Plausibility of inferred ancestral phenotypes and the evaluation of alternative models of limb evolution in scincid lizards

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Phylogenetic approaches to inferring ancestral character states are becoming increasingly sophisticated; however, the potential remains for available methods to yield strongly supported but inaccurate ancestral state estimates. The consistency of ancestral states inferred for two or more characters affords a useful criterion for evaluating ancestral trait reconstructions. Ancestral state estimates for multiple characters that entail plausible phenotypes when considered together may reasonably be assumed to be reliable. However, the accuracy of inferred ancestral states for one or more characters may be questionable where combined reconstructions imply implausible phenotypes for a proportion of internal nodes. This criterion for assessing reconstructed ancestral states is applied here in evaluating inferences of ancestral limb morphology in the scincid lizard clade *Lerista*. Ancestral numbers of digits for the manus and pes inferred assuming the models that best fit the data entail ancestral digit configurations for many nodes that differ fundamentally from configurations observed among known species. However, when an alternative model is assumed for the pes, inferred ancestral digit configurations are invariably represented among observed phenotypes. This indicates that a sub-optimal model for the pes (and not the model providing the best fit to the data) yields accurate ancestral state estimates.

Keywords: ancestral state; Bayesian inference; *Lerista*; limb reduction; Squamata

1. INTRODUCTION

Phylogeny-based inference of ancestral character states is an integral part of contemporary evolutionary biology, providing an efficient, widely applicable means of investigating patterns and rates of phenotypic evolution (Pagel 1999a). Relatively recent methodological advances, notably the development of maximum likelihood (Schluter *et al.* 1997; Pagel 1999b) and Bayesian Markov chain Monte Carlo (Pagel *et al.* 2004) approaches, have dramatically

increased the sophistication of ancestral character state estimation, enabling statistical comparisons of alternative models of character evolution and assessments of uncertainty associated with inferred ancestral traits. Nonetheless, several authors (e.g. Wiens *et al.* 2007; Ekman *et al.* 2008) have noted the potential for available methods to yield strongly supported but inaccurate ancestral state estimates, raising the issue of how the reliability of ancestral character state reconstructions should be assessed.

The consistency of ancestral states inferred for two or more characters affords a potentially useful criterion for evaluating ancestral trait reconstructions. Ancestral state estimates for multiple characters that entail plausible ancestral phenotypes when considered in conjunction may reasonably be assumed to be reliable. However, the accuracy of inferred ancestral states for one or more characters may be questionable where combined reconstructions imply implausible phenotypes for a proportion of internal nodes. This criterion for assessing reconstructed ancestral states is employed here to evaluate inferences of ancestral limb morphology in the scincid lizard clade *Lerista*. Among extant tetrapods, *Lerista* provides perhaps the best available model for studying limb evolution, comprising more than 90 species displaying an extensive array of digit configurations, from pentadactyl to limbless conditions (see Skinner *et al.* 2008). As Wiens (2009) noted, however, this exceptional variability of limb morphology poses a significant challenge for the accurate reconstruction of ancestral states, and considerable uncertainty surrounds recent inferences of evolutionary patterns within the clade (see Skinner *et al.* 2008).

2. MATERIAL AND METHODS

BEAST v. 1.4.7 (Drummond & Rambaut 2007) was employed to estimate phylogenetic relationships from nuclear intron and mitochondrial gene sequences (2868 aligned sites) for 77 species and subspecies of *Lerista* and six outgroup taxa (see electronic supplementary material). Four partitions were specified, each incorporating nucleotides presumed to experience similar functional and structural constraints (see electronic supplementary material). A relaxed-clock model in which rates for individual branches are drawn independently from a lognormal distribution (the ‘uncorrelated lognormal’ model) was assumed, with a Yule tree prior and no constraints on topology. Parameter values for nucleotide substitution models were estimated independently (i.e. parameter values were unlinked across partitions), assuming the default priors. A normal prior was placed on the age of *Lerista*, with a mean of 14.19 Myr and an s.d. of 2.069 Myr (see Skinner *et al.* 2008). Additionally, we placed a wide uniform prior (upper and lower bounds of 10 and 50 Myr) on the root of the tree. The Markov chain Monte Carlo algorithm was initiated with a UPGMA tree and run for 20 million generations, sampling every 2000 generations; the first 10 million generations were discarded as burn-in. TreeAnnotator v. 1.4.7 (Drummond & Rambaut 2007) was used to construct a maximum clade credibility tree (with branch lengths in units of 1×10^7 years), which was assumed in estimating posterior distributions for alternative ancestral states.

Ancestral numbers of digits for the forelimb (manus) and hind limb (pes) were inferred independently using BayesTraits v. 1.0 (available at www.evolution.rdg.ac.uk; see Pagel *et al.* 2004), assuming five different models of character evolution (table 1). All analyses involved running a single Markov chain for 10 million generations, sampling every 1000 generations. Uniform priors (with default upper and lower bounds of 0 and 100) were assumed for all estimated model parameters. The s.d. of successive alterations to proposed model parameter values (specified by the *ratedev* command) was adjusted so as to produce acceptance rates of 20–30%. Phylogenies were drawn from the sample of 5000 trees generated in the BEAST analysis described above; where a phylogeny did not include an internal node represented in the maximum clade

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Table 1. Models of character evolution assumed in reconstructing ancestral character states.

model A	all character state transitions occur at the same rate and are unordered (i.e. direct transitions are possible among all character states)
model B	as for model A, except that character state transitions are ordered (i.e. transitions of more than one digit are constrained to pass through all intervening states; see Brandley <i>et al.</i> 2008)
model C	forward and reverse transitions (corresponding to the acquisition and loss of digits, respectively) occur at independent rates and are unordered
model D	as for model C, except that character state transitions are ordered
model E	as for model C, with the rate of digit acquisition set to zero (prohibiting reversals of digit loss)

Table 2. BayesTraits results for the five models examined. Mean values (where applicable) are followed by 95% credible intervals in parentheses. q01, forward transition rate; q10, reverse transition rate; L, likelihood; the Bayes factors reported derive from comparisons with the model that best explains the data.

model	q01	q10	−ln L	ln harmonic mean L	2 ln Bayes factor
manus					
model A	1.120 (0.611–1.913)	1.120 (0.611–1.913)	90.773 (88.528–93.271)	−92.221	10.238
model B	9.499 (5.382–16.225)	9.499 (5.382–16.225)	100.368 (97.612–103.345)	−102.224	30.244
model C	0.345 (0.010–1.267)	1.772 (0.871–3.044)	86.767 (83.031–91.087)	−89.664	5.124
model D	5.322 (0.988–12.421)	14.726 (7.411–24.905)	98.359 (94.770–102.211)	−101.000	27.796
model E	0	1.787 (1.008–2.976)	85.340 (81.820–88.445)	−87.102	0
pes					
model A	1.478 (0.830–2.503)	1.478 (0.830–2.503)	102.140 (100.379–104.572)	−103.134	8.970
model B	6.200 (3.582–10.181)	6.200 (3.582–10.181)	97.536 (95.357–100.229)	−98.649	0
model C	1.755 (0.272–3.592)	1.495 (0.550–2.769)	102.485 (100.601–105.326)	−104.133	10.968
model D	4.477 (0.757–10.329)	8.279 (4.089–14.938)	97.385 (94.577–100.814)	−99.388	1.478
model E	0	1.817 (1.084–2.953)	103.217 (101.165–105.846)	−104.521	11.744

credibility tree, posterior probabilities for alternative character states were calculated for the most recent shared ancestor of the taxa defined by that node (see Pagel *et al.* 2004). Posterior probability distributions for alternative states were estimated for all internal nodes in the maximum clade credibility tree from posterior probabilities sampled after one million generations (the first 10% of the Markov chain was discarded as burn-in in all analyses).

The consistency of reconstructed ancestral states for the manus and pes was assessed for all 25 possible combinations of the five models of character evolution examined. Ancestral trait reconstructions for an internal node were considered to be consistent where the digit configuration implied by the most probable ancestral states for that node (i.e. the character states having the highest mean posterior probabilities) occurs among known species of *Lerista*. The proportion of internal nodes for which implied digit configurations are observed in one of more known species was employed as a measure of the overall consistency of ancestral character state reconstructions for two models (internal nodes outside *Lerista* were excluded in calculating this proportion).

3. RESULTS AND DISCUSSION

The BayesTraits analyses indicate that very different models provide the best fit to the data for the manus and pes (table 2). The data for the manus are best fit by the unordered, two-rate model in which the rate of digit acquisition is assumed to be zero (model E); when compared with the remaining models, this model yields 2 ln Bayes factor estimates of 5.124–30.244 (values greater than 6 are conventionally regarded as strong evidence for a model; see Brandley *et al.* 2005). Models in which character state transitions are unordered provide a significantly better fit than those in which character state transitions are ordered. As Brandley *et al.* (2008) noted, developmental and comparative morphological evidence indicates

that digits develop and are lost in an ordered sequence, so that models assuming ordered character state transitions may be preferred *a priori*. However, prohibitive computation time prevented us from evaluating an ordered model assuming a rate of digit acquisition of zero, so that we were unable to incorporate both character state ordering and the assumption of irreversible digit loss in a single model. The maximal consistency of ancestral states for the manus and pes inferred assuming an unordered model prohibiting digit acquisition (model E; see below) suggests that the assumption that digit loss is irreversible may be substantially more significant than character state ordering in modelling limb evolution. A single-rate model in which character state transitions are ordered (model B) provides the best fit to the data for the pes; this model yields 2 ln Bayes factor estimates of 1.478–11.744 when compared with the other models.

The consistency of reconstructed numbers of manual and pedal digits for each combination of the five models examined is presented in figure 1. Ancestral states inferred assuming the two models that provide the best fit to the data (model E for the manus and model B for the pes) are the least consistent, implying ancestral digit configurations represented among known species of *Lerista* for only 62 of 76 internal nodes; inconsistent ancestral states are generally associated with basal nodes and entail digit configurations of either four or five digits for the manus and two or three digits for the pes (figure 2). The most consistent ancestral state estimates are obtained where the best-fitting model for the manus

Figure 1. Consistency of ancestral character states for the manus and pes for all combinations of the five models examined.

(model E) is assumed for both the manus and pes. Ancestral state reconstructions for this combination of models invariably imply ancestral digit configurations observed among known species of *Lerista*.

The substantial proportion of implausible digit configurations implied by ancestral state reconstructions for the two best-fitting models (model E for the manus and model B for the pes) indicate that one or both reconstructions are inaccurate. Several considerations suggest that the inconsistent ancestral states are attributable to the reconstructions for the pes. As indicated in figure 1 and above, when the reconstructions for the manus (i.e. for the best-fitting model) are considered in combination with ancestral states for the pes inferred assuming a suboptimal model (model E), they imply digit configurations represented among known species of *Lerista* for all internal nodes. The reconstructions for the pes (i.e. for the best-fitting model), conversely, are inconsistent with one or more ancestral states for the manus in all instances (figure 1). As well as yielding ancestral states that consistently entail implausible digit configurations for internal nodes, the model that best fits the data for the pes may be regarded as inappropriate for inferring patterns of limb reduction or potential re-elaboration, as it assumes identical rates for all transitions; Brandley *et al.* (2008) recently presented evidence for significantly different rates of digit acquisition and loss across squamates, so that models assuming a single transition rate may inadequately represent the process of limb evolution. Significantly, the model producing ancestral state estimates consistent with those for the manus (model E) has a marginal likelihood differing negligibly from that for similar models (model C; table 1), and conforms well with current understanding of squamate limb evolution (which indicates a low frequency of digit acquisition).

An objective means of assessing the plausibility of implied ancestral phenotypes is essential if the criterion

of consistency among ancestral states for multiple characters is to be useful. Ancestral phenotypes were evaluated here according to whether they occur among observed states for the terminal nodes of the phylogeny (i.e. known species). This convenient criterion for assessing combinations of inferred ancestral states is only appropriate, however, if the distribution of phenotypes exhibited by known species adequately represents the range of attainable phenotypes; where this is not the case, the absence of particular phenotypes may be attributable to either anagenesis or extinction. All inconsistent ancestral states for the two models that best fit the data entail ancestors possessing well-developed forelimbs and moderately reduced hind limbs (see figure 2 and above). Although similar phenotypes are observed in the closely related clade *Anomalopus* (see Wiens 2009), known species of *Lerista* never exhibit fewer digits for the pes than the manus (indeed, the combination of well-developed forelimbs and substantially reduced hind limbs is exceedingly rare in squamates generally). The phylogenetic distribution of particular (observed) digit configurations indicates that nearly all configurations have evolved independently multiple times (figure 2). Moreover, observed digit configurations represent a continuous, relatively limited region of the possible morphospace (the shaded cells in the inset of figure 2). Accordingly, it may be assumed that the potential morphospace available to the immediate ancestor of *Lerista* is completely occupied (or nearly so). Insofar as this assumption is valid, our analyses indicate that the two-rate model with unordered character state transitions and a specified rate of digit acquisition of zero (model E) yields accurate ancestral character state reconstructions for both the manus and pes.

Comparisons of implied ancestral phenotypes with phenotypes represented among known species provides

Figure 2. Maximum clade credibility tree with implied ancestral digit configurations for the two models that best explain the data (see text). Digit configurations not represented among known species of *Lerista* are indicated by an asterisk. The frequencies of implied digit configurations are presented in (a) where shaded cells indicate configurations observed among known species (see labels for terminal nodes). Representative species of *Lerista* shown are *L. arenicola* (b) and *L. edwardsae* (c). Photographs by Trevor Peters.

an independent, potentially powerful means of evaluating ancestral character state reconstructions. Application of this criterion in assessing inferences of ancestral limb morphology in *Lerista* indicates that the best-fitting model for the pes yields inaccurate ancestral state estimates, and that alternative (suboptimal) models perform significantly better. Although our results validate concerns that ancestral state reconstructions may seriously mislead inferences of evolutionary patterns, they also demonstrate that this possibility may be substantially negated by considering ancestral state estimates for multiple characters in combination.

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