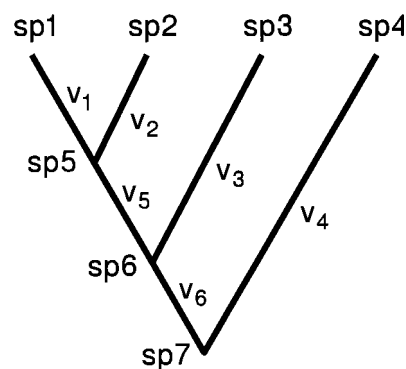
$$y_i = \beta_0 + \beta_1 x_i + \epsilon_i, \quad (1)$$

where  $\beta_0$  and  $\beta_1$  are regression coefficients and  $\epsilon_i$  is a normally distributed "error" or "residual" term with mean 0. Although this appears to be a standard regression equation, it is not, because values of  $\epsilon_i$  are correlated among different species owing to their shared phylogenetic history.

To illustrate the problem of phylogenetic correlation, consider the phylogeny of four species shown in figure 1. Branch lengths represent the expected variance of character evolution. Thus, species 1 and 2 are more likely to be similar than any other pair of species because they share evolutionary history along branches  $v_5$  and  $v_6$ , and the variance of the difference between trait values for the two species will be correspondingly low because of the short phylogenetic divergence between them, measured as  $v_1 + v_2$ . Hence, the covariance between  $\epsilon_1$  and  $\epsilon_2$  (i.e., the portion of the two species' phenotypes that are not predicted by  $x$ ) is expected to be high.

#### *Independent-Contrasts Approach*

The standard IC approach involves assigning trait values to each of the internal nodes (ancestral species) on the phylogenetic tree. For each internal node, these estimates are a weighted average of the trait values of the two daughter species, with weights proportional to the inverse of the branch lengths between mother and daughter species so that the shorter the branch length, the greater the weight. The independent contrasts are then calculated from the differences between sister taxa throughout the phylogenetic tree. Except at the root (basal) node (see Garland et



**Figure 1:** Phylogenetic relationships for four species (*sp1–sp4*) with three (*sp5–sp7*) hypothetical ancestors. Branch lengths ( $v_i$ ) are in units of expected variance of character evolution.

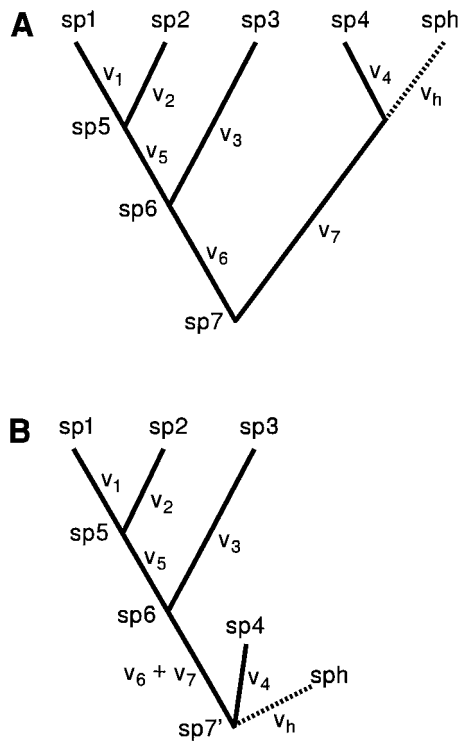
al. 1999), the estimates of ancestral trait values are not optimal estimates; instead, they are merely intermediate steps in the computation of the entire set of  $n - 1$  independent contrasts.

The formulation of regression in terms of independent contrasts removes the constant coefficient  $\beta_0$ , making it impossible to map the results from contrasts back onto the original data to obtain confidence intervals for trait  $y$  for the tip species. To obtain confidence intervals, the IC approach can be reformulated as

$$y_i = \beta_1(x_i - x_w) + y_w + (v_i')^{0.5}\epsilon_i, \quad (2)$$

where  $y_i$  and  $x_i$  denote the values of  $y$  and  $x$  for tip or ancestral species,  $y_w$  and  $x_w$  denote the values at the node directly below species  $i$ , and  $\epsilon_i$  is normally distributed with mean 0 and variance  $\sigma^2$ . Conceptually, the first term in equation (2),  $\beta_1(x_i - x_w)$ , describes the dependence of  $y_i$  on change in  $x$  between the ancestral node  $w$  and the present node  $i$ ; the second term,  $y_w$ , incorporates the dependence of  $y_i$  on the value of  $y$  at the ancestral node  $w$ ; and the last term,  $(v_i')^{0.5}\epsilon_i$ , accounts for the variance produced by Brownian motion evolution. Because evolution along sister branches occurs independently, values of  $\epsilon_i$  are independent of each other. In appendix A, we show how equation (2) can be used to obtain estimates (with standard errors) of confidence intervals for estimates of  $y$  for each tip species.

The related problem of predicting the value of  $y$  for a new species  $h$  with known value of  $x_h$  is illustrated in figure 2A. The location of species  $h$  on the phylogenetic tree will influence the prediction of  $y_h$  because (in general) species  $h$  will be more similar to closely related species than to distantly related species. The easiest way to obtain prediction intervals for  $y_h$  is to "reroot" the phylogenetic tree,



**Figure 2:** Illustration of rerooting a phylogenetic tree to predict the value for a hypothetical unmeasured species, designated as *sph*. *A* shows that the species *h* is putatively the sister of species 4. *B* shows the tree rerooted so that the last common ancestor of species 4 and *h* (*sp7*) is at the base of the phylogeny. Note that the branch labeled  $v_6 + v_7$  is not drawn to scale: it is actually equal to the lengths of the branches, as shown in *A*.

as is done in figure 2*B*. In the rerooted tree, species *h* is adjacent to the basal node. The estimate (and standard error) of trait *y* at the new basal node,  $y_7$ , can be obtained from equation (A13) of appendix A. Because species *h* is adjacent to the new basal node, the expected value of  $y_h$  equals the estimate of  $y_7$ , and the variance of the estimator of  $y_h$  equals the variance of  $y_7$  plus the variance proportional to  $v_h$  that is caused by evolutionary changes from node 7 to species *h*. The results obtained from this procedure are identical to those that could be obtained via the GLS method of Martins and Hansen (1997; see app. B). To perform these computations in PDTree, one first reroots the tree; the program will prompt the user for both  $v_h$  and  $x_h$ .

#### Generalized Least Squares Approach

The GLS approach differs from independent contrasts by explicitly dealing with correlations among  $\epsilon_i$  for extant species rather than estimating trait values for ancestral

species. The general regression problem of equation (1) can be written as (Martins and Hansen 1997)

$$Y = X\beta + \epsilon, \quad (3)$$

where *Y* is an *n*-dimensional vector of values of  $y_i$  (the measured trait that is considered as a dependent variable), *X* is an  $n \times 2$  matrix whose first column consists of ones and whose second column contains values  $x_i$  (the measured trait that is considered as an independent variable), and  $\beta = [\beta_0, \beta_1]'$  (with the prime denoting transpose). Under the assumption of Brownian motion evolution,  $\epsilon$  is an *n*-dimensional multivariate normal distribution with mean 0 and variance-covariance matrix  $\sigma^2 C$ , where  $\sigma^2$  is a scalar measuring the overall rate of evolutionary change, as with independent contrasts (matrix  $\sigma^2 C$  is called matrix *V* by Martins and Hansen [1997]). Elements of matrix *C* (which can be produced with our PDDIST program) describe the phylogenetic relationships as the lengths of the shared branches, from root to last common ancestor, between species (Martins and Hansen 1997; see also box 3 in Cunningham et al. 1998). For example, for the tree in figure 1,  $c_{11} = v_1 + v_5 + v_6$ , and  $c_{12} = v_5 + v_6$ . Thus, the greater the shared phylogenetic history between species *i* and *j*, the greater the covariance  $c_{ij}$ . Species 1 and 4 may be considered unrelated; they share no evolutionary history, so  $c_{14} = 0$ .

The GLS problem can be analyzed by converting equation (3) into the form of standard linear regression with uncorrelated error terms. Because *C* is a real symmetric nonsingular matrix, there exists another matrix *D* such that  $DCD' = I$ , the  $n \times n$  identity matrix. Matrix *D* can be used to transform values of traits *y* and *x* by letting  $Z = DY$ ,  $U = DX$ , and  $\alpha = D\epsilon$ . This gives

$$Z = U\beta + \alpha. \quad (4)$$

The variance-covariance matrix of  $\alpha$ ,  $V\{\alpha\}$ , equals  $E\{\alpha\alpha'}\} = E\{D\epsilon(D\epsilon)'\} = E\{D\epsilon\epsilon'D'\} = DE\{\epsilon\epsilon'\}D' = (D\sigma^2 C)D' = \sigma^2 I$ . Thus, no covariance terms appear in the variance-covariance matrix of  $\alpha$ , so the error terms  $\alpha_i$  are uncorrelated. Furthermore, because  $\alpha$  is a linear transformation of  $\epsilon$ ,  $\alpha$  is normally distributed. Equation (4) can, therefore, be analyzed as a standard least squares regression problem with independent errors (app. B). Unconditional confidence intervals for trait *y* for the extant species are obtained by back-transforming from *Z* to *Y*. Prediction intervals for a new species *h* can either be calculated by rerooting the phylogenetic tree, as described for the IC method, or by remaining in GLS mode (app. B). This GLS procedure assumes that the elements of *C* are known and fixed; more general procedures can be used when *C* con-

tains parameters that must be estimated (Judge et al. 1985; Hansen 1997; Martins and Hansen 1997).

### Empirical Examples

We provide three empirical examples to illustrate the inferential power of our procedures. All three examples involve the allometry of metabolic rate. This theme is chosen because metabolic rate is probably the trait most frequently studied by ecological and comparative physiologists, who in turn are heavy users of the comparative method (Calder 1984; Schmidt-Nielsen 1984; Withers 1992; Garland and Adolph 1994; Garland and Carter 1994; Bradley and Zamer 1999; Garland et al. 1999).

#### *Using Phylogenetic Information to Refine Predictions for Unmeasured Species*

We first show how predictions of values for an unmeasured species can be made more precise if the phylogenetic position of the species can be specified (see end of "Independent-Contrasts Approach"), which can lead to increased statistical power (see example in next section). This is important because many workers have complained that phylogenetically based statistical methods only seem to reduce the apparent statistical significance of various effects.

Consider Dutenhoffer and Swanson's (1996) data on summit metabolic rates of 10 species of passerine birds (fig. 3A). Figure 3B shows a scatterplot of their data along with a conventional regression line and conventional 95% prediction intervals. Figure 3C shows the regression line and 95% prediction intervals derived by independent contrasts, using the methods we have described. These are phylogenetically correct but "generic" prediction intervals, such as those that would be used if one could not specify the phylogenetic position of the hypothetical species to be predicted. In effect, the generic prediction intervals assume that the species to be predicted is attached to the root of the tree and that the branch leading to it ( $v_h$ ) is equal to the average length of the branches leading from the root to every other tip. Note that these phylogenetically correct but generic prediction intervals are wider than the conventional prediction intervals shown in figure 3B.

Figure 3D and 3E shows examples of predicting hypothetical extant species whose location on the phylogenetic tree is known. In both cases, the point estimate for the predicted  $\log_{10}$  metabolic rate is closer to the value (relative to its body mass) of related species. This effect occurs, as it should, because the computations are performed by rerooting the phylogenetic tree (as shown in fig. 2) at the node that gives rise to a three-way polytomy comprised of the hypothetical species of interest, its sister

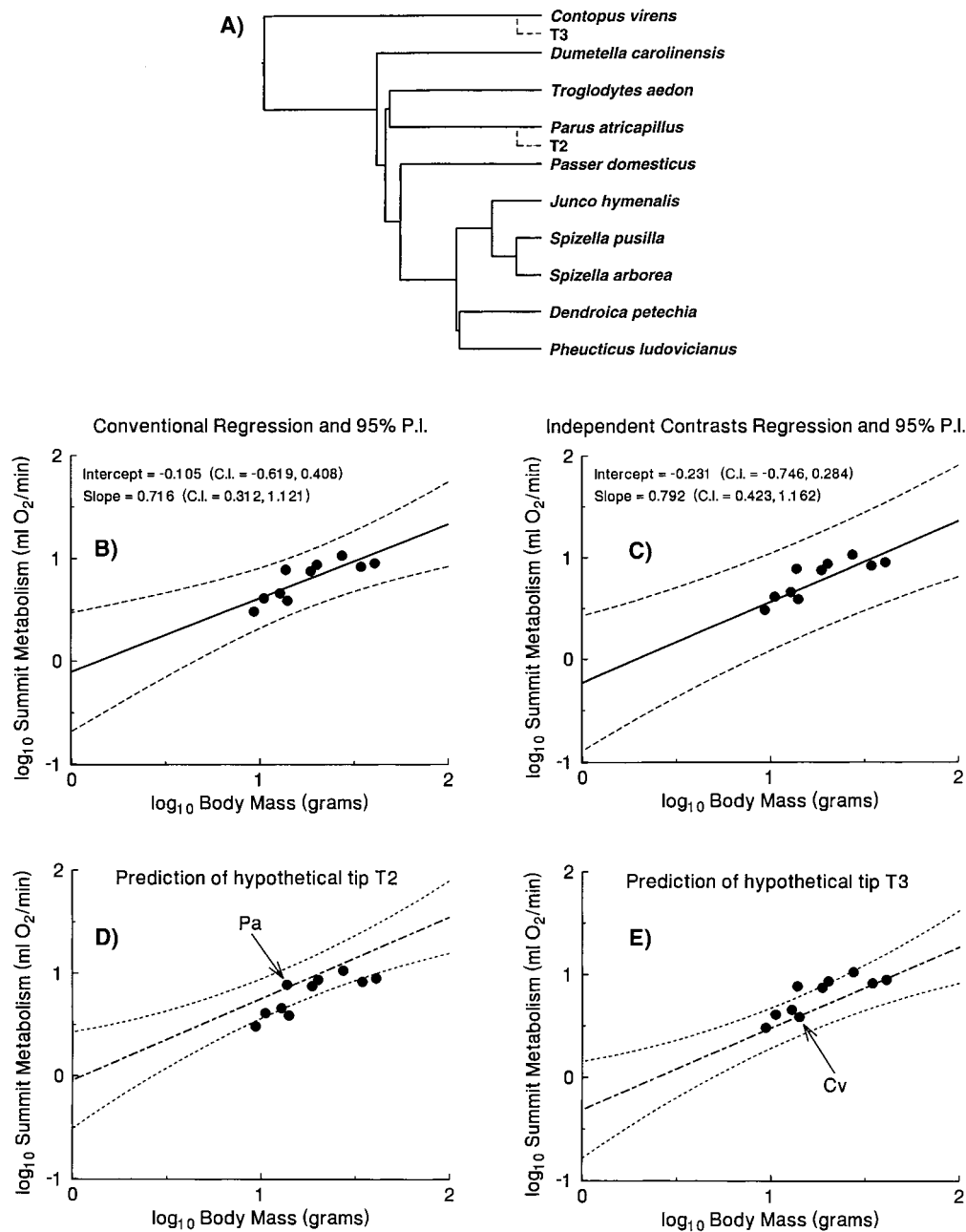
species, and the lineage containing all other species in the data set. The value estimated at this new root node is used to position the regression line vertically in the original data space. Because the value at the new root node will be relatively strongly influenced by the sister species, the elevation of the regression line will move closer to its value for the dependent variable. At the same time, the 95% prediction intervals for particular hypothetical species are considerably narrower than the phylogenetically informed but "generic" prediction intervals shown in figure 3C and are even narrower than the conventional prediction intervals shown in figure 3B. In the limit, if the hypothetical species were attached infinitely close to the sister tip, then the prediction intervals would diminish to 0.

#### *Testing Whether a Single Species Deviates from an Allometric Prediction*

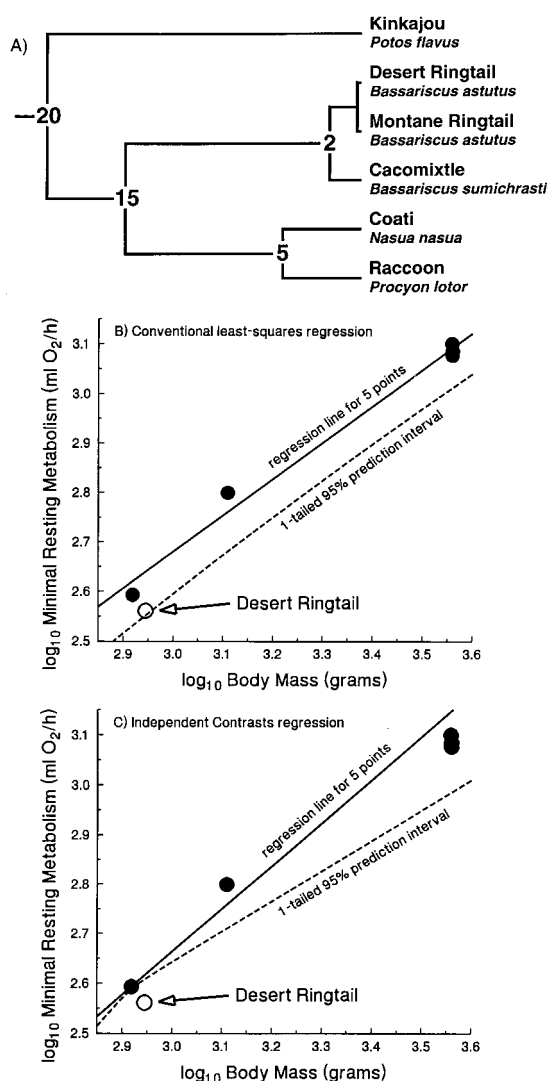
Chevalier (1991) studied metabolism in single populations of four species of procyonid mammals (fig. 4A) and in two separate populations of a fifth species, the ringtail (*Bassariscus astutus*). A main question of interest was whether the desert population of ringtails had a lower than expected minimal resting metabolic rate in the thermal neutral zone (MRM), which would be expected as an adaptation to desert conditions. As discussed in Garland and Adolph (1994), a traditional approach would have been to exclude the datum for the desert ringtail population, use conventional statistics to fit a least squares linear regression to the remaining five data points (log transformed), compute a one-tailed 95% prediction interval for a new observation, and compare the desert ringtail population with this prediction. As shown in figure 4B, the MRM of the desert ringtail population does not fall below the conventional prediction interval, so we would conclude that it does not have a significantly reduced metabolic rate.

To repeat the analysis with the methods described above for independent contrasts, the ringtail population is pruned from the tree, the tree is rerooted, and an independent-contrasts regression line is computed and mapped back onto the original data space, and the one-tailed 95% prediction interval is computed with PDTREE. As shown in figure 4C, as compared with this phylogenetically informed prediction interval, the desert ringtail does indeed have a reduced MRM for its body size.

Garland and Adolph (1994, their fig. 4) presented a similar test but remained entirely within the context of phylogenetically independent contrasts. That test yielded similar results but was less intuitive because graphs of contrasts indicate estimates of minimum rates of evolution within particular bifurcations of the phylogeny (differences between sister species or nodes, divided by square roots



**Figure 3:** A, Phylogenetic relationships of 10 species of birds studied by Dutenhoffer and Swanson (1996), as depicted in their figure 1. Branch length from root (basal node) to *Contopus virens* is 19.7 units. Splits between hypothetical tips T2 and T3 and their closest relatives (*Parus atricapillus* and *Contopus virens*, respectively) are set at 1.75 units, the same as the split between *Spizella pusilla* and *Spizella arborea*. B, Log-log plot of summit metabolism in relation to body mass for the birds studied by Dutenhoffer and Swanson (1996; data from their table 1). Least squares linear regression and 95% prediction intervals (dashed lines) are from conventional analysis. C, Same as B, but using phylogenetically independent contrasts, as described in this article and computed in our PDTREE program. D, Predictions (dashed-dotted lines) and 95% prediction intervals (dotted lines) from independent-contrasts procedures, across a range of body masses for hypothetical species T2. Pa indicates *Parus atricapillus*, the closest relative of T2 in the data set. E, As in D, but for hypothetical species T3. Cv indicates *Contopus virens*, the closest relative of T3.



**Figure 4:** Use of new methods presented here to test whether a single species (actually, a desert population of the ringtail *Bassariscus astutus*) deviates from the allometric pattern observed among related species. *A*, Cladogram (from Decker and Wozencraft's [1991] cladistic analysis of 129 morphological characters) for the six procyonid taxa studied by Chevalier (1991). Numbers at nodes indicate divergence times in millions of years before present, as estimated from fossil and biogeographic information (corrected from fig. 4 of Garland and Adolph 1994); the two ringtail populations probably began diverging about 10,000 yr ago, at the end of the last ice age. *B*, Log-log plot of minimal resting metabolism (MRM) versus body mass (from Chevalier 1991; data also shown in Garland and Adolph 1994), along with a conventional least squares linear regression fitted to the five taxa shown as closed circles (slope = 0.734, Y-intercept = 0.479) and one-tailed 95% prediction interval for a new observation. The MRM of the desert population of ringtails does not deviate significantly from this conventional allometric prediction. *C*, As in *B*, but regression (slope = 0.864, Y-intercept = 0.071) and one-tailed 95% prediction interval derived by use of methods for phylogenetically independent contrasts presented herein; the desert ringtail population has a MRM that is significantly lower than predicted for its body mass.

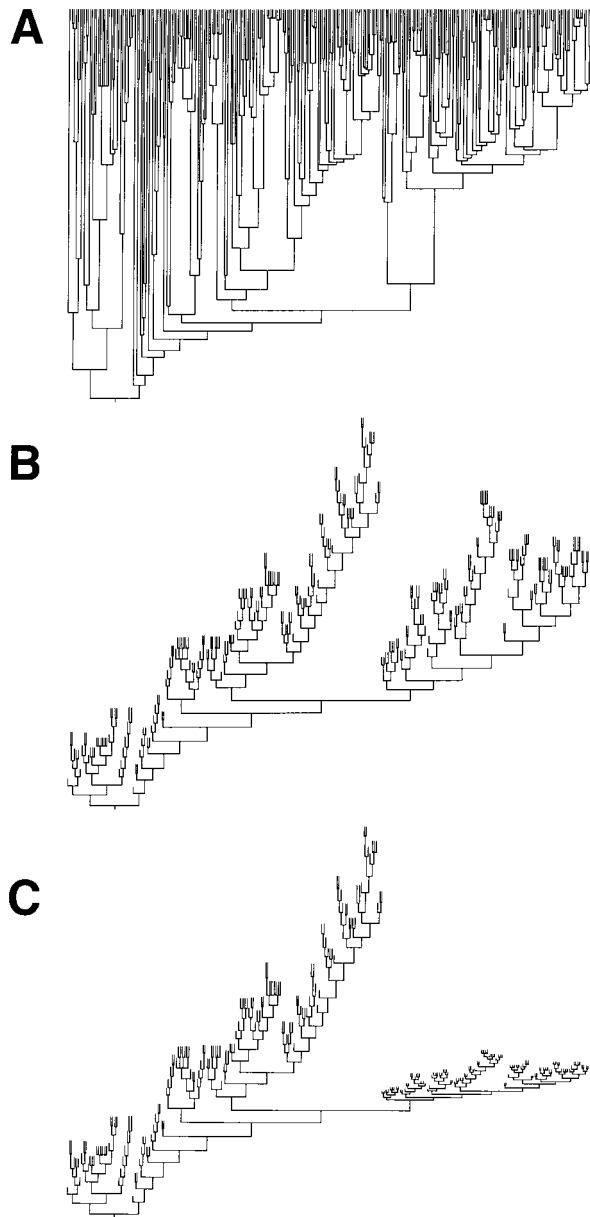
of sums of branch lengths) rather than showing actual values for species.

#### Allometry of Avian Basal Metabolic Rates

Reynolds and Lee (1996) compiled the available data for basal metabolic rates of birds. Phylogenetic relationships (topology and branch lengths, as shown in their figs. A1–A8) for the 254 species were based on Sibley and Ahlquist (1990); the taxonomic scheme in Sibley and Monroe (1990) was used to place some species within their respective genera (sometimes along with arbitrary partitioning of branch lengths). Reynolds and Lee (1996) checked the adequacy of the branch lengths derived from DNA hybridization (shown in fig. 5A) and used a modified Box-Cox procedure to arrive at an optimal branch length transformation of raising each branch segment to the power  $-0.2$  (fig. 5B). Note that this sort of inverse transformation actually converts the longest branches into the shortest. Such a transformation is difficult to reconcile with known microevolutionary mechanisms. Therefore, we also report analyses with the original untransformed branch lengths (fig. 5A) and with all branch lengths set equal to 1. Results do not change very much (see table 1), which is consistent with a number of published empirical examples and results of a simulation study that examined the effects of errors in branch lengths (Díaz-Uriarte and Garland 1998).

For all 254 species, all four of the independent-contrasts regression slopes shown in table 1 exceed the upper 95% confidence interval (0.687) of the conventional estimate. Figure 6 shows the independent-contrasts regression equation presented by Reynolds and Lee (1996, p. 741), based on DNA branch lengths raised to the  $-0.2$  power, plotted in the original data space by use of our procedures for computing a Y-intercept, confidence interval, and prediction interval. The regression line seems to fit the data for nonpasserines fairly well, but it clearly underestimates the log metabolic rate of most passerines in the data set. This is surprising, given that Reynolds and Lee (1996) applied phylogenetic ANCOVA (by both independent contrasts and computer simulation [see Garland et al. 1993]) and found no statistically significant difference between the mass-adjusted log metabolic rates of passerines and nonpasserines. What gives?

Figure 7 shows a diagnostic plot for contrasts in  $\log_{10}$  body mass. The statistical problem illustrated by this plot is the apparently lower average (minimum) rate of evolution (Garland 1992) in passerines as compared with other birds in the data set. The mean rank of the absolute values of standardized contrasts in passerines ( $n = 100$ ) is 99.52 as compared with 144.25 for contrasts within the nonpasserines ( $n = 152$ ; Mann-Whitney  $U = 4,902$ , two-



**Figure 5:** Topology and branch lengths compiled by Reynolds and Lee (1996, figs. A1–A8) for analyses of avian basal metabolic rate data. *A*, Branch lengths derived from DNA-hybridization data (from their DATA.PDI file). *B*, Branch lengths as in *A* but transformed by raising each segment to the  $-0.2$  power, as used by Reynolds and Lee (1996) for independent-contrasts analyses. *C*, As in *B* but with branch lengths within the passerine subclade rescaled so that height from its basal node to highest tip species is 4.0 (see text for explanation).

tailed  $P < .0001$  [the contrast between passerines and their sister clade is excluded from this test]). Moreover, if one examines the absolute values of the residuals from a regression of contrasts in BMR on contrasts in mass, the

passerines contrasts (mean rank = 105.84) also average smaller in magnitude than those for other birds (mean rank = 140.09; Mann-Whitney  $U = 5,534$ , two-tailed  $P = .0003$ ). The differences in rate of evolution are also apparent in figure 2 of Reynolds and Lee (1996): contrasts within passerines are clustered near the origin. Results (not shown) are very similar when all branch lengths are set equal to 1, and the pattern is also apparent with the original DNA branch lengths.

Thus, relative to DNA branch lengths raised to the  $-0.2$  power (or set equal to unity), passerines have a lower rate of  $\log_{10}$  body mass evolution and also a lower rate of mass-corrected  $\log_{10}$  BMR evolution. The overall data set is composed of (at least) two subsets, whose variances differ. Hence, fitting a single regression equation to the entire data set is inappropriate. Several solutions are possible. One would be to fit separate equations to the passerine and nonpasserine data sets. Another is to transform branch lengths differentially (as suggested by Grafen 1989, p. 146) within the passerine clade, which we illustrate.

The result that passerines have a relatively low rate of phenotypic evolution can equally well indicate that they have a high rate of DNA evolution. Indeed, Sibley and Ahlquist (1990), Bleiweiss et al. (1994), and Bleiweiss et al. (1995) have all noted that clades of birds do show significant differences in rates of DNA evolution. In either case, clade-specific variation in rates of evolution can be eliminated by rescaling all branch lengths within one or more clades. We tried several different rescalings of branches within the passerine subclade, using PDTREE, each time rechecking the diagnostic plot shown in figure 7. We found that rescaling the total height of the passerine subclade to 4.0, as shown in figure 5C, yielded absolute values of standardized contrasts that did not differ significantly between passerines (mean rank = 126.31) and nonpasserines (mean rank = 126.63; Mann-Whitney  $U = 7,581$ , two-tailed  $P = .9732$ ). For residuals from the regression of contrasts in log BMR on contrasts in log mass, the passerines contrasts (mean rank = 135.28) also do not differ significantly in magnitude from those for other birds (mean rank = 120.72; Mann-Whitney  $U = 6,722$ , two-tailed  $P = .1209$ ).

Thus, a contrast data set whose variance does not differ significantly between passerines and nonpasserines can be achieved by use of the branch lengths shown in figure 5C. Table 1 shows an allometric equation derived from these branch lengths.

Does Reynolds and Lee's (1996) conclusion that passerines and nonpasserines show no statistically significant difference in mass-corrected basal metabolic rate still hold? Yes. First, we repeated their independent-contrasts analysis (as described on both their p. 739 and fig. 2 [following Garland et al. 1993]) with the rescaled branch lengths



**Table 1:** Allometric equations for avian basal metabolic rate ( $\log_{10}$  watts; body mass in  $\log_{10}$  grams) of all 254 species

	Intercept			Slope			Diagnostic correlation	
	Lower 95% CI	Intercept	Upper 95% CI	Lower 95% CI	Slope	Upper 95% CI	Log mass	Log BMR
Conventional	−1.452	−1.411	−1.371	.652	.670	.687	−.500	−.521
Independent contrasts:								
Branch lengths = DNA hybridization	−1.884	−1.694	−1.504	.717	.759	.801	−.053	.165
Branch lengths = 1	−1.815	−1.647	−1.478	.688	.724	.759	.165	.102
Branch lengths = $\text{DNA}^{-2}$	−1.822	−1.652	−1.481	.687	.722	.757	.004	−.034
Branch lengths = $\text{DNA}^{-2}$ , passerine clade rescaled to height of 4.0	−1.827	−1.620	−1.413	.671	.709	.748	.049	.051

Source: Data from Reynolds and Lee (1996) and P. S. Reynolds and R. M. Lee, personal communication.

Note: Conventional least squares linear regressions and independent-contrasts least squares linear regressions are presented. For the latter, confidence intervals for the Y-intercept are derived by use of the new methods presented herein. For simplicity, these computations use the maximum possible degrees of freedom, as if all of the polytomies (unresolved nodes) in the phylogenetic tree ( $n = 26$  branches of 0 length) were hard (see Purvis and Garland 1993; Garland and Diaz-Uriarte 1999). “Diagnostic correlation” is the Pearson product-moment correlation (not through the origin) between the absolute values of standardized independent contrasts and their standard deviations (see text and Garland et al. 1992).

shown in figure 5C. The contrast between passerines and their sister clade was still not a significant outlier ( $P = .2935$ ). Repeating the analysis with all branch lengths set equal yielded  $P = .2187$ . We were concerned, however, that these analyses might be obscured by the inclusion of too wide a range of taxa. Therefore, we pruned the tree of all species except passerines ( $n = 101$ ) and their sister clade ( $n = 85$  [Columbiformes + Gruiformes + Charadriiformes]). For this reduced data set, examination of diagnostic plots as shown in figure 7 suggested that DNA branch lengths raised to the 0.8 power worked better than those raised to the  $-0.2$  power. Again, however, the passerines showed a significantly lower rate of evolution for both log body mass (Mann-Whitney  $U = 2,794$ , two-tailed  $P = .0001$ ) and residual log BMR ( $U = 2,947$ , two-tailed  $P = .0005$ ). Consequently, we rescaled the branches within the passerine subclade to a total height of 8.0, which eliminated the differences in evolutionary rates. We then repeated Reynolds and Lee’s (1996) analysis and again found that the contrast between passerines and their sister clade was not a significant outlier ( $P = .2549$ ).

One interesting additional result of this last analysis was that, for standardized contrasts in  $\log_{10}$  body mass, the contrast between passerines and their sisters was the fifth greatest in magnitude. The probability of this occurring by chance alone is  $5/185 = 0.0270$  (this is a two-tailed test because absolute values are analyzed). This result indicates that, on average, the passerines in this data set have significantly smaller  $\log_{10}$  body masses than their sister clade (see Garland et al. 1993, p. 283). We also performed this test for the entire data set of 254 species, using the branch lengths of figure 5C. Here, the contrast between passerines and their sister clade was only the fifty-first most extreme of 253 ( $P = .2016$ ). Thus, inclusion of all available spe-

cies in the analysis obscures statistically significant clade-specific variation in mean  $\log_{10}$  body mass.

## Discussion

We have shown how to derive both confidence and prediction intervals for regression equations derived from independent contrasts but mapped back onto the original data space. The procedures should be of considerable use in comparative studies of allometry, and so forth. They are also useful as a diagnostic in a general sense. For example, plotting the equation of Reynolds and Lee (1996) back onto the original data space clearly showed that something was amiss (fig. 6).

As explained in Felsenstein’s (1985) original presentation, when independent contrasts are used to estimate the form of a relationship between two traits, including least squares regression, reduced major axis, and major axis slopes (see Garland et al. 1992 for formulae), the line must be forced through the origin. Thus, no estimate of the Y-intercept is immediately available. Plotting the estimated slope back onto the original data space would, therefore, be impossible. However, lines describing bivariate relationships are generally constructed to pass through the point  $\bar{X}$ ,  $\bar{Y}$ . One can, therefore, position a line in the original data space by forcing it through the point  $\bar{X}$ ,  $\bar{Y}$ , where these estimates are computed as the root node estimate from independent contrasts (see also Garland et al. 1999). The first published example of this procedure is in figure 2 of Garland et al. (1993), which depicts home range areas of mammals in relation to body mass. More recently, Williams (1996) has used the procedure to derive phylogenetically correct allometric equations for evaporative water loss of birds. As in many other allometric studies, Wil-

liams (1996) used the equations to predict evaporative water loss values of hypothetical birds of various body sizes. No confidence interval was available for the  $Y$ -intercept of the regression equation, however, so prediction intervals could not be computed.

#### *Equivalency of the Independent-Contrasts and Generalized Least Squares Approaches*

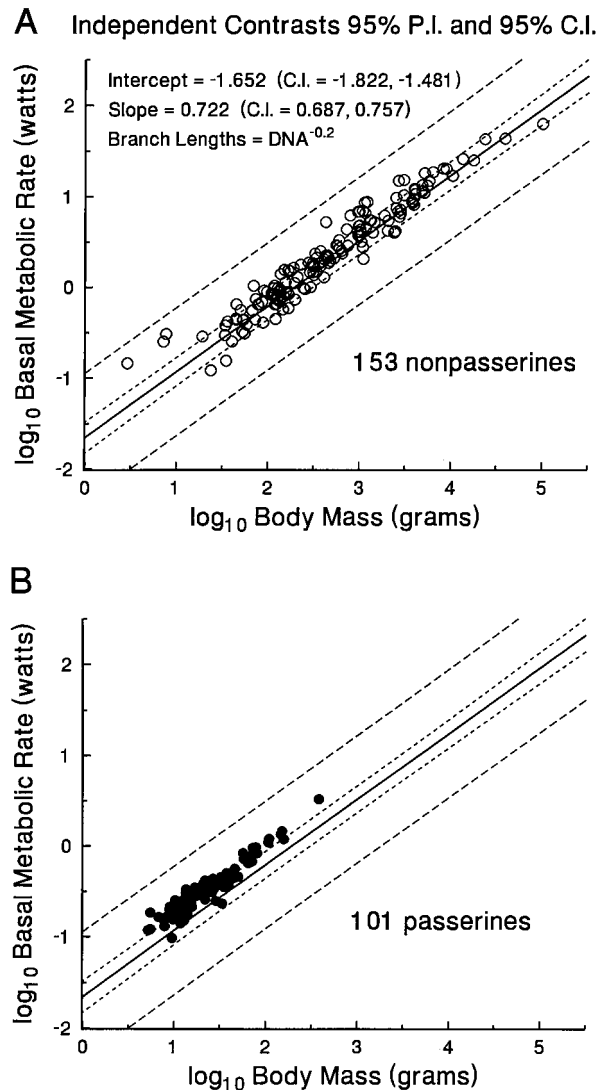
Both independent-contrasts and generalized least squares approaches start from the same statistical model (eq. [1]), use the same phylogenetic information, and give the same results. The main conceptual difference is that IC starts by estimating values of traits  $x$  and  $y$  for ancestral species; the trait value for an ancestral species is the average of its two daughter species weighted by their expected evolutionary divergence. GLS, on the other hand, uses a linear transformation of traits  $x$  and  $y$  in which a new set of variables,  $Z$  and  $U$ , are created from linear combinations of  $Y$  and  $X$ , respectively, weighted by matrix  $D$ . Thus, both approaches are weighted regression, with their difference being the procedure used to calculate weights.

The conceptual and computational differences between IC and GLS influence how they can be most effectively implemented. In the IC approach, weightings are calculated in terms of trait values of ancestral species. With this interpretation, it is easy to analyze the case in which traits show different rates of evolution (e.g., different branch lengths) across the phylogenetic tree as a whole, or even varying rates within different subsets of the tree (e.g., see fig. 5C). Although in principle these calculations are also possible with the GLS approach, the reference to branch lengths on a phylogenetic tree—rather than a weighting matrix—makes the IC approach more intuitive and visually apparent.

The strength of the GLS approach is that it transforms a regression with correlated residuals into a standard least squares regression problem. This opens the toolbox of familiar statistical diagnostics (such as tests for normality of residuals, homoscedasticity, linearity, etc.) that can be applied with standard least squares regression. Of course, corresponding diagnostics can be applied in the IC approach, which uses regression through the origin, although diagnostics for regression through the origin are less well developed.

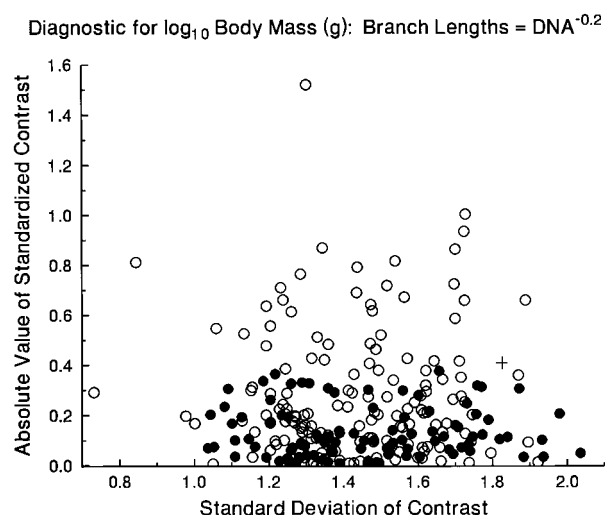
#### *Allometry of Avian Basal Metabolic Rate*

Table 1 shows that conventional and phylogenetically based estimates of allometric equations can differ significantly, even with large sample sizes ( $n = 254$  species), when several orders of magnitude in body mass are involved and when the relationship is quite tight (conven-



**Figure 6:** Independent-contrasts regression equation (see table 1) presented by Reynolds and Lee (1996, p. 741), based on DNA branch lengths raised to the  $-0.2$  power, plotted in the original data space by use of our new procedures for computing a  $Y$ -intercept, 95% confidence interval (dotted lines), and 95% prediction interval for a new observation (dashed lines). The regression line seems to fit the data for nonpasserines fairly well (A), but it clearly underestimates the log metabolic rate of most passerines (B) in the data set.

tional  $r^2 = 0.958$ ). These differences alter one's conclusions with respect to hypotheses about allometric exponents. Comparative physiologists have argued for years about the theoretical and empirical scaling exponent of basal metabolic rate. Scaling exponents of both  $2/3$  and  $3/4$  are often claimed to have special meaning (references in Calder 1984; Schmidt-Nielsen 1984; Harvey and Pagel 1991; Kozlowski and Weiner 1997). For the avian metabolic rate



**Figure 7:** Diagnostic plot of absolute values of standardized independent contrasts in  $\log_{10}$  body mass versus their standard deviations (square roots of sums of branch lengths, based on DNA distances raised to the  $-0.2$  power, as shown in fig. 5B). This plot suggests that the branch lengths are statistically acceptable in that it shows no overall trend ( $r = 0.004$ ) and approximates one-half of a normal distribution in the vertical direction (following Garland et al. 1992). However, it clearly shows that contrasts within the passerines (closed circles) are, on average, smaller in magnitude (Mann-Whitney  $U$ -test, two-tailed  $P < .0001$ ; a plus sign denotes the contrast between passerines and nonpasserines, which was excluded from the test). This indicates a difference in the average (minimum) rate of evolution (Garland 1992). Thus, the data as a whole are not “identically distributed,” as would be required for subsequent regression analyses.

data set taken as a whole, a conventional allometric equation has a slope of 0.670 and a 95% confidence interval that excludes 0.75 (table 1). Independent-contrasts equations have higher slopes, whose lower 95% confidence intervals exclude 0.67. Ironically, our preferred equation for all 254 species (table 1), which incorporates slower evolutionary change within the passerine clade, has a slope of 0.709 and confidence intervals that exclude both 0.67 and 0.75. Before too much is made of these slope estimates and confidence intervals, however, it should be remembered that if one attempted to incorporate variance caused by uncertainty about topology, branch lengths or the model of character evolution, then confidence intervals would be even wider (e.g., see Purvis and Garland 1993; Abouheif 1998; Garland and Díaz-Uriarte 1999).

The conventional and independent-contrasts equations can yield quite different predictions and prediction intervals. For a 2-g bird, for example, the conventional equation for all 254 species yields a prediction of 0.0617 w and a 95% prediction interval of 0.0344–0.1105 w; corresponding values for the independent-contrasts equation with the

passerine branch lengths rescaled are 0.0392 and 0.0061–0.2544 w. The latter may seem depressingly wide, but they can be narrowed greatly by more precise specification of the phylogenetic position of the species to be predicted, using the new methods that we present here (e.g., see fig. 3 and next section).

We do not offer the allometric equations of table 1 as final answers to problems of scaling avian metabolic rate. Only 2.6% of the world's extant avian species (9,702 in Monroe and Sibley 1993) are included in the data base. As in Reynolds and Lee's (1996) original analyses, we have not compared all possible subclades with respect to either allometric relationships or rates of evolution. We suspect that statistically significant heterogeneity of average mass-corrected metabolic rates may yet be shown to exist among clades of birds. For example, three of the four largest outliers in the nonpasserine data set are hummingbirds, which are only represented herein by four species (of 322 extant). Plus, as noted by Reynolds and Lee (1996), the available data are not a representative sample of birds. This can be illustrated even within the passerines, which consist of two major subclades, oscines (suborder Passeri: 4,580 species) and suboscines (suborder Tyranni: 1,159 species). In this data set, the oscines are represented by 92 species (2.0%), the suboscines by only nine (0.8%). Also, an analysis that accounted for variation associated with diet or ecology as additional independent variables could increase power to detect clade differences. Finally, the topology used for analyses includes species grafted onto Sibley and Ahlquist's (1990) phylogeny with little empirical support, a tree that itself almost certainly contains topological errors (but see Bleiweiss et al. 1994, 1995). As with most broad-scale comparative studies, both more (and more representative) tip data and additional phylogenetic information would be highly desirable. Ideally, a descriptive and predictive equation for an entire clade, such as Aves, should be derived from a (perhaps stratified) random sampling of its members.

As compared with other birds, we have shown that passerines have lower rates of evolution for log body mass (fig. 7), log BMR, and mass-corrected (residual) BMR. These differences were not noted by Reynolds and Lee (1996), in part because they did not have the methods to map regressions with confidence intervals back onto the original data space (e.g., fig. 6). The difference in evolutionary rates suggests that separate allometric equations are warranted, even though phylogenetic ANCOVA fails to detect a significant difference in allometric relationships between passerines and nonpasserines (Reynolds and Lee 1996; see also below). The significant difference in mean body mass between passerines (smaller) and their sister clade—reported here for the first time—also bolsters the argument for use of separate equations.

### Predictions for Unmeasured Species

Moving beyond the idea of clade-specific allometric equations (see previous section), the new methods presented here allow predictions for unmeasured species to become increasingly precise as phylogenetic information becomes more and more detailed. Consider the question, What is the predicted metabolic rate of a 2-g bird? If the hypothetical species is specified no more precisely than “a bird,” then the independent-contrasts equations for all 254 species would have to be used (and the final one listed in table 1 would be recommended). If it were specified to be a passerine bird, then an equation for the 101 passerine species alone could be used.

If the hypothetical species were specified to be a member of a particular passerine subclade, then the method illustrated in figure 3 could be used, with the species rooted at the base of the subclade. Alternatively, one could recompute the independent-contrasts equation using only the data for members of that subclade. The latter procedure would be recommended if evidence suggested heterogeneity in the mass-corrected metabolic rate of different passerine subclades. In general, however, deciding which species to include in a comparative study will often involve trade-offs among sample size, range of the independent variable, and the desire to avoid including apples with citrus (e.g., see discussions in Garland and Adolph 1994; Garland et al. 1997).

If the hypothetical species were specified as the close relative of a particular measured species, then the most precise predictions could be made, in terms of both the point estimate and the width of the prediction intervals. As in most inferential procedures, the more information we have, and the more accurate it is, the better we can

do. Note also that the phylogenetically informed prediction intervals (e.g., fig. 3D, 3E) can be narrower than those from conventional analyses (fig. 3B), leading to greatly enhanced statistical power to detect whether a particular species or population deviates from an expectation (e.g., fig. 4). Thus, what phylogeny taketh away (cf. fig. 3C to fig. 3B), phylogeny giveth back, at least with proper statistical methods.

Paleontologists often estimate body sizes of extinct forms from knowledge of the sizes of one or a few bones (Damuth and MacFadden 1990). With our procedures, the extinct form could be specified in one of three general phylogenetic positions: as a tip on a branch that terminates at some point before the present, as an internal node on the phylogenetic tree, or as a point somewhere along a given branch. The rerooting procedure is used in all three cases. Importantly, the latter two cases involve predictions for hypothetical ancestors that were directly on the line of descent to particular extant species. Previous methods for ancestor reconstruction have been limited to estimation of values at nodes (review in Garland et al. 1999).

### Acknowledgments

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## APPENDIX A

### Independent-Contrasts Approach

Before deriving statistical properties for the expectation of  $y$  given  $x$ , it is necessary to review Felsenstein's (1985) original IC method. Let  $x_i$  and  $x_j$  denote values of a trait at two adjacent nodes (or tips)  $i$  and  $j$ , and let  $y_i$  and  $y_j$  denote the values of a second trait that is assumed to depend on  $x$ . With  $\Delta x_{ij} = x_i - x_j$  and  $\Delta y_{ij} = y_i - y_j$  denoting the (nonstandardized) contrasts, the regression model of independent contrasts is

$$\Delta y_{ij} = \beta_1 \Delta x_{ij} + \sqrt{v'_i + v'_j} \epsilon_{ij}, \quad (\text{A1})$$

where  $\beta_1$  is the slope of the regression,  $v'_i$  and  $v'_j$  are the corrected branch lengths below nodes  $i$  and  $j$ , and  $\epsilon_{ij}$  is a normal random variable with mean 0 and variance  $\sigma^2$ . The corrected branch length  $v'_i$  takes account of the uncertainty in values estimated for ancestral species. Specifically,  $v'_i$  is calculated recursively from  $v'_i = v'_i + (v'_k v'_l) / (v'_k + v'_l)$ , where  $v_i$  is the uncorrected branch length and  $v'_k$  and  $v'_l$  are the corrected branch lengths above species  $i$  on the phylogenetic tree. In this particular formulation, the branch lengths for both  $x$  and  $y$  are assumed to be the same (Garland et al. 1992; Díaz-Uriarte and Garland 1996). In general, however, the branch lengths for  $x$  and  $y$  need not be the same,

which in equation (A1) is equivalent to transforming  $\Delta y_{ij}$  by multiplying by the ratio  $(\sqrt{v'_i + v'_j})/(\sqrt{u'_i + u'_j})$ , where  $v_i$  and  $u_i$  are branch lengths for  $x$  and  $y$ , respectively. This would be done following any other branch-length transformations.

The estimator of  $\beta_1$ ,  $\hat{\beta}_1$ , is calculated using regression through the origin (Garland et al. 1992), standardizing contrasts by  $1/\sqrt{v'_i + v'_j}$ :

$$\hat{\beta}_1 = \frac{\sum_{\text{contrasts}} \left[ (\Delta x_{ij}) \left( \frac{1}{\sqrt{v'_i + v'_j}} \right) \right] \left[ (\Delta y_{ij}) \left( \frac{1}{\sqrt{v'_i + v'_j}} \right) \right]}{\sum_{\text{contrasts}} \left[ (\Delta x_{ij}) \left( \frac{1}{\sqrt{v'_i + v'_j}} \right) \right]^2}. \quad (\text{A2})$$

The estimator of the variance of  $\epsilon_{ij}$  is

$$\hat{\sigma}^2 = \frac{1}{N-2} \sum_{\text{contrasts}} \left( \frac{\Delta y_{ij} - \hat{\beta}_1 \Delta x_{ij}}{\sqrt{v'_i + v'_j}} \right)^2 \quad (\text{A3})$$

for a phylogenetic tree with  $N$  tips (Neter et al. 1989, pp. 167–168). The  $1 - 2\alpha$  confidence interval for  $\hat{\beta}_1$  is

$$\hat{\beta}_1 \pm t_{\alpha, N-2} \sqrt{\frac{\hat{\sigma}^2}{\sum_{\text{contrasts}} \left[ (\Delta x_{ij}) \left( \frac{1}{\sqrt{v'_i + v'_j}} \right) \right]^2}}. \quad (\text{A4})$$

The estimate and the confidence interval for the value of  $y_i$  at tip  $t$  depend on the mean  $E\{y_i|x_i\}$  and variance  $V\{y_i|x_i\}$  of the distribution of  $y_i$  given  $x_i$ . The confidence interval is calculated presuming that tip  $t$  is rooted directly to the base of the tree with branch length equal to the sum of branch lengths leading to tip  $t$ . Formulae for  $E\{y_i|x_i\}$  and  $V\{y_i|x_i\}$  are derived below.

For nodes (and tips) other than the basal node, the regression model for independent contrasts can be written

$$y_i = \beta_1(x_i - x_w) + y_w + \sqrt{v'_i}\epsilon_i, \quad (\text{A5})$$

where  $x_w$  and  $y_w$  denote the values of  $x$  and  $y$  at the node immediately preceding node  $i$  and  $\epsilon_i$  is a normal random variable with mean 0 and variance  $\sigma^2$ . The regression model of equation (A5) is formally identical to the model of equation (A1).

The values of  $y_i$  at the tips of the phylogenetic tree can be expressed in terms of the basal value of  $y$  (denoted  $y_z$ ) by recursively working down the phylogenetic tree by the relationships

$$\begin{aligned} y_i &= \beta_1(x_i - x_w) + y_w + \sqrt{v'_i}\epsilon_i \\ &= \beta_1(x_i - x_w) + \beta_1(x_w - x_{2w}) + y_{2w} + \sqrt{v'_i}\epsilon_i + \sqrt{v'_w}\epsilon_w \\ &\vdots \\ &= \beta_1(x_i - x_z) + y_z + \sum_{\text{lineage}} \sqrt{v'_k}\epsilon_k, \end{aligned} \quad (\text{A6})$$

where the subscript  $2w$  denotes the node two below node  $i$ , and the summation is taken over all nodes  $k$  in the lineage between node  $i$  and the basal node. Incidentally, this formula demonstrates the need for the independent-contrasts method. The value of  $y_i$  depends on the values of  $\epsilon_k$  along the lineage from the basal node. For tips that partially share a lineage, values of  $y_i$  share some of the same values of  $\epsilon_k$ , making them nonindependent.

The estimator of  $y_i$  as a function of  $x_i$  at all nodes (and tips) other than the basal node is defined recursively as

$$\hat{y}_i = \hat{\beta}_1(x_i - x_w) + \hat{y}_w, \quad (\text{A7})$$

where  $\hat{\beta}_1$  is given by equation (A2). The estimator of the basal node is derived using the usual independent-contrasts method (Felsenstein 1985; Garland et al. 1999):

$$\hat{y}_z = \frac{v'_2 \hat{y}_1 + v'_1 \hat{y}_2}{v'_1 + v'_2}, \quad (\text{A8})$$

where  $\hat{y}_1$  and  $\hat{y}_2$  are the estimates of values of  $y$  at nodes 1 and 2 above the basal node, which themselves are obtained recursively from the preceding nodes by use of the formula

$$\hat{y}_k = \frac{v'_i \hat{y}_i + v'_j \hat{y}_j}{v'_i + v'_j}. \quad (\text{A9})$$

The value of  $x$  at the basal node,  $x_z$ , is calculated similarly.

For tip  $t$ , the random variable  $\hat{y}_t$  is normally distributed because it is the sum of normal random variables  $\hat{\beta}_1$  and  $\hat{y}_z$ . The expectation of  $\hat{y}_t$  given  $x_t$  is

$$\begin{aligned} E\{\hat{y}_t|x_t\} &= E\{\hat{\beta}_1(x_t - x_w) + \hat{y}_w\} \\ &= E\{\hat{\beta}_1(x_t - x_z) + \hat{y}_z\} \\ &= \beta_1(x_t - x_z) + y_z, \end{aligned} \quad (\text{A10})$$

and its variance is

$$\begin{aligned} V\{\hat{y}_t|x_t\} &= V\{\hat{\beta}_1(x_t - x_w) + \hat{y}_w\} \\ &= V\{\hat{\beta}_1(x_t - x_z) + \hat{y}_z\} \\ &= V\{\hat{\beta}_1\}(x_t - x_z)^2 + V\{\hat{y}_z\} \\ &= \frac{(x_t - x_z)^2}{\sum_{\text{contrasts}} [(\Delta x_{ij})/(\sqrt{v'_i + v'_j})]^2} \sigma^2 + \frac{v'_1 v'_2}{v'_1 + v'_2} \sigma^2. \end{aligned} \quad (\text{A11})$$

In equation (A11), the expression for  $V\{\hat{\beta}_1\}$  derives from equation (A2). From equation (A8), the value of  $V\{\hat{y}_z\}$  is obtained from

$$V\{\hat{y}_z\} = \left( \frac{v'_2}{v'_1 + v'_2} \right)^2 v'_1 \sigma^2 + \left( \frac{v'_1}{v'_1 + v'_2} \right)^2 v'_2 \sigma^2 = \frac{v'_1 v'_2}{v'_1 + v'_2} \sigma^2.$$

Finally, independence of  $\hat{\beta}_1$  and  $\hat{y}_i$  follows from Fisher's lemma in a manner analogous to standard linear regression (Larsen and Marx 1981).

From regression through the origin,  $[(N-2)\hat{\sigma}^2]/\sigma^2$  is a  $\chi^2_{N-2}$  distribution. From equations (A10) and (A11),

$$\frac{\hat{y}_t - [\hat{\beta}_1(x_t - x_z) + y_z]}{\sigma \sqrt{\frac{(x_t - x_z)^2}{\sum_{\text{contrasts}} [(\Delta x_{ij})/(\sqrt{v'_i + v'_j})]^2} + [(v'_1 v'_2)/(v'_1 + v'_2)]}}$$

is normally distributed with mean 0 and variance 1. Therefore,

$$\frac{\hat{y}_t - [\hat{\beta}_1(x_t - x_z) + y_z]}{\sqrt{\hat{\sigma}^2(x_t - x_z)^2 \left\{ \sum_{\text{contrasts}} \left[ (\Delta x_{ij}) \left( \frac{1}{\sqrt{v'_i + v'_j}} \right)^2 \right] + [(v'_1 v'_2)/(v'_1 + v'_2)] \right\}}} \quad (\text{A12})$$

is a Student  $t_{N-2}$  distribution, and the  $1 - 2\alpha$  confidence interval for  $E\{\hat{y}_t | x_i\}$  is

$$\hat{y}_z + \hat{\beta}_1(x_t - x_z) \pm t_{\alpha, N-2} \sqrt{\frac{1}{N-2} \sum_{\text{contrasts}} \left( \frac{\Delta y_{ij} - \hat{\beta}_1 \Delta x_{ij}}{\sqrt{v'_i + v'_j}} \right)^2 \left\{ (x_t - x_z)^2 \left[ \sum_{\text{contrasts}} \left( \frac{\Delta x_{ij}}{\sqrt{v'_i + v'_j}} \right)^2 \right] + \frac{v'_1 v'_2}{v'_1 + v'_2} \right\}}. \quad (\text{A13})$$

Equation (A13) leads directly to the confidence interval for the  $y$ -intercept of the independent-contrasts regression by setting  $x_t = 0$ .

To obtain an estimate and prediction interval of  $y_h$  for a new species  $h$ , first suppose that species  $h$  is rooted to the base of the phylogenetic tree on a branch of length  $v_h$ . Then the model for the estimator of  $y_h$  is

$$\hat{y}_h = \hat{\beta}_1(x_h - x_w) + \hat{y}_w + v_h \epsilon_h, \quad (\text{A14})$$

where  $\epsilon_h$  is a normally distributed random variable with mean 0 and variance  $\sigma^2$ . Following from the derivation of equation (A13), the prediction interval for  $\hat{y}_h$  is

$$\hat{y}_h + \hat{\beta}_1(x_h - x_z) \pm t_{\alpha, N-2} \sqrt{\frac{1}{N-2} \sum_{\text{contrasts}} \left( \frac{\Delta y_{ij} - \hat{\beta}_1 \Delta x_{ij}}{\sqrt{v'_i + v'_j}} \right)^2 \left\{ (x_h - x_z)^2 \left[ \sum_{\text{contrasts}} \left( \frac{\Delta x_{ij}}{\sqrt{v'_i + v'_j}} \right)^2 \right] + \frac{v'_1 v'_2}{v'_1 + v'_2} + v_h \right\}}. \quad (\text{A15})$$

To obtain prediction estimates of  $\hat{y}_h$  when species  $h$  is not rooted to the base of the tree, the tree can be rerooted as illustrated in figure 2B and the analysis performed as described above. The actual selection of the branch length  $v_h$  depends on the application. If  $v_h$  is known for a particular species, then it can be used in equation (A15). For the prediction interval for a species whose location on the phylogenetic tree is unknown, the species could be rooted to the base of the tree, and  $v_h$  could be given the average base-to-tip distance for all known species in the phylogeny.

PDTREE calculates the estimates of  $\hat{\beta}_1$  (eq. [A2]),  $\hat{\sigma}^2$  (eq. [A3]), confidence intervals for  $\hat{y}_i$  (eq. [A13]), and predictions  $\hat{y}_h$  for species rooted to the base of the phylogenetic tree (eq. [A15]). PDTREE can also reroot the phylogenetic tree, thus allowing computation of prediction intervals for hypothetical new species anywhere.

## APPENDIX B

### Generalized Least Squares Approach

Rather than restrict the analysis to a single independent variable, we will consider  $P$  independent variables. Thus, let  $\mathbf{X}$  be the  $n \times P$  matrix with elements  $x_{ik}$ , where  $i = 1, \dots, n$  denotes the species, and  $k = 1, \dots, P$  denotes the independent variable. To include a constant (intercept), the first independent variable is given the value of 1 for all species. The regression problem can be written

$$\mathbf{Y} = \mathbf{X}\boldsymbol{\beta} + \boldsymbol{\epsilon}, \quad (\text{B1})$$

where the vectors  $\mathbf{Y} = [y_1, y_2, \dots, y_n]'$ ,  $\boldsymbol{\epsilon} = [\epsilon_1, \epsilon_2, \dots, \epsilon_n]'$ , and  $\boldsymbol{\beta} = [\beta_1, \beta_2, \dots, \beta_p]'$ . Following Martins and Hansen (1997), let  $\sigma^2\mathbf{C}$  be the variance-covariance matrix of  $\boldsymbol{\epsilon}$ ;  $\sigma^2\mathbf{C} = E\{\boldsymbol{\epsilon}\boldsymbol{\epsilon}'\}$ . (Note that Martins and Hansen [1997] denote our matrix  $\sigma^2\mathbf{C}$  as  $\mathbf{V}$ .) The  $i - j^{\text{th}}$  element of  $\mathbf{C}$  is the sum of branch segment lengths that species  $i$  and species  $j$  share in common. The total branch lengths from root to tips are not constrained to be the same for all species.

As described in the text, let  $\mathbf{D}$  be an  $n \times n$  matrix such that  $\mathbf{D}\mathbf{C}\mathbf{D}' = \mathbf{I}$ . Obtaining  $\mathbf{D}$  involves singular value decomposition of  $\mathbf{C}$ , which is performed by statistical packages such as MatLab with the `SVD()` function. Transforming variables as  $\mathbf{Z} = \mathbf{D}\mathbf{Y}$ ,  $\mathbf{U} = \mathbf{D}\mathbf{X}$ , and  $\boldsymbol{\alpha} = \mathbf{D}\boldsymbol{\epsilon}$  converts the GLS problem given by equation (B1) into a standard least squares problem given by equation (4). Statistical inference can be performed for  $\mathbf{Z}$  and then back-transformed to  $\mathbf{Y}$ . Below is a set of standard formulae (Judge et al. 1985; Neter et al. 1989): estimate of  $\boldsymbol{\beta}$ ,  $\hat{\boldsymbol{\beta}}$ :

$$\hat{\boldsymbol{\beta}} = (\mathbf{U}'\mathbf{U})^{-1}(\mathbf{U}'\mathbf{Z}) = (\mathbf{X}'\mathbf{C}^{-1}\mathbf{X})^{-1}(\mathbf{X}'\mathbf{C}^{-1}\mathbf{Y}); \quad (\text{B2})$$

unbiased estimate of variance  $\sigma^2$ ,  $\hat{\sigma}^2$ :

$$\hat{\sigma}^2 = (\mathbf{Z} - \mathbf{U}\hat{\boldsymbol{\beta}})'(\mathbf{Z} - \mathbf{U}\hat{\boldsymbol{\beta}})/(n - P) = (\mathbf{Y} - \mathbf{X}\hat{\boldsymbol{\beta}})'(\mathbf{Y} - \mathbf{X}\hat{\boldsymbol{\beta}})/(\mathbf{C}^{-1}(n - P)); \quad (\text{B3})$$

variance-covariance matrix of  $\boldsymbol{\beta}$ ,  $V\{\boldsymbol{\beta}\}$ :

$$V\{\boldsymbol{\beta}\} = \sigma^2(\mathbf{U}'\mathbf{U})^{-1} = \sigma^2(\mathbf{X}'\mathbf{C}^{-1}\mathbf{X})^{-1}; \quad (\text{B4})$$

estimated variance-covariance matrix of  $\boldsymbol{\beta}$ ,  $s^2\{\boldsymbol{\beta}\}$ :

$$s^2\{\boldsymbol{\beta}\} = \hat{\sigma}^2(\mathbf{U}'\mathbf{U})^{-1} = \hat{\sigma}^2(\mathbf{X}'\mathbf{C}^{-1}\mathbf{X})^{-1}; \quad (\text{B5})$$

estimates of mean responses of  $\mathbf{Y}$ ,  $\hat{\mathbf{Y}}$ :

$$\hat{\mathbf{Y}} = \mathbf{D}^{-1}(\mathbf{D}\mathbf{X}\hat{\boldsymbol{\beta}}); \quad (\text{B6})$$

estimated variance-covariance matrix of mean responses of  $\mathbf{Y}$ ,  $s^2\{\hat{\mathbf{Y}}\}$ :

$$s^2\{\hat{\mathbf{Y}}\} = \mathbf{D}^{-1}s^2\{\hat{\mathbf{Z}}\}(\mathbf{D}^{-1})' = \mathbf{X}s^2\{\boldsymbol{\beta}\}\mathbf{X}'. \quad (\text{B7})$$

Note that with the variance-covariance of mean responses,  $s^2\{\hat{\mathbf{Y}}\}$ , it is possible to calculate the joint confidence intervals for the mean responses  $\hat{\mathbf{Y}}$ .

These formulae are exact under the assumption of Brownian motion evolution and can be used for statistical inference in the usual least squares regression fashion. Thus,  $n\hat{\sigma}^2/\sigma^2$  follows a  $\chi^2$  distribution with  $n - P$  degrees of freedom. Similarly,  $(\beta_k - \hat{\beta}_k)/s\{\hat{\beta}_k\}$  and  $(y - \hat{y})/s\{\hat{y}\}$  follow  $t$ -distributions with  $n - P$  degrees of freedom. Even though these expressions are exact only under Brownian motion evolution, by the Central Limit Theorem they are asymptotically correct for large sample sizes when error terms are nonnormal, provided the errors are identically distributed and have covariance structure given by  $\mathbf{C}$ . The general expression for the (nonestimated) variance-covariance matrix,  $\sigma^2(\mathbf{X}'\mathbf{C}^{-1}\mathbf{X})^{-1}$ , can be used to analyze cases in which  $\mathbf{C}$  is unknown; this is the case discussed broadly by Martins and Hansen (1997, pp. 659–662) and explains the difference between their discussion and the estimation procedure presented here. Note also that the estimates and standard errors for  $b_0$  and  $b_1$  given in figure 2 of Martins and Hansen (1997) are incorrect because of a computational error (T. F. Hansen, personal communication).

It is instructive to compare these results with those obtained from independent contrasts in appendix A. For the case of only one independent variable, the following identities hold: estimate of variance  $\sigma^2$ ,  $\hat{\sigma}^2$ :



$$\begin{aligned}\hat{\sigma}^2 &= (\mathbf{Z} - \mathbf{U}\hat{\boldsymbol{\beta}})'(\mathbf{Z} - \mathbf{U}\hat{\boldsymbol{\beta}})/(n - 2) \\ &= \frac{1}{N - 2} \sum_{\text{contrasts}} \left( \frac{\Delta y_{ij} - \hat{\beta}_1 \Delta x_{ij}}{\sqrt{v'_i + v'_j}} \right)^2;\end{aligned}\quad (\text{B8})$$

estimated unconditional variances of  $\mathbf{Y}$ ,  $\text{diag}(s^2\{\hat{\mathbf{Y}}\})$ :

$$\begin{aligned}\text{diag}(s^2\{\hat{\mathbf{Y}}\}) &= \text{diag}(\mathbf{X}s^2\{\boldsymbol{\beta}\}\mathbf{X}') = \hat{\sigma}^2 \text{diag}[\mathbf{X}(\mathbf{X}'\mathbf{C}^{-1}\mathbf{X})^{-1}\mathbf{X}'] \\ &= \hat{\sigma}^2 \left( \left( (x_t - x_z)^2 \left[ \sum_{\text{contrasts}} (\Delta x_{ij}) \left( \sqrt{v'_i + v'_j} \right)^2 \right] \right) + [(v'_1 v'_2)/(v'_1 + v'_2)] \right).\end{aligned}\quad (\text{B9})$$

This last expression leads to the further identity that

$$(\mathbf{1}'\mathbf{C}^{-1}\mathbf{1})^{-1} = \frac{v'_1 v'_2}{v'_1 + v'_2}, \quad (\text{B10})$$

where  $\mathbf{1}$  is an  $n \times 1$  vector of ones. These expressions show the clear relationships between weightings in terms of  $v'_i$  in the IC approach and weightings in terms of  $\mathbf{C}$  in the GLS approach.

Prediction intervals for a new species  $h$  can either be calculated by rerooting the tree, as described for the IC method, or by explicitly calculating the conditional mean and variance of the error term  $\epsilon_h$  for the new species. For new species  $h$ , the value of  $y_h$  is given by

$$y_h = \beta_0 + \beta_1 x_h + \epsilon_h, \quad (\text{B11})$$

where  $\epsilon_h$  is normally distributed with mean  $\mu$  and variance  $\sigma^2 c_h$ . Both  $\mu$  and  $c_h$  depend on the error terms obtained for all related species on the phylogenetic tree (here, "related" means that the amount of shared branch length is  $>0$ ). In particular, if  $c_{ih}$  gives the sum of branch lengths shared by species  $i$  and  $h$ , and  $\mathbf{C}_{ih}$  is the  $n \times 1$  vector of values of  $c_{ih}$  for all species  $i$  other than  $h$ , then  $\mu = \mathbf{C}'_{ih} \mathbf{C}^{-1}(\mathbf{X} - \bar{x})$ , and  $c_h = c_{hh} - \mathbf{C}'_{ih} \mathbf{C}^{-1} \mathbf{C}_{ih}$  (Box et al. 1994, pp. 282–285; see also Martins and Hansen 1997, p. 661). The computations used in appendix B are not performed by PDTree, but they can be performed using such software packages as MatLab (MathWorks 1996).

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