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Running head: INFERRING PHYLOGENIES FROM CONTINUOUS CHARACTERS

Title: Continuous Characters Outperform Binary Discrete Characters in Phylogenetic Inference

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ABSTRACT

The recent surge in enthusiasm for simultaneously inferring relationships from extinct and extant species has reinvigorated interest in statistical approaches for modelling morphological evolution. Current statistical methods use the Mk model to describe substitutions between discrete character states. Although representing a significant step forward, the Mk model presents challenges in biological interpretation, and its adequacy in modelling character evolution has not been well explored. Another major hurdle toward increasing objectivity and reproducibility in morphological phylogenetics is the often subjective process of character coding of discrete characters. Assignment of discrete characters by different researchers can often yield discordant phylogenetic hypotheses. One potential solution to issues may be the employment of continuous measurements to infer phylogenies. Although not widely used in the inference of topology, models describing the evolution of continuous characters have been well examined, and their statistical behaviour is well understood. Also, continuous measurements avoid the substantial ambiguity often associated with the assignment of discrete characters to states. I present a set of simulations to determine whether use of continuous characters is a feasible alternative to discrete for inferring phylogeny. I compare relative reconstruction accuracy by inferring phylogenies from continuous characters simulated under unbounded Brownian motion and discrete characters simulated under the Mk model of morphological evolution. These tests demonstrate significant promise for continuous traits by demonstrating their higher overall accuracy as compared to reconstruction from discrete characters under Mk. Continuous characters also perform reasonably well in the presence of covariance between sites. This study provides the first step toward recognition of the potential utility of continuous characters in phylogenetic inference through use of Brownian motion and related Gaussian models.

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The widespread development and adoption of statistical phylogenetic methods has revolutionized disparate disciplines in evolutionary biology, epidemiology, and systematics. Studies utilizing maximum-likelihood (ML) and Bayesian approaches have become the preferred means to analyse molecular data, largely eclipsing parsimony and distance methods. Despite this, approaches which draw inference from morphological data have remained comparatively underdeveloped. As a result, non-statistical tree inference methods have continued to be employed for the phylogenetic analysis of morphological characters. Nonetheless, several landmark advances in the development of statistical morphological phylogenetic methods have demonstrated the benefits of further developing this framework. This will be particularly important in the near future as burgeoning approaches enabling the rapid collection of morphological data, such as that of Chang and Alfaro (2015b,a), may begin to outstrip methods through which to analyse them. This may significantly alter and enhance our view of the tree of life, especially considering that the majority of macro-organisms, represented by fossil taxa, can only be analysed from their morphology.

A critical contribution to statistical phylogenetic methods has been the Mk model to describe discrete trait evolution (Lewis 2001). This is a version of the Jukes-Cantor model of nucleotide substitution generalised to accommodate varying numbers of character states (Jukes and Cantor 1969). Extensions to this model accommodate for biased sampling of parsimony informative characters (Lewis 2001), rate heterogeneity between sites (Wagner 2012), and asymmetric transition rates (Wright *et al.* 2015). The deployment of this model has demonstrated the utility of statistical approaches to morphological phylogenetics, both through their ability to better describe uncertainty over non-statistical approaches, and to estimate branch lengths. This has enabled a better understanding of much of the fossil tree of life (Dávalos *et al.* 2014; Pattinson *et al.* 2014;

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Dembo *et al.* 2015). The unique ability of Mk to estimate morphological branch lengths has also enabled the development of tip dating, methods, which combine morphological and molecular data to co-estimate phylogeny between living and fossil data (Nylander *et al.* 2004; Ronquist *et al.* 2012). Tip dating methods have been widely used since their introduction, and are implemented in the BEAST (Bouckaert *et al.* 2014) and MrBayes (Ronquist and Huelsenbeck 2003) packages. These have shown the potential to more resolutely understand the timing of species divergences and relationships between fossil and living taxa (Wiens *et al.* 2010; Wood *et al.* 2012; Lee *et al.* 2013, 2014; Arcila *et al.* 2015). Overall, statistical approaches to morphological phylogenetics appear to represent an improvement in accuracy compared to cladistic methods, and are indispensable in their distinct ability to allow the estimation of branch lengths and evolutionary rate. The benefits of a statistical total-evidence framework will only become clearer as more data become available and improved methods are developed (Pennell and Harmon 2013; Lee and Palci 2015).

Despite the great strides made through their development, discrete character models represent an imperfect solution. Although Bayesian inference under Mk appears to outperform parsimony, error increases at high evolutionary rates (Wright and Hillis 2014). Also, under many circumstances, phylogenetic inference under the Mk model includes imprecision and uncertainty, both in simulations (O'Reilly *et al.* 2016; Puttick *et al.* 2017) and empirical studies (Lee and Worthy 2012; Dembo *et al.* 2015). Previous researchers have also expressed concerns over the efficacy of model-based approaches in the presence of missing data (Livezey and Zusi 2007; O'leary *et al.* 2013), although these have been largely assuaged (Wright and Hillis 2014). Another potential issue is the lack of clarity in interpreting the Mk model biologically. Although transition rates have a strong theoretical and empirical basis in population genetics, their

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significance beyond serving as nuisance parameters is less straightforward when applied to morphological data. Discrete morphological characters may not undergo change in a manner analogous to nucleotides, whose change is better understood as a relatively consistent, population-based process. This leaves ambiguity in the interpretation of Mk substitution rate, and weakens biologists' ability to examine biological process from Mk rate estimation. Therefore, it is unclear whether the slightly nebulous interpretations of evolutionary rate gleaned through discrete morphological substitution models can be employed for major biological discoveries in the same way as has been done with molecular data (e.g. Smith and Donoghue 2008; Bromham 2011). This is particularly important when considering the importance of branch lengths in the total-evidence methods discussed above.

Aside from the model concerns discussed above, discrete characters themselves present a non-trivial set of challenges to phylogenetics that are distinct from those possessed by molecular data. Perhaps, foremost among these is disagreement between researchers in the categorisation, ordering, and weighing of discrete character states (Farris 1990; Hauser and Presch 1991; Pleijel 1995; Wilkinson 1995). Despite extensive discussion among comparative biologists, the interpretive nature of the process of character coding has continued to leave major palenotological questions unresolved (Upchurch 1995; Wilson and Sereno 1998; Bloch and Boyer 2002; Kirk *et al.* 2003). Character coding methods that increase the objectivity of discrete character collection may represent an improvement to these issues (Thiele 1993; Wiens 2001), but it may also be worthwhile to explore alternate sources of data for phylogenetic reconstruction.

Use of continuous characters may help to address some of the concerns with discrete traits discussed above. They can be collected more objectively than qualitative observations and do not require ordering of states. Their use in phylogenetic inference has been discussed among the

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earliest advancements in statistical phylogenetics (Cavalli-Sforza and Edwards 1967; Felsenstein 1973), and their phylogenetic informativeness has been demonstrated empirically (Goloboff *et al.* 2006; Smith and Hendricks 2013). Still, the use of continuous characters for the inference of phylogenetic topology has remained uncommon, with statistical methods for their use in phylogenetics remaining relatively poorly examined.

Another potential benefit to inferring phylogeny from continuous characters is the wealth of models developed in phylogenetic comparative methods to describe their evolution. Most comparative models of continuous trait evolution belong to the Gaussian class, which are also well utilized in disparate fields such as physics, economics, and engineering. In comparative biology, they are used to describe stochastic Markovian movement through trait space along continuous time. This class of models includes Brownian motion (BM) (Felsenstein 1973, 1985; Gingerich 1993), Ornstein-Uhlenbeck (OU) (Hansen 1997; Butler and King 2004; Beaulieu *et al.* 2012), and Lèvy processes (Landis *et al.* 2013). Under BM, evolution is described as a random walk, with phenotypic change being normally distributed with a mean displacement of zero, and variance σ^2. OU models expand upon this by introducing terms producing a stabilizing force which stabilizes movement around an optimal trait value, while Lèvy processes contain terms producing saltational jumps in character space, interspersed either by BM diffusion or stasis. Two major benefits to Gaussian models in phylogenetics are their relatively straightforward interpretability and the relative ease of deriving mathematical extensions to describe a range of biological processes.

Given the existence of well understood and clearly interpretable models describing their evolution, the use of continuous traits may offer several advantages over discrete characters in phylogenetic inference. However, their behaviour is not well understood when applied to the

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inference of phylogenetic topology, and so further investigation is needed. In addition, there are potential hurdles to their efficacy. Possibly foremost among these is the widespread covariance between continuous measurements that is expected through both genetic and morphometric perspectives (Lynch *et al.* 1998; Uyeda *et al.* 2015; Adams and Felice 2014). Nevertheless, the expected magnitude in covariance among continuous morphological measurements and the robusticity of phylogenetic methods to this violation is not known. Furthermore, it is also generally reasonable to expect evolutionary covariance between nucleotide sites, and phylogenetic methods that do not accommodate for this are routinely applied to molecular data.

In this study, I carry out simulations to compare the relative performance of binary discrete and continuous characters at reconstructing phylogenetic relationships. Simulations of continuous characters were designed to reflect a range of scenarios that may influence accuracy including overall evolutionary rate and matrix sizes. I also conduct inference on continuous traits that have undergone correlated evolution, an important violation to single-rate BM thought to be widespread in continuous character evolution.

METHODS

Simulations

I generated a set of 100 pure birth trees using the Phytools package (Revell 2012), each containing ten taxa. All trees were ultrametric and generated with a total length of 1.0 for consistency in parameter scaling for trait simulations (Fig. 1). These trees were used to simulate continuous characters evolving along an unbounded BM process in the OUwie package (Beaulieu and O'Meara 2012). This is a Markovian process in continuous time where the variance of the process can increase infinitely through time. This differs from the BM σ^2 parameter, which gives the variance in the amount of character displacement at each draw, effectively describing the

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magnitude of the random BM walk or a rate of character displacement. To assess performance across several biological scenarios, traits were simulated at σ^2 parameterizations of 0.05, 0.5, 1.0, 1.5, and 3. Since the process under which traits were simulated is unbounded, phylogenetic signal is expected to remain consistent across rates (Revell *et al.* 2008), but different rates were chosen to illustrate this consistency and to provide even comparison to discrete trait simulations. Discrete characters were simulated in the Phytools package (Revell 2012) under an Mk model with homogeneous transition probabilities. Traits were generated at transition rates 0.05, 0.5, 1.0, 1.5, and 3. All character matrices were generated without rate heterogeneity, and with all invariable sites (ie. no acquisition bias).

Matrices were generated at a length of 500 traits and subsampled to create smaller sets of 20 and 100 characters to reflect a range of sampling depths. These were chosen because many published morphological matrices fall within this range. The subsampled matrix sizes were chosen to represent reasonably sized paleontological datasets, while the 500 trait matrices were tested to assess performance in complete abundance of data. While such large datasets are uncommon in morphology, several studies have produced character matrices of this size, and for continuous characters, it may be feasible to generate such large datasets from morphometric data.

Data were also generated under a correlated BM process to mimic inference in the presence of multidimensionality. These datasets were constructed at covariance strengths of 0.1, 0.5, and 0.9 and covarying dimensions of 5 and 25 traits. These were chosen to represent situations where traits range from being loosely to tightly correlated to each another, and where the number of correlated dimensions is large to small. Although differing, these values were chosen to loosely follow the scheme of Adams and Felice (2014).

Estimation of Phylogenies and Reconstruction Accuracy

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I estimated Bayesian phylogenetic trees from continuous data under single rate BM in RevBayes (Höhna *et al.* 2016). Tree likelihoods were computed from the phylogenetic independent contrasts (Felsenstein 1985) using reduced maximum likelihood (REML) as implemented in RevBayes. MCMC simulations were run for 150,000 generations. Trees were inferred from discrete data in MrBayes version 3.2.6 (Ronquist and Huelsenbeck 2003), simulating for 1,000,000 generations. Example configuration files for RevBayes and MrBayes analyses are provided as supplementary data. Trees were summarized using TreeAnnotator version 2.4.2 (Rambaut and Drummond 2013) to yield maximum clade credibility (MCC) topologies. MCC trees maximize the posterior probability of each individual clade, summarizing across all trees sampled during MCMC simulation. Once summarised, all trees were rescaled to match inferred tree lengths to the true trees using Phyx (https://github.com/FePhyFoFum/phyx).

I assessed topological accuracy from simulated trait data using the symmetric (Robinson-Foulds) distance measure (Robinson and Foulds 1981), giving the topological distance between true trees and inferred trees. Symmetric distance is calculated as a count of the number of shared and unshared partitions between compared trees. As such, the maximum symmetric distance between two unrooted trees can be calculated as $2(N-3)$. These values were then scaled to the total possible symmetric distance for interpretability. Additionally, I measured error in branch length reconstruction using the branch length distance (BLD) measure of Kuhner and Felsenstein, also referred to as Euclidean distance (Kuhner and Felsenstein 1994). This is calculated as the sum of the vector representing the individual differences between the branch lengths of all shared bipartitions. The scale of this value depends on the lengths of the trees under comparison. If trees of different lengths are compared, BLD can be very high. However, in this study, all trees are scaled to a root height of 1 to allow comparison of topological and internal

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branch length reconstruction error. All distances were calculated using the DendroPy Python package (Sukumaran and Holder 2010).

RESULTS

Reconstruction from Independently Evolving Traits

Reconstruction error is lower overall for trees estimated from continuous characters than from binary discrete (Fig. 2a, Supp. Fig, 1a). For discrete characters, symmetric distance increases significantly at high evolutionary rates, likely due to saturation and loss of phylogenetic signal. Distance also increases in discrete characters when rate is very slow, due to lack of time for phylogenetic signal to develop. This pattern is similar to that recovered by (Wright and Hillis 2014) in their test of Bayesian inference of Mk, which revealed highest topological error at very low and high rates. As expected, continuous characters perform consistently across rates because saturation cannot occur, even at very fast rates. Because of the differing sensitivities of each data type to evolutionary rate, topological error should also be compared using the most favourable rate class for discrete characters, 0.5 substitutions per million years (Fig. 2b, Supp. Fig. 1b). Even at this rate, continuous reconstruction performs more consistently than discrete, with error more tightly distributed around a slightly lower mean. It is possible that this occurs because of the relative lack of informativeness of binary characters compared to continuous. The small state space of the binary character model likely causes phylogenetic signal to become saturated more quickly at fast rates, and develop too slowly at slow rates than multi-state characters. BM and Mk appear to perform fairly similarly in reconstructing branch lengths (Fig. 2; Supp. Fig. 1). The pattern across rates and matrix sizes are very similar between BLD and symmetric distances, with the fastest rates producing the most error. This likely results from increased saturation at fast rates, causing underestimation of hidden character changes.

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Matrix size has a major impact on tree reconstruction accuracy. Estimations from both discrete and continuous traits improve substantially at each increasing matrix size (Fig. 2). Estimates from 20-character matrices possess fairly high error in both data types, with approximately 1 in 5 bipartitions being incorrectly estimated from continuous characters, and 2 in 5 incorrectly being incorrectly estimated from discrete data. Increasing matrix size to 100 traits improves accuracy significantly, with both data types estimating approximately 1 in 10 bipartitions incorrectly. Although at several rates, mean symmetric distance compared between data types is close, continuous characters tend to be less widely distributed, and thus appear to reconstruct trees with more consistent accuracy. When matrix size is increased to 500 characters, both continuous and discrete characters are able to recover phylogeny with very high accuracy, except for at very fast rates, where discrete characters estimate approximately half of all bipartitions incorrectly on average.

Simulation of Covarying Continuous Characters

Tree inference under BM appears relatively robust to the violation of co-evolving continuous characters. Although error is recognisably greater with strong covariance and many trait dimensions, symmetric distance is remains close to values from uncorrelated traits at lower covariance strengths and/or fewer trait dimensions (Fig. 3). When correlated traits are of low dimensionality and covariance strength, reconstruction appears to be nearly as accurate as uncorrelated traits, with all bipartions estimated correctly on average. As covariance strength and dimensionality are increased to intermediate values, topological error increases such that between 0 and 17% of bipartitions are estimated incorrectly, with a wider distribution than is present at the lowest values. Accuracy is most diminished when covariance and dimensionality are strongest, with most reconstructions estimating between 17-29% of bipartitions incorrectly. Although

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statistical significance cannot be estimated for BLD and symmetric distance, estimation under low to intermediate trait covariance appears at least qualitatively similar, albeit slightly worse, to uncorrelated continuous and binary discrete characters. The decreases in accuracy observed can likely be attributed to the decrease in total information content caused by covariance. This reduces the effective amount of data from which to draw inference. This is reflected in the results, with higher covariances and dimensionalities reconstructing trees with a similar magnitude of error as is shown for the 100 character datasets.

DISCUSSION

These results suggest that, although imperfect, phylogenetic reconstruction from continuous trait data may provide a reasonable supplement or alternative to inference from discrete characters. Continuous characters appear to perform better in phylogenetic inference than binary discrete overall. Their resilience to high evolutionary rate is expected, because continuous characters evolving under an unbounded BM process will continue to increase in variance through time. Therefore, such characters are able to retain phylogenetic information at high evolutionary rates that may cause rampant saturation in discrete characters (Fig. 4). Although bounded evolutionary models should experience diminished phylogenetic signal at high evolutionary rates and/or long timescales in comparison, the larger amount of information contained in continuous datasets may allow longer retention of signal. Further adding to this, temporal variation in evolutionary regimes and model parameters can interact in complex ways, sometimes extending the maintenance of phylogenetic signal through time (Revell *et al.* 2008). Therefore, although real continuous characters are undoubtedly bounded in their evolution, the added information contained in continuous character datasets may lessen the extent of saturation relative to discrete. More empirical and simulation work is needed to better understand realistic

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conditions for loss of signal in continuous traits.

The susceptibility of discrete characters to the loss of phylogenetic signal at high evolutionary rates and deep timescales has long been recognised (Hillis and Huelsenbeck 1992; Yang 1998). Although this effect is understood to affect molecular data, discrete morphological datasets may possess increased susceptibility to this effect because of the frequent use of binary character coding schemes. Discrete characters constrained to fewer states increases signal loss at high evolutionary rates due to increased levels of homoplasy, saturation, and lower information content overall (Donoghue and Ree 2000). This is ultimately a result of the fewer number of possible evolutionary transitions in binary characters than those with larger state spaces. Although continuous characters are expected to exhibit more severe loss of phylogenetic signal through time in empirical datasets than the simulated examples generated in this study, the greater information contained in continuous characters suggests the possibility that they are more resilient to saturation than discrete characters.

Error in branch length estimation was fairly high with the 20-trait matrices, but decreased substantially when matrix size was increased to 100 traits. Although BM and Mk achieve similar accuracy in estimating branch lengths in this study, careful thought should continue to be applied when relying upon Mk branch length estimates in the future. Branch length error may be higher when inferring under Mk from empirical datasets, since many discrete morphological matrices are constructed to include only parsimony informative characters. In these cases, characters are expected to have undergone only single synapomorphic changes. Although this issue is addressed through the ascertainment bias correction developed by (Lewis 2001), it is unclear how meaningfully single character changes can inform evolutionary rates. This mode of change, which may characterise much of discrete character evolution, differs from the population

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dynamics of nucleotide substitution. This raises questions surrounding the interpretability of rates estimated from discrete morphology. Molecular data matrices (ie. nucleotides and amino acids) share ontologies between sites. And so, substitution rates estimated across sites are easily interpretable. By contrast, discrete morphological character matrices differ in ontology between sites, rendering substitution rates estimated across sites non-interpretable. One solution would be to calculate per-site rates in morphology. However, the problem related to single state changes, described above, makes this problematic. Continuous characters may be expected in many cases to evolve at fairly consistent rates through time (Simpson 1944), lending an intuitive biological interpretation to branch lengths estimated from continuous characters as the amount of character displacement over time. This interpretation remains consistent even in cases where traits may not evolve gradually or are bounded by physical limitations and/or attraction to selective optima, with the only difference being in the parameters describing the process of character displacement. Of course, this is stated cautiously, as further studies addressing the interpretability and adequacy of both discrete and continuous trait models are needed.

Although continuous measurements may often follow covarying evolutionary trajectories in nature, this appears to have a relatively minor impact on reconstruction. Accuracy was only greatly lowered in the simultaneous presence of very high dimensionality and covariance strength. Offering further support to the ability of continuous characters to reconstruct phylogeny despite evolutionary covariance, Adams and Felice (2014) also report the presence of phylogenetic information in multidimensional characters, even when the number of dimensions is greater than the number of taxa. Despite these generally positive findings, it should be noted that inference may be mislead if sampling is significantly biased to include relatively small numbers of strongly correlated measurements. In these cases, it would be beneficial to examine the

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correlation structure and information content of the dataset to assess the amount of biased redundancy in signal.

Are Continuous Characters a Feasible Data Source for Phylogenetics?

Use of continuous traits has the benefit of reducing subjectivity in the construction of data matrices. While categorizing qualitative characters often requires subjective interpretation, quantitative measurements can be taken without this source of human error. This would allow biologists to assess uncertainty statistically instead of attributing discordance in tree estimates to differences in opinion. Translation of morphological observations into data that can be analysed can present serious complications in discrete characters. Steps such as the determination of whether or not to order states, the total number of states chosen to describe characters, and the assignment of character states states can vary greatly and often yield widely different results (Hauser and Presch 1991; Pleijel 1995; Wilkinson 1995; Hawkins *et al.* 1997; Scotland and Pennington 2000; Scotland *et al.* 2003). Continuous measurements avoid many of these issues because they can be measured, by definition, objectively and quantitatively. In addition, they may better describe variation than discrete characters. Several workers have suggested that the majority of biological variation is fundamentally continuous (Thiele 1993; Rae 1998; Wiens 2001). Although continuous characters have long been employed in phylogenetic analysis, they are generally artificially discretised, either using quantitative approaches, or through gross categorisations such as 'large' and 'small. The major disadvantage to this approach is the loss of valuable biological information. Several researchers have condemned with the use of continuous characters in phylogenetics, arguing that intraspecies variation may be too great for clear phylogenetic signal to exist (Pimentcl and Riggins 1987; Chappill 1989). However, these arguments have been largely undermined by studies demonstrating the phylogenetic

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informativeness of continuous measurements (Goloboff *et al.* 2006; Smith and Hendricks 2013).

The expectation of correlated evolution between continuous characters has been a major argument against their use in phylogenetic reconstruction in the past (Felsenstein 1985). However, evolutionary covariance between sites is not a phenomenon that is restricted to continuous morphological characters. Population genetic theory predicts tight covariance between nucleotide sites under many conditions (e.g. Hill and Robertson 1968; Reich *et al.* 2001; Palaisa *et al.* 2004; Schlenke and Begun 2004; McVean 2007). Such covariance also may be expected to occur in discrete morphological characters in cases where traits are genetically linked. While it is difficult to assess the relative magnitude of sitewise covariance between continuous, discrete, and molecular data, examination of the correlation structure of traits may be more straightforward in continuous characters using standard regressional techniques. This would ease the identification of biased and positively misleading signal among continuous characters, enabling correction through common transformation approaches such as principal components analyses or by weighting likelihood calculations by the amount of overall variance contributed by covarying sets of characters.

The fundamentally continuous nature of many biological traits is supported by differential gene expression and quantitative trait loci mapping studies, which demonstrate their quantitative genetic basis (Andersson *et al.* 1994; Hunt *et al.* 1998; Frary *et al.* 2000; Valdar *et al.* 2006). Nevertheless, there remain well known instances where traits are truly discrete. Studies in evolutionary developmental biology have shown that many traits can be switched on or off in response to single genes controlling genetic cascades (e.g. Wilkinson *et al.* 1989; Burke *et al.* 1995; Cohn and Tickle 1999). Such traits may be incorporated as separate partitions into integrated analyses along with continuous measurements, using Mk or parsimony approaches.

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Characters under the control of developmental expression pathways may also exhibit very deep phylogenetic signal (De Rosa *et al.* 1999; Cook *et al.* 2001). Thus, such integrated analyses may enable the construction of large phylogenies from morphology by use of datasets containing phylogenetic signal at multiple taxonomic levels.

Accuracy in both character types increases with dataset size, but continuous characters outperform discrete at each matrix length. Practically, the performance disparity between the two may be most important when analysing small datasets, when error is highest for both character types. Use of continuous characters may help in in paleontological studies. Since paleontologists are often restricted to analysing small datasets, use of continuous characters may improve confidence and resolution of phylogeny by increasing the information content of character matrices. Although many paleontological datasets many never achieve sufficient size to resolve relationships with high confidence, use of continuous characters may increase the efficiency of data use. Also, the measurement of continuous characters may be easier to automate than recognition of discrete characters. This would enable the collection of large datasets that are impractical to assemble manually. Improved ability to infer phylogeny among fossil taxa would also benefit molecular phylogenetics because the incorporation of fossils into total evidence matrices can improve both inference of molecular dates and alleviate long branch attraction (Huelsenbeck 1991; Wiens 2005; Ronquist *et al.* 2012). These findings should urge paleontologists to consider data collection schemes that give greater consideration to continuous measurements. Overall, this may improve efficiency in the use of hard-won paleontological data by maximizing the amount of information gleaned from specimens.

Moving forward, several extensions to Gaussian models should be explored for application to phylogenetic inference. For example, further work is needed to determine the extent and

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distribution of rate heterogeneity between sites in continuous alignments. Since its presence has been well documented in molecular and discrete morphological data, it is likely that such rate heterogeneity is present in continuous measurements, and should be accommodated in empirical studies. Since traits can evolve under a broad range of processes, the fit of alternative models of continuous character evolution to empirical data and their adequacy in describing variation among them should also be examined.

Is Mk a reasonable model for discrete character evolution?

Although Mk and its extensions have been increasingly adopted in morphological phylogenetics, it is unclear whether it provides a reasonable approximation of the evolutionary process. Although there are explicit theoretical links between Markovian substitution models and population genetic processes (Jukes and Cantor 1969), such theory does not exist in morphology. In addition, morphological evolution might be intuitively expected to evolve in a fashion more idiosyncratic to individual taxa and traits. Parsimony accounts for this idiosyncrasy, being mathematically equivalent to the no common mechanisms (NCM) model which treats each character individually (Tuffley and Steel 1997), while Mk approaches do not. There have been extensions to Mk that address several of the most egregious model violations, but fundamental issues remain concerning the applicability of substitution processes to morphological character change. In particular, the difficulty of interpreting rates of character change in empirical datasets, discussed above, begs deeper discussion of the application of branch lengths estimated under Mk to larger biological questions. This is especially important in total-evidence tip dating methods employing Mk, as poor branch length estimates may weaken the ability to infer branching times. Although presenting a unique set of challenges, the use of continuous characters may mitigate some of these issues through the more straightforward interpretability of models describing their

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change. Nonetheless, further work is needed to address the relative adequacy of discrete and continuous trait models in describing the evolution of phenotypic data. These questions will be of critical importance moving forward as advances in morphological data and new fossil discoveries usher in an age of unprecedented discovery in morphological phylogenetics.

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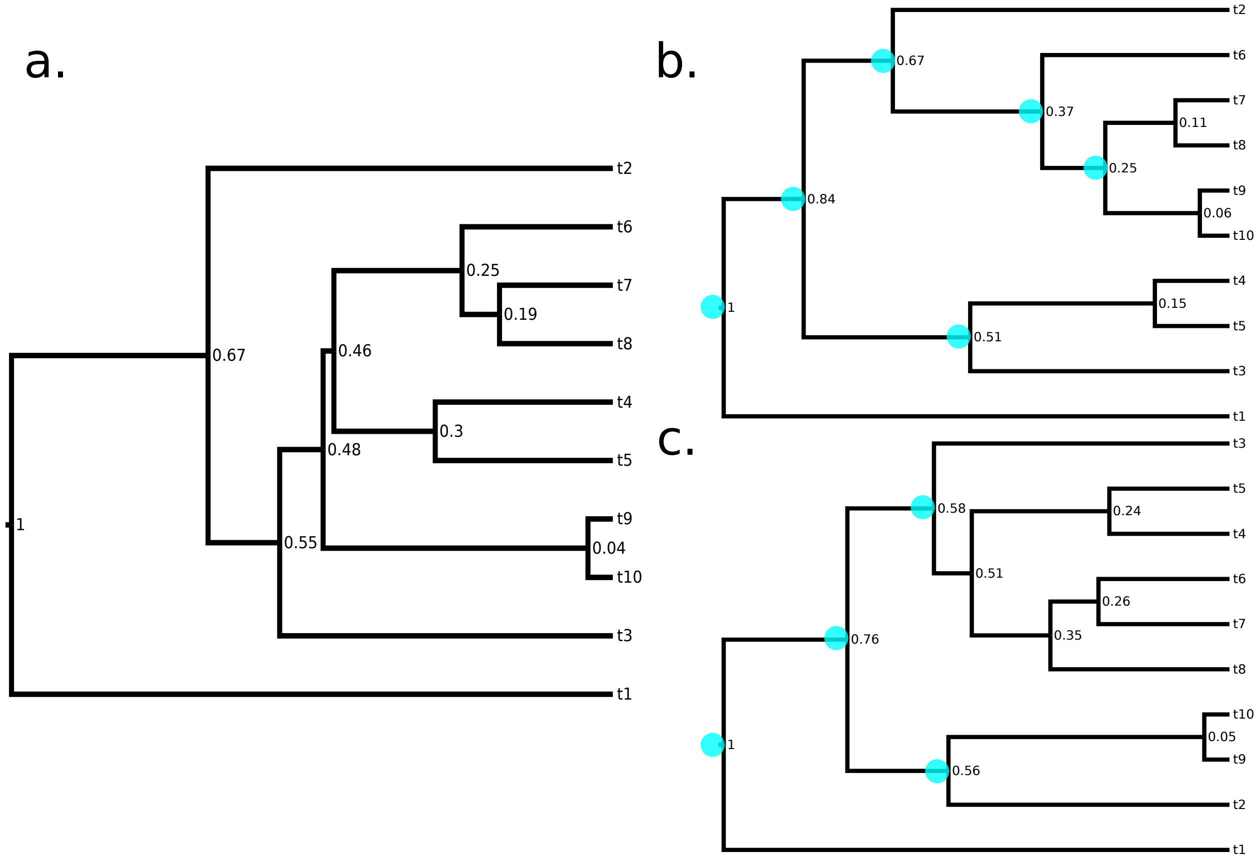

Figure 1: a. Exemplar true simulated tree. **b.** Tree inferred from 20 discrete characters simulated under Mk from true tree. **c.** Tree inferred from 20 continuous characters simulated under Brownian motion. Node labels correspond to node heights. Blue dots represent bipartitions not present in the true tree.

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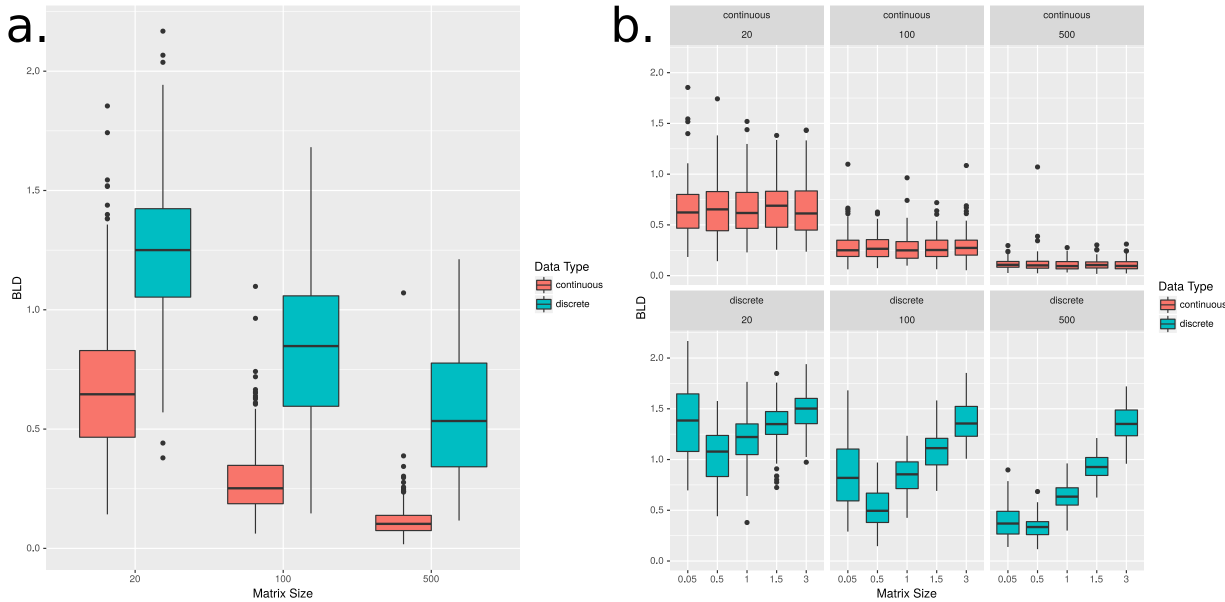

Figure 2: Branch length distance (BLD) across trees estimated from independently evolving continuous characters. **a.** BLD averaged across all rates except for the highest rate category, which resulted in the highest error when inferring under Mk. **b.** BLD across all matrix sizes and rates.

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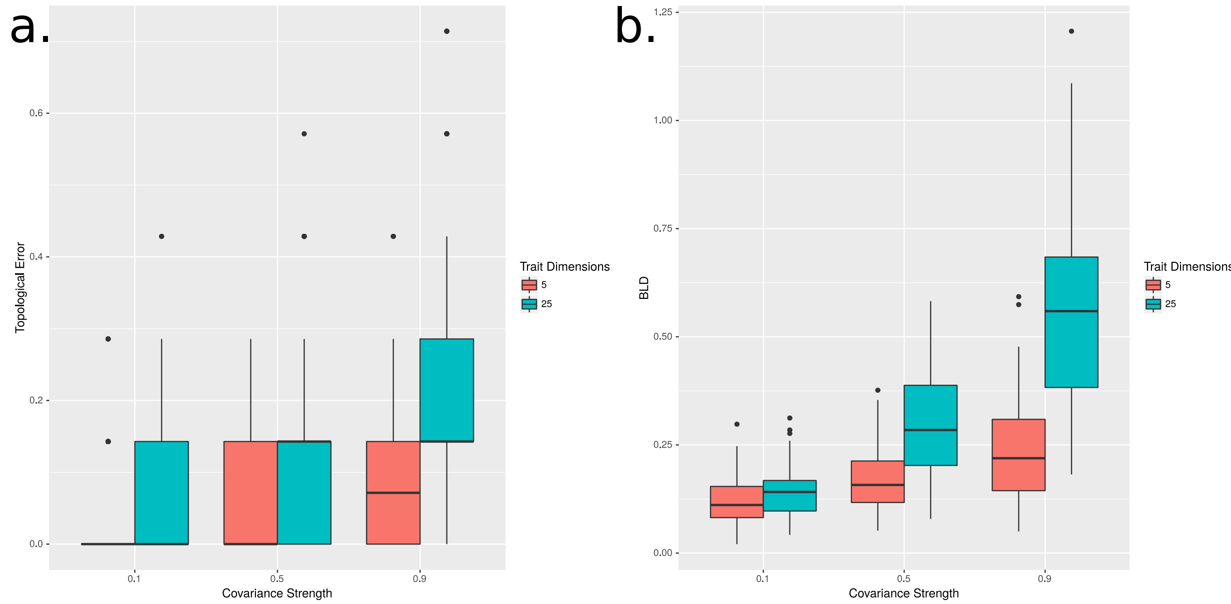

Figure 3: a. Topological error, calculated as proportion of maximum symmetric distance across trees estimated from covarying continuous characters. **b.** Branch length distance (BLD) across trees estimated from covarying continuous characters. Dimensions refers to the number of traits within covarying blocks. Covariance strength refers to the strength of the correlation between covarying characters, with 1 describing perfect correlation.

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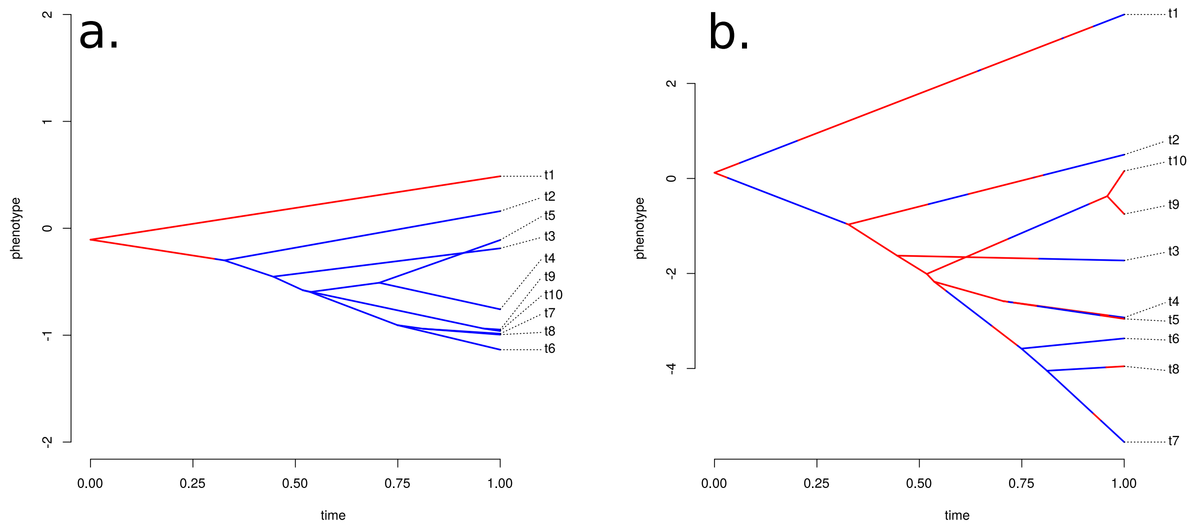

Figure 4: Discrete and continuous characters simulated **a.** at slow evolutionary rate and **b.** fast evolutionary rate. Y axis represents continuous phenotype. Changes in colour represent changes in discrete character state. Note how continuous characters retain phylogenetic signal at fast rates, while discrete characters saturate.

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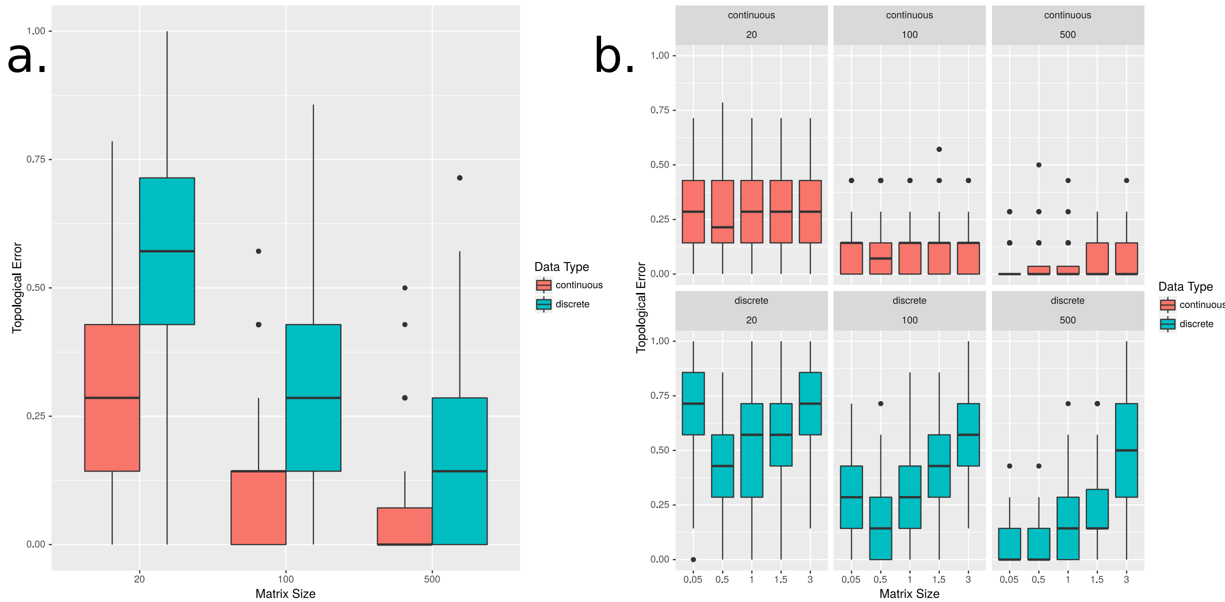

Figure S1: Topological error calculated as the proportion of maximum symmetric distance across trees estimated from independently evolving continuous characters. **a.** Error averaged across all rates except for the highest rate category, which resulted in the highest error when inferring under Mk. **b.** Error across all matrix sizes and rates.