POINT OF VIEW

Ultrametric trees or phylograms for ancestral state reconstruction: Does it matter?

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Supplementary Material The Electronic Supplement (Appendix S1: Detailed Methods; Appendix S2: Detailed Results; Figs. S1–S13) is available in the Supplementary Data section of the online version of this article at http://www.ingentaconnect.com/content/iapt/tax

INTRODUCTION

Reconstructing character states on a molecular phylogeny is a powerful tool for investigating trait evolution. Traits being "reconstructed" range from morphology and ancestral areas to ancestral ecologies and chromosome numbers. The factors determining state reconstructions are the statistical framework used, whether maximum parsimony, maximum likelihood, or Bayesian methods (D.R. Maddison, 1994; W.P. Maddison, 1995; Maddison & Maddison, 2006; Pagel, 1994, 1999a; Pagel & al., 2004), the model of state change (Cunningham & al., 1998; Mooers, 2004), the density of taxon sampling (Salisbury & Kim, 2001; Mooers, 2004; Gascuel & Steel, 2010), and the extent of rate heterogeneity in a dataset (Skinner, 2010). Over the past ten years, the mathematical, statistical, and biological difficulty, or indeed the impossibility, of ancestral state reconstruction (ASR) on trees has been explored and become increasingly clear (Mossel, 2003; Ekman & al., 2008; Losos, 2011; Royer-Carenzi & al., 2013). Nevertheless, trying to infer ancestral states remains an important activity in comparative biology and can set up strong hypotheses for further testing. In this note, we focus on an underappreciated problem, namely the lack of a criterion by which to choose the best branch length model for an ASR problem at hand.

The effect of branch length on ASR. — An important parameter in ASR is the way the branch lengths in the tree are modeled. Traditionally, workers have preferred ultrametric trees, and we have repeatedly experienced reviewers and editors who insist that ultrametric trees must be used for ASR. The first maximum likelihood ASRs were all carried out on ultrametric trees (Schluter & al., 1997; Mooers & Schluter, 1999; Pagel, 1999a), reflecting the notion that in living species we expect the amount of change to be related to the amount of time that has elapsed along a branch, not the number of substitutions in the (often short) stretches of DNA sequenced for a particular study. Most ASR studies have acted on this expectation even when a few plant studies showed that phenotypic evolution and molecular branch lengths can be correlated (Davies & Savolainen, 2006; Smith & Donoghue, 2008; animal studies so far show no such correlation: Davies & Savolainen, 2006). Given this possibility, exploring the effects of doing ASR on either ultrametric trees or phylograms seems expedient, perhaps especially when working on plants or on traits likely to reflect changes in an organism's DNA organization, such as changes in chromosome number (below).

To our knowledge, only Litsios & Salamin (2012) have studied effects on ASR of using either ultrametric trees or phylograms. Their study used 5000 simulated trees with 64 tips (species) and branch lengths corresponding to time; various levels of substitution rate heterogeneity were then introduced and the initially ultrametric trees transformed into phylograms. From each of the 5000 phylograms, Litsios & Salamin (2012) produced four types of ultrametric trees, one with uncorrelated rates in BEAST (Drummond & Rambaut, 2007) and three with correlated rates using the penalized likelihood (PL) approach of Sanderson (2002) with the smoothing factor ranging from 0 (complete parametric estimation) over 10 to 10,000 (nearly strict clock). On all tree types, they simulated the evolution of continuous characters using a Brownian motion model as well as characters that evolved in a manner uncorrelated to branch length and they then reconstructed the characters on the phylograms and chronograms using maximum likelihood. For each simulated dataset, they summed the differences between the inferred and the true ancestral states at each node to obtain one value per tree as a measure of the error in the character state reconstructed. They also measured the phylogenetic signal using the K (Blomberg & al., 2003) and λ (Pagel, 1999b) indices. As expected, states were most confidently reconstructed

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on the tree type on which their evolution had been simulated. More surprisingly, characters that had been simulated on a tree with uncorrelated rates (specifically, a PL tree with low smoothing) were reconstructed equally well on phylograms or ultrametric trees. This is an important insight, suggesting that phylograms should not be automatically disregarded in ASR. Litsios & Salamin (2012) recommended that workers prefer the tree with the higher phylogenetic signal, that is, the one in which traits evolve as they would under a random walk on the phylogeny, measured with K or λ, the latter having higher power. However, the dogma that traits must be reconstructed on ultrametric trees persists (for a recent example see Pellicer & al., 2014), even when obtaining these trees means a huge analytical effort that involves forcing branch lengths using congruification (Zanne & al., 2014).

In the present study, we want to highlight the sensitivity of ASRs to branch length, extending Litsios & Salamin's (2012) exploration from continuous characters to discrete multistate unordered characters (note that tree topology is not an issue, only the way branch lengths are modeled). The specific multistate unordered character we focus on are changes in plant chromosome numbers. While multistate unordered character coding is commonly used, inferring past changes in such characters presents huge challenges because the trait space is near infinite and because of the lack of a test statistic for measuring accuracy. The test statistics used by Litsios & Salamin (2012), Pagel's (1999b) λ & Blomberg's (2003) K, were designed for continuous characters. Discrete data require different measures of phylogenetic signal, which is why Fritz & Purvis (2010) developed a test statistic for binary characters. No measure of phylogenetic signal applicable to multistate unordered characters has so far been developed. Nevertheless, the fitDiscrete function in Harmon & al.'s (2008) Geiger package v.1.3-1 for R can calculate λ also for discrete characters, and in the present study we use this to investigate how λ behaves in the case of ASRs of multistate unordered characters. A problem in Litsios & Salamin (2012) was that with the empirical data, correlations between λ (as well as K) and the extent of difference in state reconstruction were not significant (adjusted $R^2 = 0.19$ and 0.002). The model underlying the λ statistic is the simple Brownian motion, and Boettiger & al. (2013) found that in small phylogenies, λ cannot be trusted (their phylogenies ranged from 13 to 281 tips). In sum, the utility of Pagel's λ for choosing among different branch length models for ASR input trees is limited, and the statistic is probably inappropriate for multistate characters and phylograms.

The lack of a test statistic is one reason why we chose not to follow the simulation approach of Litsios & Salamin (2012). Instead, we here (1) document the extent to which branch length depiction in phylograms, ultrametric trees with correlated rates, or ultrametric trees with uncorrelated rates affects ASRs in nine datasets ranging from 7 to 113 tips (Electr. Suppl.: Table S1 in Appendix S1) and (2) explore whether λ or other tree parameters correlate with differences in ASRs (see Electr. Suppl.: Table S2 in Appendix S1). The empirical data we chose for our investigation involve ancestral state reconstructions of chromosome numbers.

Reconstruction of chromosome number evolution. — Changes in chromosome number are common, especially in ferns and flowering plants, clades in which such change is a powerful driving force of speciation. Changes can be due to chromosome fusion or fission, polyploidization, or translocation events, in which an entire chromosome becomes inserted into other chromosomes (Schubert & Lysak, 2011). The empirically documented shifts in chromosome numbers even among close relatives have led to numerous studies, especially by botanists, trying to infer the history of chromosome number change on phylogenies (reviewed in Cusimano & al., 2012). Inference can be done in a likelihood framework, using the program ChromEvol (Mayrose & al., 2010). ChromEvol also implements a Bayesian approach, and the obtained posterior probabilities around inferences yield a statistically well-understood measure of confidence in the results.

The ChromEvol approach was tested using simulated and empirical datasets in the original work by Mayrose and colleagues, and has been used in at least 11 studies (Mayrose & al., 2011: 63 clades of vascular plants; Ness & al., 2011: Pontederiaceae; Cusimano & al., 2012: Araceae; Ocampo & Columbus, 2012: *Portulaca*; Cristiano & al., 2013: leafcutter ants (tribe Attini); Harpke & al., 2013: *Crocus*; Metzgar & al., 2013: fern genus *Cryptogramma*; Soza & al., 2013: *Thalictrum*; Pellicer & al., 2014: Melanthiaceae; Sousa & al., 2014: Areae; Chacón & al., 2014: Colchicaceae, see Electr. Suppl.: Appendix S1 for information about the study groups). Regarding tree branch lengths, Mayrose & al. (2010) used either ultrametric trees or phylograms from five empirical datasets, but they did not focus on the effect of branch lengths on the reconstructions. Of the subsequent studies, Ness & al. (2011), Ocampo & Columbus (2012), and Pellicer & al. (2014) used ultrametric trees, Cristiano & al. (2013), Harpke & al. (2013), and Soza & al. (2013) a phylogram, and Cusimano & al. (2012), Metzgar & al. (2013), Chacón & al. (2014), and Sousa & al. (2014) both types of trees. In the latter four cases, inferences often differed depending on the tree type used.

To investigate the causes of different ASRs on phylograms versus ultrametric trees, we carried out a meta-analysis of all datasets that had relied on the program ChromEvol by late 2013 (including our own). The data represent different tree sizes, lengths (root-to-tip distances), and chromosome number ranges (see Electr. Suppl.: Appendix S1 for detailed Methods). Since the true history of chromosome number change in none of the datasets is known and since there is no test statistic (see previous section), it is difficult to judge the accuracy of the ASRs, although knowledge of plant cytogenetics can help assess scenario plausibility; for example, sudden jumps from high to low numbers seem implausible given what is known about polyploidy.

■ RESULTS

The nine empirical datasets are *Crocus*, *Passiflora*, *Aristolochia*, *Cryptogramma*, *Portulaca*, Areae, Araceae, Colchicaceae, and Melanthiaceae (for details see Electr. Suppl.: Appendix S2 and Figs. S1–S12), and we infer chromosome

Table 1. Reconstruction of chromosome evolution in the nine clades analyzed or re-analyzed here.

						Inferred number of events						
	Tree[a]	Total tree length	Root-tip length	Best model[b]	*a* at root node	Gains	Losses	Duplication	Demi-duplication	Total	λ	Electr. Suppl.: Fig.
Aristolochia	P	1.09	0.1	cr	8	1	2.6	1	0	4.6	1	S1A
	PL	2.85	0.1	cr	8	0.9	2.5	1	0	4.4	1	S1B
	UCLN	2.02	0.12	cr	8	0.9	6.7	1	0	8.6	1	S1C
Cryptogramma	P	0.39	0.13	crde	30	0	1	1.7	0	2.7	1	S2A
	PL	0.36	0.1	crde	30	0	1	1.7	0	2.7	1	S2B
Portulaca	P	0.63	0.16	crde	5 (4)	0	5.7	2.7	22.5	30.9	0.76	S3A
	PL	0.6	0.1	crde	4	0	6.4	2.6	21.4	30.4	0.63	S3B
Colchicaceae	P	0.79	0.1	crd	7 (8)	5.8	6.5	8	5.9	26.2	0.90	S4A
	PL	2.53	0.1	crd	7	13.5	0	7.3	9.1	29.9	0.98	S4B
	UCLN	1.55	0.13	crd	7 (6)	15.6	0	6.4	8.7	30.7	0.92	S4C
Araceae	P	1.55	0.22	crd	18 (17–19)	0	77.9	8.4	7.8	94.1	1	S5A
	PL	6.03	0.1	crd	15	0	64.5	10.5	8.1	83.1	1	S5B
	PL	4.12	0.1	crd	15	0	65.8	9.7	6.6	82.1	0.99	S5C
	UCLN	1.78	0.1	crd	18/19	0	81.6	8.9	8.3	98.8	0.98	S5D
Areae	P	2.17	0.17	crde	14	1.8	38.3	27.1	6.3	73.5	0.84	S6A
	PL^{exp-10}	3.86	0.1	crde	7	6.8	16.7	39.2	5.4	68.1	0.96	S6B
	PL^{exp+10}	3.39	0.1	crde	7	4.3	12.4	36.2	6	58.9	0.96	S6C
	UCLN	4.71	0.2	crde	14	2.7	36	31.6	4	74.3	0.87	S6D
Crocus + outgroups	P	1.23	0.14	crd/crde/**lrd**/lrde	9/10	44.4	92.3	13.3	6.7	156.7	0.98	S7A
	PL	7.07	0.1	crd/ crde	5	13.1	14.5	19.4	19	66	1	S7B
Crocus	P	2.24	0.15	crd/**lrd**/lrde	5	34.9	63.8	18.3	13.5	130.5	0.96	S8A
	PL	10.26	0.14	**crde**/lrd	5	11.2	15.3	17.6	20.4	64.6	1	S8B
Passiflora+outgroups	P	0.6	0.12	crnd	12/13	0	14.3	0	0	14.3	1	S9A
	PL	2.43	0.1	crd	6	1	0	4.8	2	7.8	1	S9B
	UCLN	2.03	0.15	crd	6	1	0	4.9	2	7.9	1	S9C
Passiflora	P	0.77	0.13	crnd	12	0	13.3	0	0	13.3	1	S10A
	PL	3.48	0.13	crd	6	1	0	3.8	2	6.8	1	S10B
Melanthiaceae+outgroups	P	0.45	0.1	cr/crd/**crde**	10	0	10.2	10.5	5.1	25.8	1	S11A
	PL	2.67	0.1	crde	5	2.1	0	13.3	5.6	21	1	S11B
Melanthiaceae	P	0.61	0.09	crde	5	5.3	0	11.4	3.7	20.4	1	S12A
	PL	2.46	0.09	crde	5	2.9	0.5	11.1	3.8	18.3	1	S12B

[a] Tree: P, phylogram; PL, ultrametric tree with correlated rates, the superscript shows the penalty lambda (smoothing factor), where only one ultrametric tree was used, its lambda was that with the lowest cross-validation value; UCLN, ultrametric tree with uncorrelated rates.

[b] Models: cr, constant rates with duplication; crd, constant rates with demi-duplication rate = duplication rate; crde, constant rates with demi-duplication rate ≠ duplication rate; crnd, constant rates without duplications; lrd, linear rates with duplication; lrde, linear rates with demiduplication rate ≠ duplication rate; if AIC of different models did not differ significantly, all best-fitting models are reported (in all cases inferring the same root number and similar numbers of events); the parameters shown are of the best fitting model, which is indicated in bold.

a, inferred ancestral chromosome number; λ, Pagel's lambda indicating the phylogenetic signal.

number change on maximum likelihood phylogenies and ultrametric trees with correlated rates as obtained with PL (Sanderson, 2002) and, for five of the datasets, also with uncorrelated rates as obtained with BEAST (Drummond & al., 2012, see Electr. Suppl.: Appendix S1 for detailed Methods). Comparison of the resulting ASRs on phylograms versus ultrametric trees shows four patterns (Table 1): The reconstructions for three of the datasets (Electr. Suppl.: Figs. S1–S3) were essentially unaffected by the type of input tree used, for another three (Electr. Suppl.: Figs. S4–S6), ASRs differed mostly in terms of the changes inferred along the backbones of the trees. For one of these, the reconstructions on the two kinds of ultrametric trees were identical, whereas in the other two, they differed on every tree used. In the last three cases (Electr. Suppl.: Figs. S7, S9, S11), both the inferred node numbers and scenarios of chromosomal change differed completely depending on whether they were inferred on an ultrametric tree or a phylogram. The three cases in which tree type made no difference included a tree with 67 tips, one with 9 tips, and one with 29 tips, with percentages of known tip states ranging of 17%, 88%, and 26% (Electr. Suppl.: Table S1 in Appendix S1). The datasets in which tree type had the greatest effect on ASRs were *Crocus* with 88 tips and chromosome counts representing 88% of the species in that clade, and *Passiflora* with 59 tips and chromosome counts representing 11% of the species. Pagel's λ is uncorrelated with the characteristics of our data (Table 1; see Electr. Suppl.: Appendix S2 for detailed Results). Regression analyses to test whether differences in total tree length, tree imbalance, or stemminess correlate with reconstruction results, did not yield any significant result, probably due to the few, and partly dependent, data (Electr. Suppl.: Fig. S13, but see Conclusions).

■ CONCLUSIONS

Our results show that branch lengths can have large effects in ASRs of multistate unordered characters just as is the case for continuous characters (Litsios & Salamin, 2012). The good news is that some ASRs are insensitive to the way branch lengths are modeled. On the other hand, in our "worst" dataset, the Areae, ASRs differed on every one of the four input trees. Given these findings, the essential questions concern (1) ways to predict which kinds of datasets are prone to yielding different ASRs depending on branch lengths models, and (2) which ASRs to prefer in those cases where tree type changes the reconstructions. Regarding the first question, tree parameters influencing the reconstructions are total tree length, tree imbalance, and stemminess, all of which are interrelated (Electr. Suppl.: Table S2 in Appendix S1). A factor setting apart the three datasets for which tree type had essentially no effect on ASRs from those where it did, is internal stemminess. The reason probably is that in stemmy trees, the proportional lengths of branches are changed especially drastically as one changes from a phylogram to an ultrametric depiction of ones data. On our stemmiest trees, the total tree length was six or four times higher in ultrametric trees compared to phylograms (Table 1). By contrast, in cases where tree type hardly affected ASRs, the lengths of the ultrametric tree and the phylogram were nearly the same or differed only by a factor of three. Where the stemminess is due to a distant outgroup, the problem might sometimes be overcome by excluding that outgroup from the analysis (Electr. Suppl.: Figs. S8, S10, S12), which often reduces total tree length drastically. When applying this "workaround" to the *Crocus* and Melanthiaceae datasets, the inferred haploid number at the root became the same (ancestral chromosome number $a = 5$) in the different types of trees (Electr. Suppl.: Fig. S9). However, if a long stem is in the ingroup, as is the case in the Colchicaceae (Electr. Suppl.: Fig. S4), exclusion of a single long branch is clearly not an option.

Regarding the second question, which ASRs to prefer, there does not appear to be a ready answer apart from data consilience when knowledge is available from other fields of science, in our case cytogenetics. For example, comparative FISH analyses can reveal if a decrease in chromosome number is gradual by end-to-end fusion of chromosomes, which can leave interstitial telomere signals (Souza & al., 2014). Such external evidence may often be the only way to choose among contrasting ASRs (Skinner, 2010), because no statistical measure of phylogenetic signal is available for multistate discrete characters (first section of this Point of View), and Pagel's λ in our study did not relate to whether or not trees yielded drastically different ASRs (Table 1). Even if λ were suitable for phylograms, there is clearly a continuous range of possible branch length depictions (for the same tree topology) ranging from phylograms with various levels of rate smoothing to ultrametric trees.

Our recommendation then is to run ASRs on more than one type of branch length depiction. In the absence of external evidence, the simpler scenarios, explaining the data with fewer inferred steps, should probably be preferred, and this might be one way to decide in cases where phylograms and ultrametric trees yield models of different complexity (but see Pirie & al., 2012, who showed that this is only true when rates are low).

Remaining aware of the limits of ASR. — Inferring the evolution of multistate unordered characters is difficult because of the immense trait space. It is important to be aware of the limits of what can be inferred rather than getting carried away by the positively loaded name of the game, namely "reconstruction". Mathematical and computational studies show that it is impossible to reconstruct ancestral states at the root of "deep" phylogenetic trees with high mutation rates and suggest that it may not be possible to predict the best method of ASR reconstruction on a particular tree (Mossel, 2003; Royer-Carenzi & al., 2013). The reason is the stochastic nature of trait change. Besides branch length, taxon sampling density, character coding, and the model of trait change (cf. Introduction), molecular trees lack information about extinct species, which may be the single largest problem in ASR. Expecting simple rules, or worse, defending dogmatic attitudes about this or that tree type or inference approach always deserving preference, is naïve and inappropriate. As concluded by Ekman & al. (2008: 153): "Obviously, ancestral state reconstructions need to be conducted with more than one method while awaiting clarification of the statistical properties of each method."

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