Syst. Biol. 62(2):181–192, 2013

DOI:10.1093/sysbio/sys083
Advance Access publication September 27, 2012

Comparing Evolutionary Rates for Different Phenotypic Traits on a Phylogeny Using Likelihood

DEAN C. ADAMS*

Department of Ecology, Evolution, and Organismal Biology and Department of Statistics, Iowa State University, Ames, IA 50011, USA
**Correspondence to be sent to: E-mail: dcadams@iastate.edu.*

Received 31 May 2012; reviews returned 14 August 2012; accepted 17 September 2012
Associate Editor: Luke Harmon

Abstract.—In recent years, likelihood-based approaches have been used with increasing frequency to evaluate macroevolutionary hypotheses of phenotypic evolution under distinct evolutionary processes in a phylogenetic context (e.g., Brownian motion, Ornstein-Uhlenbeck, etc.), and to compare one or more evolutionary rates for the same phenotypic trait along a phylogeny. It is also of interest to determine whether one trait evolves at a faster rate than another trait. However, to date no study has compared phylogenetic evolutionary rates between traits using likelihood, because a formal approach has not yet been proposed. In this article, I describe a new likelihood procedure for comparing evolutionary rates for two or more phenotypic traits on a phylogeny. This approach compares the likelihood of a model where each trait evolves at a distinct evolutionary rate to the likelihood of a model where all traits are constrained to evolve at a common evolutionary rate. The method can also account for within-species measurement error and within-species trait covariation if available. Simulations revealed that the method has appropriate Type I error rates and statistical power. Importantly, when compared with existing approaches based on phylogenetically independent contrasts and methods that compare confidence intervals for model parameters, the likelihood method displays preferable statistical properties for a wide range of simulated conditions. Thus, this likelihood-based method extends the phylogenetic comparative biology toolkit and provides evolutionary biologists with a more powerful means of determining when evolutionary rates differ between phenotypic traits. Finally, I provide an empirical example illustrating the approach by comparing rates of evolution for several phenotypic traits in *Plethodon* salamanders. [Evolutionary rates; macroevolution; morphological evolution; phenotype; phylogenetic comparative method; phylogeny.]

Describing the pace of evolutionary change is essential for understanding how morphological, and ultimately, biological diversity is generated and maintained. Biologists have often observed that rates of phenotypic evolution differ across taxonomic groups (e.g., Simpson 1944; Gingerich 1993; Harmon et al. 2003; Hulsey et al. 2010). Furthermore, differences or changes in evolutionary rates may play a significant role generating large-scale phenotypic trends (Foote 1997; Sidlauskas 2008; Ackerly 2009). Indeed, several evolutionary models posit a direct link between contemporary selection pressures, rates of phenotypic evolution, and macroevolutionary patterns of phenotypic diversification (see Hansen and Martins 1996; Schluter 2000; Mahler et al. 2010). However, testing these hypotheses has remained a challenge, in part because methods for assessing rates of phenotypic evolution in a phylogenetic context have only recently been developed (e.g., Garland 1992; O'Meara et al. 2006; Thomas et al. 2006).

Frequently, evolutionary rates are quantified in terms of the amount of phenotypic change between ancestor and descendent lineages, standardized by time. One approach, the "darwin" (Haldane 1949), measures evolutionary rates in terms of the difference of log-trait values between ancestors and descendents per million years, and is often used in paleontological studies. Another measure, the "haldane" (Gingerich 1983), estimates the evolutionary rate as the change in phenotypic trait values across generations (standardized for variation within traits) and is more commonly used in neontological studies. For sets of traits, Mahalanobis distance has been used as a multivariate analog of the haldane (Lerman 1965; Gingerich 2009; Arnegard et al. 2010), although this has sometimes been applied to pairs of extant species rather than ancestor–descendent lineages (e.g., Arnegard et al. 2010; Carlson et al. 2011). Both darwins and haldanes have been used extensively to estimate the pace of evolutionary change in a wide variety of taxa and traits (for reviews, see Gingerich 1983, 1993, 2009; Hendry and Kinnison 1999; Hendry et al. 2008). However, a shortcoming of these measures is that they only estimate evolutionary rates between pairs of taxa. When the rate of phenotypic evolution for an entire clade of organisms is of interest, methods that incorporate the phylogenetic relatedness among taxa are required.

Over the past several years, likelihood-based approaches have been developed for estimating the rate of phenotypic change along a phylogeny based on a particular model of evolution. Typically, a Brownian motion (BM) model of evolution is utilized (Edwards and Cavalli-Sforza 1964; Felsenstein 1973, 1985), although other models describing alternative scenarios of phenotypic evolution, such as Ornstein-Uhlenbeck (OU: Lande 1976; Hansen and Martins 1996; Hansen 1997; Butler and King 2004) and early-burst (EB) models (Blomberg et al. 2003), can also be examined. In addition, evolutionary models that include distinct evolutionary rates on different parts of the phylogeny can be assessed. Here, likelihood approaches are used to compare the fit of an evolutionary model with a single rate to a model containing multiple evolutionary rates (e.g., O'Meara et al. 2006; Thomas et al. 2006) to

determine whether the tempo of phenotypic evolution has changed on particular branches of the phylogeny (see also Eastman et al. 2011; Revell et al. 2012). Such approaches can provide evidence for differences in evolutionary rates among clades, or shifts in evolutionary rates within clades (e.g., Collar et al. 2009; Thomas et al. 2009; Hulsey et al. 2010). An increasing number of studies have used this approach to examine the tempo of evolution in various phenotypic traits within and among lineages (e.g., Collar et al. 2009; Thomas et al. 2009; Harmon et al. 2010; Mahler et al. 2010; Price et al. 2010; Valenzuela and Adams 2011).

Another important question concerning the tempo of evolution is whether one phenotypic trait evolves at a faster rate than another. Numerous studies have examined trait evolution in both fossil and extant lineages, revealing considerable variation in the rate of phenotypic evolution among traits and clades (Gingerich 1983, 1993; Hendry and Kinnison 1999; Hunt 2007; Ackerly 2009). Furthermore, several authors have suggested that some phenotypic traits, such as behavioral characteristics, evolve at a faster rate than other traits, such as morphology (Huey and Bennett 1987; Gittleman et al. 1996; Blomberg et al. 2003). Indeed, comparisons of phylogenetically independent contrasts for different traits have revealed that some traits do evolve faster than others (Garland 1992; Katzourakis et al. 2001; Hone et al. 2005; Ackerly 2009), lending support to this hypothesis. In addition, several recent studies have quantified evolutionary rates for multiple traits using likelihood approaches (Adams et al. 2009; Revell and Collar 2009; Harmon et al. 2010; Martin and Wainwright 2011). Thus, at first glance it appears conceptually straightforward to employ likelihood approaches to identify differences in evolutionary rates among traits. However, no study has explicitly compared evolutionary rates between traits in a phylogenetic framework using likelihood, because methods have not been developed for testing the hypothesis that different traits evolve at distinct evolutionary rates. In this article, I extend existing methods by describing a likelihood procedure that allows the comparison of evolutionary rates for two or more phenotypic traits on a phylogeny. With the approach, within-species measurement error and within-species trait covariation can also be accounted for when these are available. I then compare the statistical performance of this method to several alternative approaches, and show that the likelihood approach has preferable statistical properties. I provide a biological example demonstrating the utility of the approach, and computer code written in R for implementing the procedure.

Comparing Evolutionary Rates For Different Traits

For a single phenotypic trait, the evolutionary rate of change under a BM model of evolution can be estimated using several analytical approaches, such as phylogenetic generalized least squares (PGLS: Grafen 1989; Martins and Hansen 1997), independent contrasts (e.g., Garland and Ives 2000), and maximum likelihood (e.g., O'Meara et al. 2006). Using the PGLS formulation, the least squares and maximum likelihood estimates (MLE) of the evolutionary rate parameter are equivalent (Felsenstein 1973; see also Garland and Ives 2000; O'Meara et al. 2006), and can be found as:

$$\sigma^2 = \frac{\left[\mathbf{Y} - E(\mathbf{Y})\right]^{\mathbf{t}} \mathbf{C}^{-\mathbf{1}} \left[\mathbf{Y} - E(\mathbf{Y})\right]}{N} \quad (1)$$

where $\mathbf{Y}$ is a $N \times 1$ column vector containing the phenotypic values for the N species, and $E(\mathbf{Y})$ is a $N \times 1$ column vector of phylogenetic means, found as $\widehat{a} = \left(\mathbf{1}^{\mathbf{t}}\mathbf{C}^{-\mathbf{1}}\mathbf{1}\right)^{-1}\left(\mathbf{1}^{\mathbf{t}}\mathbf{C}^{-\mathbf{1}}\mathbf{Y}\right)$ (Rohlf 2001; Blomberg et al. 2003; O'Meara et al. 2006) [note: matrix notation is retained here for consistency with previous authors (e.g., O'Meara et al. 2006), and because $\mathbf{Y}$ could also represent a matrix of phenotypic values, in which case, $\widehat{a}$ would be a vector, see Revell and Collar 2009]. The phylogenetic relationships among taxa are encoded by the phylogenetic covariance matrix, $\mathbf{C}$, which is an $N \times N$ matrix constructed from the phylogenetic tree (Garland and Ives 2000; Freckleton et al. 2002; O'Meara et al. 2006; Thomas et al. 2006). Notice that equation (1) represents the MLE of σ^2, which is slightly downwardly biased (see O'Meara et al. 2006). To obtain the unbiased estimate of σ^2, the numerator of equation (1) is divided by $(N-1)$ rather than N. A numerically equivalent estimate of σ^2 can also be obtained using phylogenetically independent contrasts rather than PGLS (see e.g., Felsenstein 1985; Garland 1992; Garland and Ives 2000; Ackerly 2009). Finally, for a univariate trait and under a BM model of evolution, the log likelihood of σ^2, given the phenotypic data ($\mathbf{Y}$), the ancestral state ($\widehat{a}$), and the phylogeny ($\mathbf{C}$) is derived from the multivariate normal distribution (Felsenstein 1973; O'Meara et al. 2006), and is given by:

$$\log(L) = -\frac{1}{2}\left\{\left[\mathbf{Y} - E(\mathbf{Y})\right]^{\mathbf{t}}\left(\sigma^2\mathbf{C}\right)^{-\mathbf{1}}\left[\mathbf{Y} - E(\mathbf{Y})\right]\right\} - \log\left|\sigma^2\mathbf{C}\right| \Big/ 2 - N\log(2\pi)/2. \quad (2)$$

For a set of traits treated simultaneously (i.e., a multivariate $\mathbf{Y}$ matrix), equation (1) results in an evolutionary rate matrix ($\mathbf{R}$: sensu Revell and Harmon 2008), which is the algebraic extension of σ^2 (Freckleton et al. 2002; McPeek et al. 2008; Revell and Harmon 2008; Adams et al. 2009). Here, the diagonal elements (σ^2_{ii}) represent the evolutionary rates for each of the individual traits and are identical to the evolutionary rates estimated for each trait when it is analyzed separately. The off-diagonal elements (σ^2_{ij}) describe the evolutionary covariation between traits, which have been interpreted as evolutionary correlations between characters (e.g., Revell and Collar 2009). Under a BM model of evolution, the log likelihood of $\mathbf{R}$, given the

phenotypic data (**Y**), the ancestral state for each of the traits ($\widehat{a}$), and the phylogeny (**C**) is obtained from a generalization of equation (2):

$$\log(L) = -\frac{1}{2}\left\{[\mathbf{Y}-E(\mathbf{Y})]^{\mathbf{t}}(\mathbf{R}\otimes\mathbf{C})^{-\mathbf{1}}[\mathbf{Y}-E(\mathbf{Y})]\right\} - \log|\mathbf{R}\otimes\mathbf{C}|/2 - N\log(2\pi)/2 \quad (3)$$

where the expected evolutionary covariance matrix is found as the Kronecker product of the evolutionary rate matrix and the phylogenetic covariance matrix ($\mathbf{R}\otimes\mathbf{C}=\mathbf{V}$, see Revell and Harmon 2008).

To determine whether two or more phenotypic traits differ in their evolutionary rates, one can compare the log likelihoods from two alternative models that describe distinct ways in which phenotypic variation evolves. In the first model, each trait evolves according to its own evolutionary rate, whereas in the second model, all phenotypic traits are constrained to evolve at a common evolutionary rate. This procedure is conceptually similar to methods that evaluate changes in evolutionary rates for a single trait on a phylogeny by comparing a model containing one rate to models containing two or more rates in different parts of the tree (e.g., O'Meara et al. 2006; Thomas et al. 2006; Revell 2008; Revell and Harmon 2008; Thomas et al. 2009). For the model where all traits evolve at their own separate rates, the evolutionary rate matrix (**R**) for all traits is obtained using equation (1), and the log likelihood of this evolutionary model is found using equation (3). Next, the alternative evolutionary scenario is examined, where all phenotypic traits are constrained to evolve at a common rate. For this model, there is a common evolutionary rate along the diagonal of the evolutionary rate matrix (**R**), but all trait covariances are left unconstrained. Determining the log likelihood of this alternative model requires an optimization routine that searches over possible rate matrices containing a common evolutionary rate σ^2, and selects the rate matrix **R** that maximizes the joint likelihood for the traits as described in equation (3). Unfortunately, implementing this procedure is not straightforward due to a number of computational issues. First, the rate matrix must be positive semidefinite, as **R** is a covariance matrix. This criterion can be satisfied by optimizing over parameters of the Cholesky decomposition of **R**, rather than the rates and evolutionary covariances that comprise the rate matrix itself (see Revell and Collar 2009; Revell 2012). Second, the optimization is further complicated by the fact that the rate matrix **R** must be constrained to have equal values along its diagonals. Typically, parameters from matrix decomposition do not guarantee this property. However, this mathematical constraint may be imposed in the Cholesky decomposition of **R** by adjusting the manner in which the Cholesky matrix is derived. Appendix 1 provides the algebraic details for obtaining this constrained Cholesky decomposition, which is utilized here.

Using parameters from the constrained Cholesky decomposition, likelihood optimization may proceed in the usual fashion. First, an initial set of parameters are provided, and used to generate the rate matrix **R**, which, by construction, is constrained to contain equal diagonal elements. The likelihood of this model is then found using equation (3). Next a quasi-Newton method is used to search an approximation of the log likelihood surface to identify the parameters that maximize the log likelihood as described in equation (3). This approach thus identifies the MLE found under the constraint that the diagonal elements of the rate matrix **R** are equal. The two evolutionary models are then statistically compared using a likelihood ratio test $\left[\text{LRT} = -2\left(\log L_{\text{constrained}} - \log L_{\text{obs}}\right)\right]$, which is approximately chi-square distributed with $p-1$ degrees of freedom (the difference in estimated parameters between the observed and constrained models). A significant LRT provides support for the hypothesis that the phenotypic traits evolve at different rates. Additionally, AIC values can be obtained and compared for the two models. Computer code written in R for implementing the above procedures is found in Appendix 2.

Finally, an alternative implementation of the above procedure may be used to model the scenario where no evolutionary covariation between traits is assumed. This is accomplished by setting the off-diagonal elements of the rate matrix to zero and maximizing the likelihood of this model, which is identical to the sum of the log likelihoods for each trait treated separately (results not shown). In this case, the alternative model of equal rates for all traits is optimized for the common evolutionary rate σ^2, and the value of σ^2 that maximizes the joint likelihood for the traits as described in equation (3) is treated as the common evolutionary rate for all traits. Note that although models assuming trait independence are biologically unrealistic (as phenotypic traits are frequently evolutionarily correlated), this approach is analogous to rate comparisons based on phylogenetically independent contrasts (Garland 1992), which are obtained for each trait individually and without incorporating evolutionary covariation between traits.

Trait Scale

One important consideration when comparing evolutionary rates among traits is the effect of trait scale. Trait variation is dependent on both units and scale, with larger traits generally displaying larger variances (Sokal and Rohlf 2011). Evolutionary rates (which are phylogenetically standardized variances) are also scale-dependent (Gingerich 1993; Arnegard et al. 2010); for instance, a trait represented in millimeters will have a σ^2 that is 100 times higher than σ^2 for the same data represented in centimeters. Similarly, traits that differ greatly in their means will have different per-unit changes, because traits with larger values are expected to have larger changes per unit time. As a consequence, comparisons of evolutionary rates

between traits may be compromised if the traits under investigation are not in commensurate units. A number of authors have advocated log-transforming the data prior to the estimate of evolutionary rates (Felsenstein 1985; O'Meara et al. 2006; Ackerly 2009; Gingerich 2009). This provides a scale-free estimate of evolutionary rates, and has the desirable property that evolutionary rates are described as the relative rate of change in proportion to the mean for each trait. Furthermore, log-transformation permits the comparison of traits measured in different units, because the resulting evolutionary rates are expressed in terms of the relative change in proportion to the mean for each trait. In accord with these suggestions, I recommend that when traits are in different scales, they should be log-transformed prior to the comparison of evolutionary rates. However, for instances, when scale dependence cannot be accounted for by log-transformation, alternative transformations may be required.

Within-Species Variation and Measurement Error

Another important consideration when comparing evolutionary rates is measurement error. Most phylogenetic comparative studies assume that within-species variation is negligible. However, within-species variation and measurement error can have a profound effect on parameters estimated from evolutionary models (Ives et al. 2007; Felsenstein 2008). For instance, increased within-species variation (or sampling error) will cause evolutionary rates to be overestimated (Ives et al. 2007). This can complicate comparisons of evolutionary rates between traits, as differences in sampling error can lead to perceived differences in evolutionary rates. Several methods for incorporating within-species variation in phylogenetic comparative analyses have recently been proposed (Ives et al. 2007; Felsenstein 2008; also O'Meara et al. 2006; Harmon et al. 2010). For instance, for a single trait, the within-species measurement error can be accounted for by adding this variation to the diagonal of the expected evolutionary covariance matrix (**V**) before calculating the likelihood (Harmon et al. 2010; also O'Meara et al. 2006). In this case, however, **V** is of dimension $(pN \times pN)$, as it is found through the Kronecker product of the evolutionary rate matrix and the phylogenetic covariance matrix ($\mathbf{R} \otimes \mathbf{C} = \mathbf{V}$). Therefore, a generalization of the approach described earlier is utilized. Here, the within-species measurement error vectors for each trait are first concatenated into a vector of length pN. This vector is then added to the diagonal of **V** prior to the likelihood calculations. Additionally, if within-species trait covariation is available, this can also be incorporated by adding it to the appropriate subdiagonals of **V** (i.e., those elements of **V** that represent the trait covariation within each species for each pair of traits). The approach allowing for within-species measurement error and within-species trait covariation is implemented in the computer code in Appendix 2.

Additional Tests and Flow of Computations

With all analytical approaches, sound biological inferences are only possible when alternative explanations for the observed patterns have been investigated. In this regard, several alternatives should be considered when testing for differences in evolutionary rates. First, perceived differences in evolutionary rates may arise simply because the phenotypic traits are in different scales; as larger traits are expected to have higher rates (see above). As such, log transforming the data prior to the estimate of evolutionary rates is recommended for traits that differ notably in scale. Second, measurement error may generate perceived differences in evolutionary rates, so when possible, within-species measurement error should be examined and accounted for (see above). Finally, comparisons of evolutionary rates may be compromised if the traits differ in their degree of phylogenetic structure (Blomberg et al. 2003), or if their patterns of diversification follow distinct models of evolution (e.g., BM vs. EB). As distinct modes of trait evolution are possible, it is recommended that model-fitting procedures first be used to determine whether a BM model of evolution best describes the observed patterns of phenotypic diversification for the set of traits, or whether some alternative model is favored. However, it should also be noted that while such procedures allow the best fitting model to be identified, BM still provides a general overall measure of the evolutionary rate of change of a trait along the phylogeny, and thus remains useful for identifying traits that differ greatly in their evolutionary rates. In other words, σ^2 represents the "effective rate" of trait evolution (see discussion in Ackerly 2009), although when models differ markedly between traits, comparisons of σ^2 must be evaluated more cautiously.

Statistical Performance

To evaluate the performance of the proposed likelihood procedure, I executed a series of computer simulations. For each simulation, 1000 random phylogenies containing 32 taxa were generated (results from a wider set of phylogenies under different conditions are found in the Supplemental Material, available on Dryad at http://datadryad.org, doi:10.5061/dryad.1117q). Two continuous phenotypic traits were then evolved along each phylogeny under a variety of conditions. Changes in both traits followed a BM model of evolution, with a rate parameter for the first trait of $\sigma_1^2 = 1.0$, and the rate of the second trait equal to or exceeding that of the first trait, depending upon simulation conditions. Evolutionary rates for both traits were then estimated as described earlier, and a likelihood ratio test was used to determine whether or not the traits differed in their evolutionary rates (both implementations of the likelihood approach were evaluated: Supplemental Material). Additionally,

two alternative approaches were used to test for differences in evolutionary rates. First, phylogenetically independent contrasts were obtained for each trait, and evolutionary rates for the two traits were compared using a *t*-test on the absolute value of the contrasts (Garland 1992). Second, 95% confidence intervals (CIs) were estimated from the standard errors of each evolutionary rate as obtained from the Hessian matrix, and these CIs were examined for potential overlap (for details, see Supplemental Material). For all approaches, the number of significant results (out of 1000) was treated as an estimate of Type I error (when $\sigma_1^2 = \sigma_2^2$) or statistical power (when $\sigma_2^2/\sigma_1^2 > 1.0$). Results from additional computer simulations for a wider range of conditions are found in the Supplemental Material.

Using this procedure, I found that the likelihood-based approach displayed appropriate Type I error rates, and its statistical power increased rapidly as the relative difference in evolutionary rates between traits increased (Fig. 1). Furthermore, the likelihood-based approach displayed higher statistical power than the method based on independent contrasts (Fig. 1), and this result was consistent across all simulation conditions evaluated (Supplemental Figures 1 and 2). Similarly, the likelihood-based approach outperformed the method based on 95% CI. Here, the latter displayed Type I error rates lower than expected, and had low power when differences between evolutionary rates were small (Fig. 1). Finally, simulations revealed that the method performed equally well when traits were simulated with or without evolutionary covariation between them (Supplemental Figure 3), and parameter estimates obtained from the likelihood approach adequately represented input rates for the simulations (Supplemental Figure 4). Overall, these results demonstrate that the likelihood-based method exhibits superior statistical properties as compared with alternative approaches, and thus provides a powerful means of detecting differences in evolutionary rates between traits. This represents an important analytic advance over existing approaches.

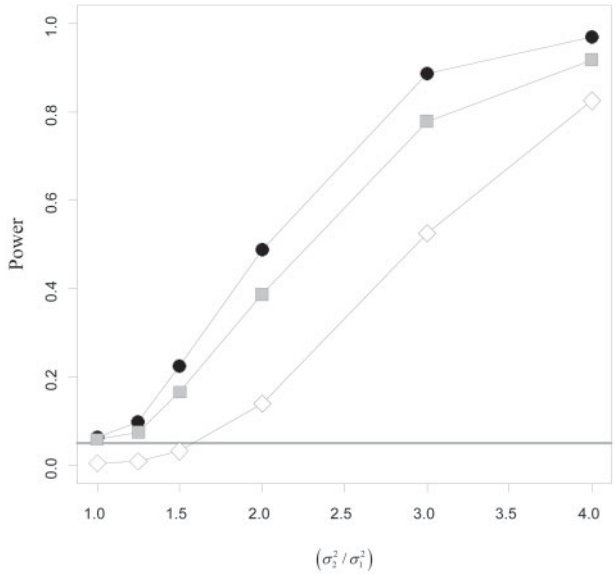

FIGURE 1. Statistical power curves for tests to compare rates of evolution revealed by computer simulations based on a phylogeny containing 32 taxa. The abscissa shows the relative difference in evolutionary rates between the 2 traits, whereas the ordinate displays the relative power based on the percentage of 1000 simulations found to be significant. Results for the likelihood-based approach are shown as black dots, results for the independent contrasts approach are shown as gray squares, and results from a comparison of 95% CIs of model parameter are shown as white diamonds. Results from simulations conduced over a wider set of conditions are found in the Supplemental Material.

A Biological Example

To illustrate the methods described earlier, I provide a biological example comparing evolutionary rates among multiple phenotypic traits in *Plethodon* salamanders. *Plethodon* are long-lived, direct-developing terrestrial salamanders found in North American forests (Highton 1995). Forty-five species inhabit eastern North America, and extensive field collecting at thousands of geographic localities has rigorously documented their geographic distributions. Considerable behavioral and ecological research has shown that interspecific competition is widespread (e.g., Jaeger 1971; Hairston 1980; Anthony et al. 1997; Deitloff et al. 2008), and likely influences community structure at both a local and regional scale (Adams 2007). In *Plethodon*, interspecific competition is often mediated through aggressive encounters, in which agonistic displays (e.g., all-trunk raised: Jaeger and Forester 1993) and biting (Anthony et al. 1997) frequently determine which individual is competitively superior. In some instances, interspecific competition has resulted in the evolution of morphological differences, particularly in head shape (e.g., Adams 2000, 2004, 2010; Adams and Rohlf 2000; Adams et al. 2007; Arif et al. 2007). Further, body size differences are prevalent between species (Adams and Church 2008, 2011), and because *Plethodon* are generalist predators (Petranka 1998), species of similar size may compete more intensively for similar prey resources (see Kozak et al. 2009). Together, these observations suggest the hypothesis that morphological traits associated with species interactions, such as aggressive encounters or differential food acquisition, may have elevated rates of evolution as compared with other phenotypic attributes.

I obtained linear measurements for three phenotypic traits (head length, forelimb length, and body width) from 311 adult individuals, representing 44 of the 45 species of eastern *Plethodon* (Fig. 2a; data from Adams et al. 2009). For these traits, head length is expected to be related to competitive interactions between species, as an increased head length is correlated with increased jaw length, which in turn is related to both aggressive biting and prey acquisition (see e.g., Maglia and Pyles 1995; Adams and Rohlf 2000). Similarly, forelimb length is expected to be related to competitive interactions, as greater forelimb length results in greater perceived body size during aggressive displays (Jaeger and Forester

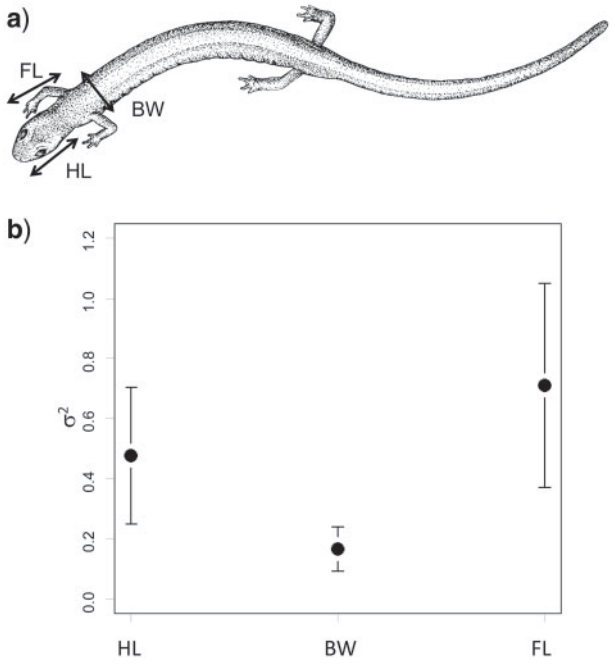

FIGURE 2. a) Locations of 3 linear measurements obtained from adult *Plethodon* salamanders (HL, head length; FL, forelimb length; BW, body width). b) Estimates of evolutionary rates for 3 linear traits used in this study, with their 95% CIs.

1993). For these species body width has no known association with selection mediated through competitive interactions. For each species, the mean value of each trait was obtained, and because all the 3 traits were measured in the same units (mm) and of the same scale (similar means and ranges), untransformed values were used for all analyses. AIC comparisons of evolutionary models (BM, OU, and EB) revealed that a BM model was the best model (i.e., lowest AIC) for 2 of the 3 phenotypic traits (forelimb length and body width), and had a ΔAIC <4.0 from the best model for head length. As such, a BM model was used in all subsequent analyses (see Burnham and Anderson 2002, p. 70).

From the species means I estimated the rate of evolution for each trait under a BM model of evolution (σ^2) using a multigene, time-calibrated molecular phylogeny (Wiens et al. 2006) for the genus (Fig. 3). Rate assessments were performed on both the set of mean trait values as described here, as well as the set of mean trait values standardized by the mean body size for each species. To determine whether or not the phenotypic traits evolved at similar rates, I used the procedure described earlier by comparing evolutionary rates among traits while allowing for evolutionary covariation between traits. In addition, analyses were performed for all pairs of traits to determine which morphological traits evolved at different rates. Because estimates of within-species variation and within-species trait covariation were available, I repeated the primary analysis above while accounting for measurement error in the likelihood calculations. Finally, for comparison, I examined the evolutionary rates among the traits under the model where no evolutionary covariation between traits was included. All calculations were performed in R 2.15 (R Development Core Team 2012) using the APE library (Paradis et al. 2004; Paradis 2012) and new routines written by the author (Appendix 2).

RESULTS

Likelihood ratio tests indicated that the three morphological traits were not evolving at a common evolutionary rate (Fig. 2b; Table 1). Further, pairwise comparisons revealed that both head length and forelimb length displayed considerably higher evolutionary rates than did body width, but the evolutionary rates for head length and forelimb length were not distinguishable from one another (Table 1). Analyses of traits standardized for body size yielded similar results to those described above (results not shown). AIC results were consistent with results found using likelihood, and showed that head length and forelimb length did not have appreciably distinct evolutionary rates, as the preferred model for this comparison was one with a single evolutionary rate for the two traits (Table 1). Incorporating within-species measurement error did not alter the results described above, as the likelihood ratio test under this scenario also revealed differences in evolutionary rates among the traits (Table 1). Similarly, analyses incorporating within-species trait covariation yielded consistent results with those reported here (not shown). Finally, for this example, statistical results obtained from a model assuming no evolutionary covariation between traits were concordant with results obtained from a model where evolutionary trait covariation was incorporated (Table 1). Therefore, biological conclusions from both approaches were equivalent. As expected, the model incorporating trait covariation had considerably better AIC and likelihood scores as compared to the model with no trait covariation, indicating that it provided a better description of phenotypic variation and evolutionary change in these phenotypic traits (Table 1). Biologically, the results here suggest that in *Plethodon*, different morphological traits evolve at distinct evolutionary rates. Further, because head length and forelimb length are related to competitive interactions, these observations are consistent with the hypothesis that selection resulting from interspecific interactions may increase the evolutionary rate of those traits important for such encounters, which may further enhance the morphological diversification in *Plethodon* (see discussion in Adams 2011).

DISCUSSION

In this article, I described an approach for comparing evolutionary rates for two or more phenotypic traits using likelihood. The approach extends existing procedures for comparing one or more evolutionary

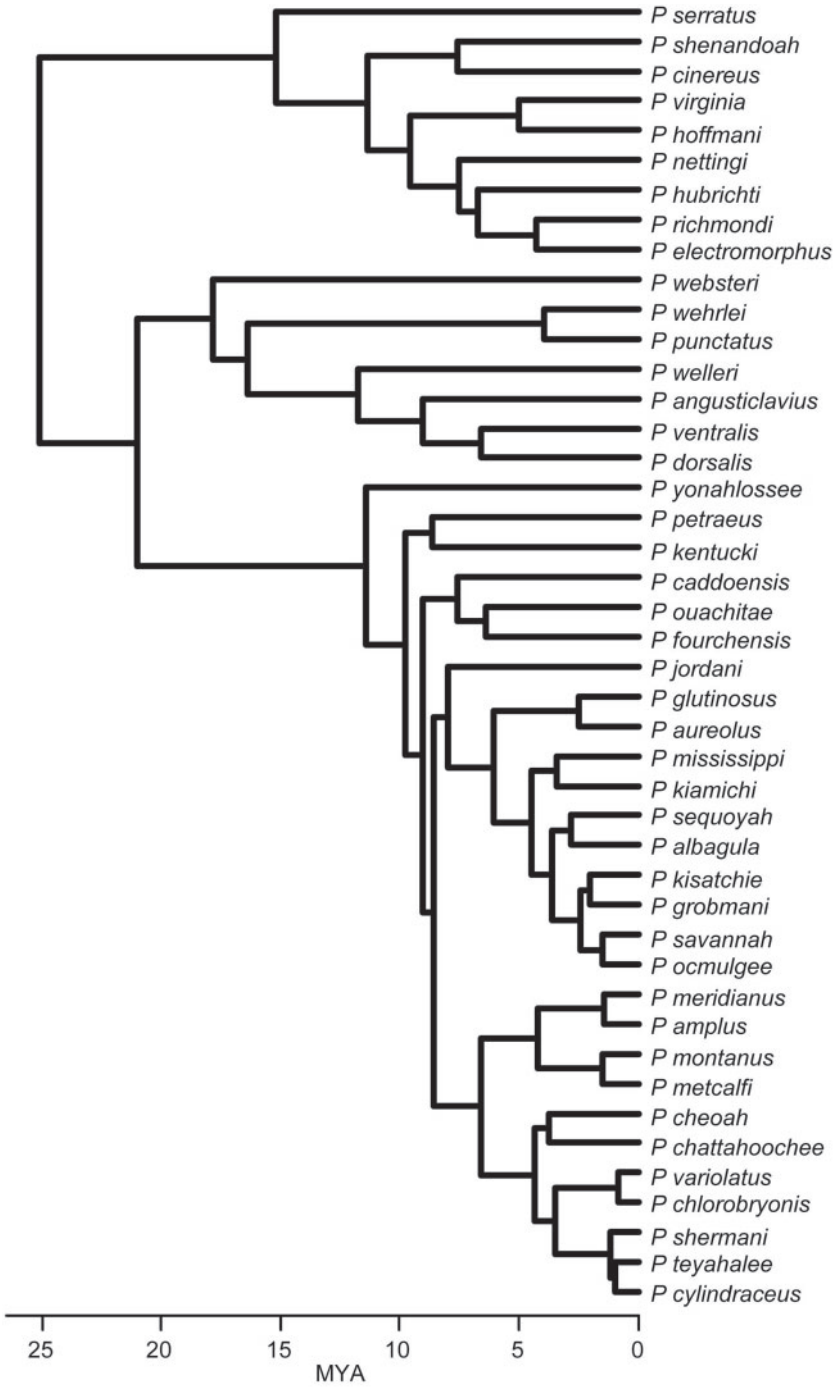

FIGURE 3. Fossil-calibrated molecular phylogeny displaying the estimated phylogenetic relationships among *Plethodon* species in eastern North America (from Wiens et al. 2006).

rates for the same phenotypic trait along a phylogeny (e.g., O'Meara et al. 2006; Thomas et al. 2006; Revell 2008) by allowing the evolutionary rate of multiple traits to be compared directly to one another. Additionally, within-species measurement error can be incorporated into the analysis when it is available. Using computer simulations, I demonstrated that the method had appropriate Type I error rates and statistical power. Further, when compared with alternative approaches based on phylogenetically independent contrasts, and methods that compare CIs for model parameters, the likelihood method displayed preferable statistical properties for a wide range of simulated conditions. Therefore, this new approach provides a powerful means of detecting when evolutionary rates differ between traits, thereby extending the likelihood framework for

TABLE 1. Empirical results comparing evolutionary rates for 3 phenotypic traits for 44 species of *Plethodon*.

A:σ^2_{obs}				$Log(L)_{obs}$	$Log(L)_{common}$	$LRT_{df=2}$	P	AIC_{obs}	AIC_{common}
HL	0.4765	0.2001	0.5109	−202.83					
BW	0.2001	0.1641	0.2784						
FL	0.5109	0.2784	0.7099						
σ^2_{common}									
HL	0.4177	0.2744	0.3422		−227.31	48.95	< 0.0001	417.67	462.63
BW	0.2744	0.4177	0.2658						
FL	0.3422	0.2658	0.4177						
Pairwise analyses						$LRT_{df=1}$	P	AIC_{obs}	AIC_{common}
HL versus BW						21.74	< 0.0001	294.95	314.69
BW versus FL						46.96	< 0.0001	295.93	340.89
HL versus FL						7.14	0.007	325.98	331.12
B: Incorporating measurement error				−209.93					
σ^2_{common}									
HL	0.326	0.302	0.286		−222.18	24.50	< 0.0001	431.86	452.36
BW	0.302	0.326	0.266						
FL	0.286	0.266	0.326						
C: Trait independence									
	σ^2_{obs}	σ^2_{common}		$Log(L)_{obs}$	$Log(L)_{common}$	$LRT_{df=2}$	P	AIC_{obs}	AIC_{common}
HL	0.4765	0.4502		−259.37	−270.29	21.85	< 0.0001	530.74	548.59
BW	0.1641								
FL	0.7099								

Notes: (A) Evolutionary rate comparisons performed while incorporating trait covariation. Here, the observed rate matrix, the rate matrix with a constant rate for all traits, their log(L) and AIC values, and likelihood-ratio tests are shown. Likelihood-ratio tests for pairs of traits are also shown. (B) The same analysis incorporating within-species measurement error. (C) Rate comparisons performed under the assumption of trait independence.
HL = head length; BW = body width; FL = forelimb length.

evaluating comparative patterns in evolutionary biology using a phylogenetic perspective.

Importantly, the method proposed here extends the phylogenetic comparative biology toolkit by expanding the type of evolutionary hypotheses that can be examined with phylogenetic comparative methods using likelihood. At present, existing approaches permit the evaluation of alternative hypotheses of trait evolution by comparing the fit of the data to the phylogeny under distinct evolutionary scenarios (such as BM, OU, or EB models: Butler and King 2004; O'Meara et al. 2006; Harmon et al. 2010). Other methods gauge whether or not a phenotypic trait displays one or more evolutionary rates on distinct regions of the phylogeny (e.g., O'Meara et al. 2006; Thomas et al. 2006; Revell 2008), which can be used to test hypotheses of rate shifts within lineages. The approach developed here complements these procedures, and extends them across trait boundaries, so that comparisons of evolutionary rates among phenotypic traits can also be assessed. Therefore, long-standing hypotheses concerning the relative rate of evolution between traits (such as the rate of size vs. shape evolution across taxa) can now be evaluated using a likelihood framework. Finally, the method proposed here can be additionally extended (in theory) to evaluate other distinct evolutionary models, by adjusting the phylogenetic covariance matrix according to alternative evolutionary processes (see Blomberg et al. 2003), or by scaling the phylogeny relative to the observed phylogenetic signal for the traits (see Pagel 1999). Incorporating the approach described here with existing approaches would therefore provide researchers greater flexibility in evaluating evolutionary hypotheses of character change, and the accumulation of phenotypic diversity within and across lineages.

SUPPLEMENTARY MATERIAL

Data files and/or other supplementary information related to this paper have been deposited on Dryad at http://datadryad.org under doi: 10.5061/dryad.1117q.

FUNDING

This work was partially supported by National Science Foundation [grant DEB-1118884].

ACKNOWLEDGEMENTS

I thank M. Collyer, A. Kaliontzopoulou, B. O'Meara, and J. Serb for discussion and comments on the manuscript, and J. Niemi for discussions on Cholesky

decomopositions of constrained covariance matrices. M. Alfaro, A. Purvis, G. Thomas, and several anonymous reviewers provided extensive constructive comments on previous versions of this manuscript and offered many useful suggestions. Jessica Gassman kindly provided the salamander illustration in Figure 2.

Appendix 1

Cholesky Decomposition of an Equi-Diagonal Covariance Matrix

For a set of p traits, a $p \times p$ covariance matrix Σ can be constructed, which is a positive semi-definite matrix. For any covariance matrix Σ, the Cholesky decomposition is defined as:

$$\Sigma = \mathbf{L}\mathbf{L}^t \tag{A.1}$$

where $\mathbf{L}$ is a lower-triangular matrix containing real entries. Suppose that Σ is constrained such that the diagonal elements of Σ are equal ($\sigma^2_{11} = \sigma^2_{22} = \cdots = \sigma^2_{pp}$). Algebraically, this constraint may be represented in the Cholesky decomposition of Σ. For example, when $p = 2$, $\mathbf{L} = \begin{bmatrix} l_{11} & 0 \\ l_{21} & l_{22} \end{bmatrix}$. Thus Σ can be represented as:

$$\begin{bmatrix} l_{11} & 0 \\ l_{21} & l_{22} \end{bmatrix}\begin{bmatrix} l_{11} & l_{21} \\ 0 & l_{22} \end{bmatrix} = \begin{bmatrix} l^2_{11} & l_{11}(l_{21}+l_{22}) \\ l_{11}(l_{21}+l_{22}) & l^2_{21}+l^2_{22} \end{bmatrix} \tag{A.2}$$

Under the constraint, $\sigma^2_{11} = \sigma^2_{22}$ and thus: $l^2_{11} = l^2_{21} + l^2_{22}$. It therefore follows that given l_{21} and l_{22}, l_{11} can be determined algebraically as:

$$l_{11} = \sqrt{l^2_{21} + l^2_{22}} \tag{A.3}$$

For $p > 2$, the diagonal elements of $\mathbf{L}$ can be found recursively, such that the constraint of equality of the diagonal elements of Σ remains satisfied. Required to obtain the diagonal elements of $\mathbf{L}$ are the $p(p-1)/2$ off-diagonal elements, plus one diagonal element (e.g., l_{pp}). Generalizing equation A.3 to more than 2 traits, l_{11} is found as the square-root of the sum of squared elements in the p^{th} row:

$$l_{11} = \sqrt{\sum_{i=1}^{p} l^2_{pi}} \tag{A.4}$$

Then, for $i = 2 \rightarrow (p-1)$, the remaining diagonal elements are found as:

$$l_{ii} = \sqrt{l^2_{11} - \sum_{j=1}^{i-1} l^2_{ij}} \tag{A.5}$$

Numerical Optimization: The Cholesky decomposition above describes the expected algebraic relationships among the diagonal elements in $\mathbf{L}$ given the constraint of equal diagonal elements of Σ. Algorithmically, this relationship can be utilized to obtain a Σ for which the diagonal elements are similarly constrained. To accomplish this, a vector (b) of length $p(p-1)/2$ of initial input parameters is first defined. Here, $b_1 = \log(l_{pp})$, and the remaining $p(p-1)/2$ elements of b are set to zero (these represent the initial starting values of the off-diagonal elements of $\mathbf{L}$). Next, $\mathbf{L}$ is assembled from b following equations A.2–A.5, and Σ is obtained using equation A.1. By construction Σ will contain equal diagonal elements. For the present application, Σ is then incorporated into equation 3 of the main text to obtain the likelihood of Σ given the data and the phylogeny. Finally, the parameters in b are optimized until the maximum likelihood estimate (MLE) is obtained for equation 3. This MLE is found under the constraint that the diagonal elements of Σ are equal.

Appendix 2

Computer Code for R

The function below estimates the Brownian motion evolutionary rate parameter (σ^2) for two or more phenotypic traits, as well as their combined likelihood. A second likelihood is then obtained, where the evolutionary rate of each trait is constrained to be the same. The two models are compared to determine whether or not the traits exhibit the same evolutionary rate. The method can be implemented with or without within-species measurement error. The code requires as input data a phylogeny (read into R using the APE library), a data matrix containing species values for the traits (where the row names of the data matrix match the species names in the phylogeny), and optionally a matrix of within-species measurement estimates for all species for each trait.

```
CompareRates.multTrait<-function(phy,x,TraitCov=
T,ms.err=NULL,ms.cov=NULL){
#Compares LLik of R-matrix vs. LLik of R-matrix with
constrained diagonal
#TraitCov = TRUE assumes covariation among traits
(default)
#ms.err allows the incorporation of within-species
measurement error. Input is a matrix of species (rows)
by within-species variation for each trait (columns).
#ms.cov allows the incorporation of within-species
covariation between traits. Input is a matrix of species
(rows) by within-species covariation for each pair of
traits (columns). These must be provided in a specific
order, beginning with covariation between trait 1 and
the rest, then trait 2 and the rest, etc. For instance, for 4
traits, the columns are: cov_12, cov_13, cov_14, cov_23,
cov_24 cov_34.
#Some calculations adapted from 'evol.vcv' in
phytools (Revell, 2012)
library(MASS)
  x<-as.matrix(x)
  N<-nrow(x)
  p<-ncol(x)
```

```
    C<-vcv.phylo(phy)
        C<-C[rownames(x),rownames(x)]
    if (is.matrix(ms.err)){
      ms.err<-as.matrix(ms.err[rownames(x),])}
    if (is.matrix(ms.cov)){
      ms.cov<-as.matrix(ms.cov[rownames(x),])}


  #Cholesky decomposition function for diagonal-
constrained VCV
  build.chol<-function(b){
    c.mat<-matrix(0,nrow=p,ncol=p)
    c.mat[lower.tri(c.mat)] <- b[-1]
    c.mat[p,p]<-exp(b[1])
    c.mat[1,1]<-sqrt(sum((c.mat[p,])^2))
    if(p>2){
    for (i in 2:(p-1)){
      c.mat[i,i]<-ifelse((c.mat[1,1]^2-sum((c.mat[i,])^2))
>0,
      sqrt(c.mat[1,1]^2-sum((c.mat[i,])^2)), 0)
    }}
  return(c.mat)
  }


  #Fit Rate matrix for all traits: follows code of L. Revell
(evol.vcv)
    a.obs<-colSums(solve(C))%*%x/sum(solve(C))
    D<-matrix(0,N*p,p)
      for(i in1:(N*p))for(j in1:p)if((j-1)*N<i&&i<=
j*N)D[i,j]=1.0
    y<-as.matrix(as.vector(x))
    one<-matrix(1,N,1)
    R.obs<-t(x-one%*%a.obs)%*%solve(C)%*%
(x-one%*%a.obs)/N
    if (TraitCov==F) #for TraitCov = F
    {R.obs<-diag(diag(R.obs),p)}
  #Calculate observed likelihood with or without
measurement error
    LLik.obs<-ifelse(is.matrix(ms.err)==TRUE,
    -t(y-D%*%t(a.obs))%*%ginv((kronecker(R.obs,C)+
diag(as.vector(ms.err))))%*%(y-D%*%t(a.obs))/2-
N*p*log(2*pi)/2-
      determinant((kronecker(R.obs,C)+
  diag(as.vector(ms.err))))$modulus[1]/2 ,
    -t(y-D%*%t(a.obs))%*%ginv(kronecker(R.obs,C))%
*%(y-D%*%t(a.obs))/2-N*p*log(2*pi)/2-
      determinant(kronecker(R.obs,C))$modulus[1]/2
    )


  #Fit common rate for all traits; search over parameter
space
    sigma.mn<-mean(diag(R.obs)) #reasonable start
value for diagonal


  #Within-species measurement error matrix
  if(is.matrix(ms.err)){m.e<-diag(as.vector(ms.err))}

  #Within-species measurement error and trait
covariation matrix
  if (is.matrix(ms.err) && is.matrix(ms.cov)){
    within.spp<-cbind(ms.err,ms.cov)
    rc.label<-NULL
    for (i in 1:p){ rc.label<-rbind(rc.label,c(i,i)) }
      for (i in 1:p){
      for(j in2:p){if(i!=j&&i<j){rc.label<
-rbind(rc.label,c(i,j))}}}}
    m.e<-NULL
    for (i in 1:p){
      tmp<-NULL
      for (j in 1:p){
        for (k in 1:nrow(rc.label)){
          if(setequal(c(i,j),rc.label[k,])==T)
{tmp<-cbind(tmp,diag(within.spp[,k]))}
        }
      }
    m.e<-rbind(m.e,tmp)
    }
  }


  #likelihood optimizer for no trait covariation
    lik.covF<-function(sigma){
      R<-R.obs
      diag(R)<-sigma
    LLik<-ifelse(is.matrix(ms.err)==TRUE,
    -t(y-D%*%t(a.obs))%*%ginv((kronecker(R,C)+
m.e))%*%(y-D%*%t(a.obs))/2-N*p*log(2*pi)/2-
      determinant((kronecker(R,C)+
m.e))$modulus[1]/2 ,
    -t(y-D%*%t(a.obs))%*%ginv(kronecker(R,C))%*%(y-
D%*%t(a.obs))/2-N*p*log(2*pi)/2-
      determinant(kronecker(R,C))$modulus[1]/2
    )
      if (LLik == -Inf) { LLIkk <- -1e+10 }
      return(-LLik)
    }


  #likelihood optimizer with trait covariation
    lik.covT<-function(sigma){
      low.chol<-build.chol(sigma)
      R<-low.chol%*%t(low.chol)
    LLik<-ifelse(is.matrix(ms.err)==TRUE,
      -t(y-D%*%t(a.obs))%*%ginv((kronecker(R,C)+
m.e))%*%(y-D%*%t(a.obs))/2-N*p*log(2*pi)/2-
        determinant((kronecker(R,C)+
m.e))$modulus[1]/2 ,
    -t(y-D%*%t(a.obs))%*%ginv(kronecker(R,C))%*%(y-
D%*%t(a.obs))/2-N*p*log(2*pi)/2-
      determinant(kronecker(R,C))$modulus[1]/2
    )
      if (LLik == -Inf) {LLIkk <- -1e+10 }
      return(-LLik)
    }
```

```
  ##Optimize for no trait covariation
    if (TraitCov==F)
      { model1<-optim(sigma.mn,fn=lik.covF,method
="L-BFGS-B",hessian=TRUE,lower=c(0.0))}
  ##Optimize with trait covariation
    R.offd<-rep(0,(p*(p-1)/2))
    if (TraitCov==T)
    {model1<-optim(par=c(sigma.mn,R.offd),fn
=lik.covT,method="L-BFGS-B")}


  #### Assemble R.constrained
    if (TraitCov==F){R.constr<-diag(model1$par,p)}
    if (TraitCov==T){
    chol.mat<-build.chol(model1$par)
    R.constr<-chol.mat%*%t(chol.mat)}


    if(model1$convergence==0)
      message<-"Optimization has converged."
    else
      message<-"Optim may not have converged.
Consider changing start value or lower/upper limits."
    LRT<- (-2*((-model1$value-LLik.obs)))
    LRT.prob<-pchisq(LRT, (p-1),lower.tail=FALSE)
#df = Nvar-1
    AIC.obs<- -2*LLik.obs+2*p+2*p #(2p twice: 1x for
rates, 1x for anc. states)
    AIC.common<- -2*(-model1$value)+2+2*p #(2*1:
for 1 rate 2p for anc. states)
    return(list(Robs=R.obs,Rconstrained=R.constr,
Lobs=LLik.obs,Lconstrained=(-
model1$value),LRTest=LRT,Prob=LRT.prob,
      AICc.obs=AIC.obs,AICc.constrained
=AIC.common,optimmessage=message))
  }
```

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