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Identical inferences about correlated evolution arise from ancestral state reconstruction and independent contrasts



Michael G. Elliot

Human Evolutionary Studies Program, Simon Fraser University, British Columbia, Canada V5A 1S6

HIGHLIGHTS

- Independent contrasts are expressible in terms of reconstructed ancestral states.
- Both approaches yield identical inferences about correlated evolution.
- Independent contrasts can be defined for any model with a local likelihood function.
- Formulae for independent contrasts with directional drift are defined.
- Directional contrasts outperform standard contrasts in simulation studies.

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ABSTRACT

Inferences about the evolution of continuous traits based on reconstruction of ancestral states have often been considered more error-prone than analysis of independent contrasts. Here we show that both methods in fact yield identical estimators for the correlation coefficient and regression gradient of correlated traits, indicating that reconstructed ancestral states are a valid source of information about correlated evolution. We show that the independent contrast associated with a pair of sibling nodes on a phylogenetic tree can be expressed in terms of the maximum likelihood ancestral state function at those nodes and their common parent. This expression gives rise to novel formulae for independent contrasts for any model of evolution admitting of a local likelihood function. We thus derive new formulae for independent contrasts applicable to traits evolving under directional drift, and use simulated data to show that these directional contrasts provide better estimates of evolutionary model parameters than standard independent contrasts, when traits in fact evolve with a directional tendency.

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1. Introduction

Statistical methods for the detection of correlated evolution have been divided into two broad classes. Directional methods involve reconstruction of ancestral states followed by statistical inference based on the deviation in trait values along each branch of a phylogenetic tree, while nondirectional or cross-sectional methods involve comparisons of trait values across taxa rather than along branches (Harvey and Pagel, 1991; Pagel, 1993). Methods arising from the Brownian motion model, in which traits evolve over time by accumulating increments drawn from a symmetrical zero-centered distribution with fixed variance, include both directional approaches such as reconstruction of ancestral states under maximum likelihood or squared-change parsimony criteria (Maddison, 1991; Pagel, 1993; McArdle and Rodrigo, 1994; Schluter et al., 1997) and nondirectional

approaches such as independent contrasts (Felsenstein, 1985; Garland et al., 1992) and phylogenetic generalized least squares (Grafen, 1989; Martins and Hansen, 1997).

It is well known that all methods based on the Brownian motion model are ultimately means of estimating the same model parameter, namely the variance of the Brownian process underlying trait evolution Pagel (1993, 1997), Rohlf (2001), Freckleton (2012). The mean squared standardized independent contrast across the internal nodes of a phylogeny is an estimator of this parameter, while the mean squared deviation of reconstructed trait value across the branches of a phylogeny is an estimator of half this parameter (Ackerly, 2009). The close association of methods based on Brownian motion is further indicated by the facts that the phylogenetic mean trait value inferred under independent contrasts is identical to the global maximum likelihood estimate of the root's trait value (Garland et al., 1999; Revell et al., 2008), that independent contrasts and phylogenetic generalized least squares models yield identical regression estimators for the slope and gradient of two correlated traits

E-mail address: micke@sfu.ca

(Blomberg et al., 2012), and that regression coefficients of bivariate data estimated under directional and nondirectional approaches are highly correlated (Pagel, 1997).

The primary reason to select one class of method over another is thus not that they measure different things but that their estimators exhibit different statistical properties that may be more or less desirable (Pagel, 1993). In this sense, independent contrasts and phylogenetic generalized least squares models are generally favored over ancestral state reconstruction. Pagel (1993) argues that independent contrasts are best suited to the problem of identifying evolutionary correlation coefficients, since directional methods based on a tree with n tips count evolutionary changes on $2(n-1)$ internal branches, meaning that “half of the variation that a directional method calculates is redundant because it overlaps with variation already calculated” yielding “results that seem more stable than they actually are”, whereas independent contrasts, based on values calculated at $n-1$ internal nodes, “make use of all the variance in the data, but in a way that does not count any of it twice”. Ackerly (2009) concurs with this view and adds that deviations in trait value occurring on internal branches of a phylogeny are not independent, since trait deviations associated with each sibling pair of branches depend on the value of the ancestral state at the pair’s common ancestor. Based on an analysis of phenotypic change in a bacteriophage colony with known evolutionary history, Oakley and Cunningham (2000) advocate “the use of independent contrasts in addition to or instead of the more error-prone ancestral estimation procedures”, error they ascribe to the existence of a directional bias in the polarity of trait change over time in their dataset. Directional tendencies in the evolutionary process have been shown to reduce the accuracy of ancestral state estimation in studies using fossil calibration to assess reconstruction quality (Finarelli and Flynn, 2006) and the quality of ancestral state reconstruction has been challenged in general (Donoghue et al., 1989; Webster and Purvis, 2002; Slater et al., 2012).

It is shown here that independent contrasts and maximum likelihood ancestral state reconstruction not only estimate the same underlying Brownian rate parameter for a univariate trait, but also – in studies of correlated evolution – yield numerically identical regression estimators for the gradient and correlation coefficient of bivariate traits. As a consequence, inferences about correlated evolution derived from maximum likelihood ancestral state estimation are as valid as, and indeed identical to, those derived from independent contrasts procedures. We show that the independent contrast associated with a pair of sibling nodes in a phylogenetic tree can be expressed in terms of the Gaussian local likelihood function of the node that is the direct common ancestor of the pair. It thus transpires that the numerical calculations carried out in generating independent contrasts are identical to those carried out in maximum likelihood ancestral state estimation in both univariate and multivariate situations. One consequence of this finding is that novel formulae for independent contrasts can be derived for any model of trait evolution for which a local likelihood function can be defined, including non-standard models that deviate from classical neutral assumptions. As a demonstration we derive new formulae for independent contrasts appropriate for a Brownian motion model of trait evolution with directional drift, which, in a bivariate context, are shown to yield more accurate estimates of correlation coefficient and slope than standard independent contrasts when the underlying evolutionary process does in fact exhibit a directional tendency. These findings are discussed in the context of claims that ancestral state estimation is in some sense more error-prone than independent contrasts.

2. Methods

Our primary results depend on the standard Brownian motion likelihood function for a trait \mathbf{X} evolving over a rooted bifurcating

phylogenetic tree such that the deviation in trait value along a branch of length t is normally distributed with variance proportional to t Felsenstein (1985). Our formulae refer to a general node n whose child nodes are denoted i and j connected by branches of length t_i and t_j respectively, and whose parent node p is connected by a branch of length t_n . Trait \mathbf{X} takes value x_n at node n . The likelihood of an ancestral state assignment is given by

$$L(\mathbf{X}; \mathbf{T}) \propto \prod_n \phi(x_n - x_p; 0, \sqrt{t_n}) \quad (1)$$

where $\phi(x; \mu, \sigma)$ is the density of the Gaussian distribution $N(\mu, \sigma^2)$ evaluated at x . Following Maddison (1991), each node n is associated with a Gaussian global maximum likelihood function which describes the maximized likelihood of the tree conditional on the value of x_n , denoted $N(\hat{\mu}_n, \hat{\sigma}_n)$, and also with a Gaussian local maximum likelihood function describing the maximized likelihood of the subtree rooted at n conditional on the value of x_n , denoted $N(\tilde{\mu}_n, \tilde{\sigma}_n)$. All of the results presented arise from algebraic manipulation of these first principles, making particular use of convenient formulae for Gaussian product and convolution (Bromiley, 2003).

To give an example of the benefits of our ancestral states-based formulation of independent contrasts (that is, the value of modeling trait values directly), we use our value-based results to easily derive new formulae for independent contrasts accommodating traits evolving under Brownian motion with directional drift. In order to assess the performance of these directional independent contrasts in comparison with standard independent contrasts in identifying the slope and correlation coefficient of a pair of continuous characters evolving with a directional tendency, simulation studies were performed on one thousand random Yule trees, each with a number of tips drawn uniformly from 40 to 400, generated using the APE package in R (Paradis et al., 2004). For each tree, evolution was simulated under a bivariate Brownian motion model with random reduced major axis regression slope (drawn uniformly from 0.2 to 2), random correlation coefficient (drawn uniformly from 0.2 to 1) and random drift parameters M_X and M_Y (drawn uniformly from $0-2\sigma_X^2$ and $0-2\sigma_Y^2$). To be specific, for each branch of a random phylogeny, the random mean vector and covariance matrix were scaled proportionately to branch length and a bivariate random variate was picked from the resultant distribution (using the GNU Scientific Library; Gough, 2009). By accumulating these increments in a preorder traversal of the phylogeny, states for the ancestors and tips of the phylogeny were generated. The realized reduced major axis RMA regression slope and correlation coefficient were recorded, and then re-estimated, on the basis of tip data only, using standard independent contrast and directional independent contrasts as defined in Eq. (6) below.

3. Results

Supplement S1 shows that the standardized independent contrast between nodes i and j , $IC_{(i,j)}$ can be expressed as the sum of the squared directional (*sensu* Pagel, 1993) standardized deviations in local maximum likelihood ancestral state as follows:

$$IC_{(i,j)}^2 = \frac{(\tilde{\mu}_i - \tilde{\mu}_n)^2}{t_i + \tilde{\sigma}_i^2} + \frac{(\tilde{\mu}_j - \tilde{\mu}_n)^2}{t_j + \tilde{\sigma}_j^2} \quad (2)$$

An estimator for the variance of an evolving trait \mathbf{X} based on global maximum likelihood ancestral state reconstruction is given by

$$\text{var}[\mathbf{X}] = 2E \left[\frac{(\hat{\mu}_n - \hat{\mu}_p)^2}{t_n} \right] \quad (3)$$

while the covariance of traits **X** and **Y** is given by

$$\text{cov}[\mathbf{X}, \mathbf{Y}] = 2E \left[\frac{(\hat{\mu}_{\mathbf{X}_n} - \hat{\mu}_{\mathbf{X}_p})(\hat{\mu}_{\mathbf{Y}_n} - \hat{\mu}_{\mathbf{Y}_p})}{t_n} \right] \quad (4)$$

Supplement S2 demonstrates that these variance and covariance estimators are numerically identical to the variance and covariance of the set of independent contrasts generated from the same phylogeny and data.

An estimator for the RMA regression slope between **X** and **Y** based on maximum likelihood ancestral state reconstruction is given by

$$|\beta| = \sqrt{\frac{E[(\hat{\mu}_{\mathbf{Y}_n} - \hat{\mu}_{\mathbf{Y}_p})^2 / t_n]}{E[(\hat{\mu}_{\mathbf{X}_n} - \hat{\mu}_{\mathbf{X}_p})^2 / t_n]}} \quad (5)$$

Supplement S3 demonstrates that this regression gradient estimator is numerically identical to the RMA regression estimator based on independent contrasts, and that this identity also holds for ordinary least squares regression.

Eq. (2) can be used to generate formulae for independent contrasts appropriate for any model of trait evolution for which a local likelihood function can be defined. Supplement S4 derives formulae for a model of trait evolution with a directional tendency:

$$\text{directional IC}_{(ij)} = \frac{(\tilde{\mu}_i - \tilde{\mu}_j) - M(t_i - t_j)}{\sqrt{t_i + \tilde{\sigma}_i^2 + t_j + \tilde{\sigma}_j^2}} \quad (6)$$

where M is the mean directional drift per unit time (with $M=0$ under standard independent contrasts) and where $\tilde{\mu}_i$ and $\tilde{\sigma}_i^2$ are estimated recursively from the tips to the root of the phylogeny

according to

$$\tilde{\mu}_n = \frac{(\tilde{\mu}_i - t_i M)(\tilde{\sigma}_j^2 + t_j) + (\tilde{\mu}_j - t_j M)(\tilde{\sigma}_i^2 + t_i)}{(\tilde{\sigma}_i^2 + t_i) + (\tilde{\sigma}_j^2 + t_j)} \quad (7)$$

$$\tilde{\sigma}_n = \sqrt{\frac{(\tilde{\sigma}_i^2 + t_i)(\tilde{\sigma}_j^2 + t_j)}{(\tilde{\sigma}_i^2 + t_i) + (\tilde{\sigma}_j^2 + t_j)}} \quad (8)$$

Supplement S4 also includes formulae for calculating the maximum likelihood ancestral state reconstruction under this model. The parameter M is typically not known *a priori* but is easily estimated by a linear search maximizing the likelihood of an ancestral state assignment (or, identically, minimizing the sum of squared contrasts). Results of simulation studies comparing performance of directional independent contrasts to standard independent contrasts in estimating correlation coefficient, RMA gradient and M are illustrated in [Figs. 1 and 2](#). While the directional model has generally been regarded

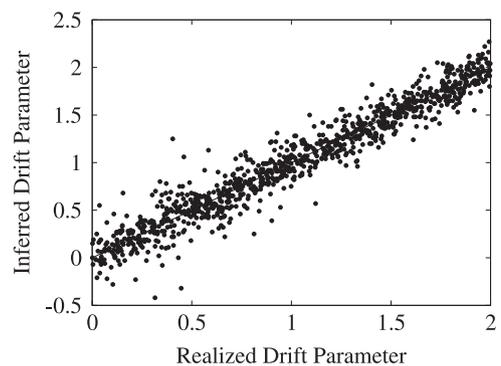


Fig. 2. Accuracy in estimation of the directional drift parameter under directional independent contrasts.

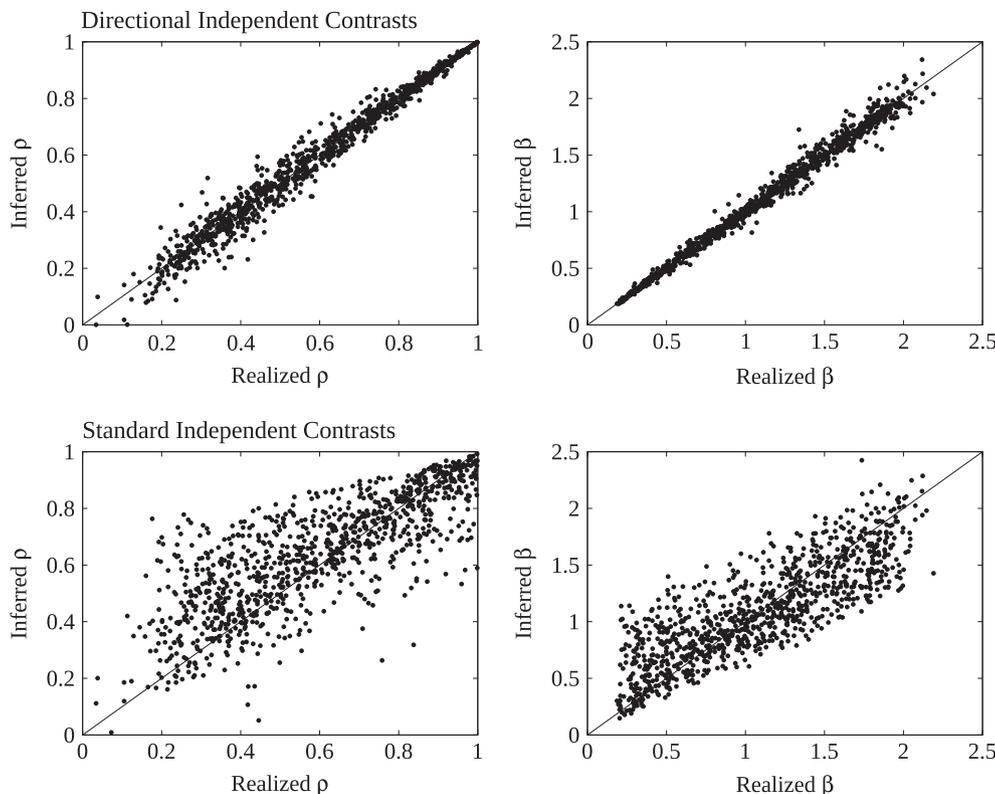


Fig. 1. Accuracy in estimation of correlation coefficient (ρ , left column) and regression slope (β , right column) of two traits evolving under bivariate Brownian motion with directional drift, using directional independent contrasts (top row) and standard independent contrasts (bottom row). The reference line for perfect estimates is included on each panel.

as underidentified (Grafen, 1989) we find maximum likelihood estimates of M to be identifiable for phylogenies that are not ultrametric; for ultrametric trees, a highly similar model with directional tendency on selected branches is feasible. Software for maximum likelihood estimation of M along with standardized directional independent contrasts has been made available at (<http://www.sfu.ca/~micke/dirpic.html>).

4. Discussion

Maximum likelihood ancestral state reconstruction has often been regarded as a poor second cousin to nondirectional analysis of correlated evolution using independent contrasts of phylogenetic generalized least squares (Pagel, 1997; Oakley and Cunningham, 2000). Ancestral reconstruction has been regarded as more error-prone (Oakley and Cunningham, 2000), requiring fossil calibration to improve accuracy, especially in the reconstruction of directional deviations in trait value (Donoghue et al., 1989; Webster and Purvis, 2002; Slater et al., 2012). Independent contrasts, being nondirectional, have been considered more robust with respect to such sources of bias and error. In this paper we have shown that, to the contrary, regression estimators based on maximum likelihood ancestral state reconstruction are numerically identical to estimators based on independent contrasts. Previous authors have considered the calculation of ancestral states using independent contrasts to yield identical results as direct methods such as those of Schluter et al. (1997) but “without the use of maximum likelihood”. We have shown that the numerical calculations involved in calculating independent contrasts are in fact identical to those involved in fitting the maximum likelihood model and that the standardized independent contrast associated with a pair of nodes of a phylogeny can be expressed directly in terms of the Gaussian likelihood function at those nodes and their common parent. It has previously been proposed that maximum likelihood estimates yield too narrow confidence intervals, since there are twice as many branches in a phylogeny than there are internal nodes (Pagel, 1993, 1997). We agree with (Ackerly, 2009) that this apparent overconfidence is wholly remedied by reducing the number of degrees of freedom in the calculation of confidence bounds by a factor of two when using ancestral state reconstruction, though this manipulation is not necessary to guarantee the identity of point estimates made by regression estimators under ancestral state reconstruction and independent contrasts. In the light of findings that regression estimators based on independent contrasts are also identical to those based on phylogenetic generalized least squares (Blomberg et al., 2012), we conclude that all comparative methods based on the Brownian motion model of evolution yield identical inferences about the parameters of correlated evolution and are conceptually indistinguishable. Our response to claims that ancestral state reconstruction is error-prone (Donoghue et al., 1989; Webster and Purvis, 2002; Slater et al., 2012) is to point out that the ancestral states themselves are merely nuisance parameters of the model formulation. Cross-sectional methods simply embed this error into the values of independent contrasts themselves. In estimating summary statistics of these nuisance parameters, such as evolutionary rate or correlation coefficient, independent contrasts offers no advantage over ancestral state-based methods.

One useful implication is that ancestral states under non-standard models of trait evolution contain useful information about correlation structure. Given the evidence that directional tendencies may give rise to biased estimates of evolutionary model parameters (Finarelli and Flynn, 2006), it may be useful to incorporate such tendencies directly into the model likelihood function. For those wedded to the idiom of independent contrasts, we have shown that

Eq. (2) can be used to generate novel formulae for independent contrasts when an appropriate likelihood function can be formulated. Specifically, we here present formulae for independent contrasts under directional drift (Eqs. (6)–(8)) and show that these “directional independent contrasts” markedly improve estimation of correlation coefficient and slope (Figs. 1 and 2). The fact that independent contrasts and ancestral state reconstruction are interchangeable means that the likelihood scores of models based on nested forms of Brownian motion (including directional models, in which the standard model has directional component $M=0$) can be compared using likelihood ratio tests, in order to assess the improvement in model fit arising from incorporation of additional parameters that are easily implemented in the ancestral states framework. For this reason, the software accompanying this paper generates likelihoods for directional and standard independent contrasts. More generally, we note that the Brownian assumption is that cumulative increments to an evolving trait are normally distributed, rather than the value of the trait value itself. Indeed, many traits of biological interest are constrained to be positive and are log-transformed prior to analysis. By this means, normally distributed evolutionary increments are made consistent with the constraint of positivity by imposing a logarithmic scale on the evolutionary process, with lower trait values exhibiting smaller evolutionary increments that can never result in the trait value becoming negative. While mathematical treatments of value-constrained Brownian motion exist (reviewed in Morse, 2004), classic comparative methods have rarely incorporated these stochastic models other than through implementation of Ornstein-Uhlenbeck processes (Beaulieu et al., 2012), which we note also admits of an ancestral state interpretation (Harmon et al., 2008). Dealing with independent contrasts does not promote thinking in terms of absolute constraints of trait values, but working directly in terms of these values under ancestral state reconstruction is expected to greatly facilitate the implementation of more complex constrained and autocorrelated models of continuous character evolution.

Significant progress has been made in phylogenetic comparative methods through the modification of cross-sectional independent contrasts, or direct analysis of covariance structured by the phylogeny, to calculating the likelihood of relatively complex evolutionary models, including REML estimates of evolutionary rate and heterogeneous models in which evolutionary rate varies across the phylogeny (O'Meara et al., 2006; Eastman et al., 2011; Revell, 2012). When Brownian evolutionary models can be expressed in terms of directional formulae – for example Brownian motion with drift – our results suggest that it should in general be possible to derive cross-sectional measures similar to independent contrasts that express those models successfully. However, translation of a directional model to its cross-sectional counterpart is not strictly necessary: our findings show that statistical methods operating directly in trait-space, that is, directly in terms of ancestral states, appear to be perfectly valid means of fitting evolutionary models to comparative data, yielding identical results. This is an important finding given increasing interest in the development of wholly non-Brownian stochastic processes such as Lévy jumps (Landis et al., 2013), which have undefined variance and are thus unsuited to fitting through minimizing squared contrasts, and invalidate the assumptions of both independent contrasts and phylogenetic generalized least squares as currently formulated. For example, Elliot and Mooers (2013) recently proposed a method for fitting an infinite-variance model of continuous character evolution, intractable under any approach based on manipulation of covariance matrices under Brownian assumptions, based solely on a Markov chain Monte Carlo estimation of ancestral states, from which model parameters were recoverable. Working directly in terms of ancestral states rather than in terms of the covariance matrix of a phylogeny under Brownian assumptions may offer significant computational benefits in such situations, and in other cases where real trait values are constrained or autocorrelated in complex ways at internal nodes of a phylogeny.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version at <http://dx.doi.org/10.1016/j.jtbi.2014.09.033>.

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