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Accommodating Phylogenetic Uncertainty in Evolutionary Studies

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Many evolutionary studies use comparisons across species to detect evidence of natural selection and to examine the rate of character evolution. Statistical analyses in these studies are usually performed by means of a species phylogeny to accommodate the effects of shared evolutionary history. The phylogeny is usually treated as known without error; this assumption is problematic because inferred phylogenies are subject to both stochastic and systematic errors. We describe methods for accommodating phylogenetic uncertainty in evolutionary studies by means of Bayesian inference. The methods are computationally intensive but general enough to be applied in most comparative evolutionary studies.

The processes of mutation, selection, and genetic drift that underlie evolutionary change operate very slowly in most species. Many questions in evolutionary biology are therefore addressed by comparing molecular, morphological, ecological, or behavioral characteristics among groups of species (*1*). The comparative method, for example, uses comparisons of the states at two or more characters among extant species as a tool for detecting the evidence of adaptation. Correlated change among characters is the footprint of natural selection. Similarly, comparative analyses of DNA sequences have transformed molecular evolutionary studies, indicating evidence of strong purifying selection and, more rarely, evidence for positive selection at the molecular level. Many evolutionary biologists, however, are concerned with much simpler questions than whether characters have changed in a correlated manner. For example, a question commonly asked in comparative evolutionary studies is whether certain traits are capable of rapid evolutionary change and whether they change in a biased manner over evolutionary time. Such studies typically examine the number of times that a trait has been gained and lost over the evolutionary history of a group of related species.

Most evolutionary biologists now recognize that phylogenetic history must be accommodated in comparative analyses. Otherwise, the covariation among characters induced by their common phylogenetic history can compromise a statistical analysis (*2*). Accordingly, the "gold standard" of between-species studies today includes a phylogenetic analysis. However, phylogenies used in comparative studies are typically treated as known (*1*). Although wary of this assumption, in the absence of any clear alternative the usual response of biologists has been to "hedge bets" by performing the analysis with only the well-supported parts of a phylogeny, or with phylogenies obtained by several methods with the implicit assumption that the conclusions of the analysis are well founded if they are robust to the method of phylogenetic inference used. Recent attempts to accommodate phylogenetic uncertainty seek to ascertain the sensitivity of a comparative study to the phylogeny used (*3*) or to average over all possible phylogenies generated by a stochastic model of cladogenesis (*4*). Such approaches do not make efficient use of information about the relative probabilities of potential phylogenies that can be obtained from a sample of DNA sequences. Developing a formal method for dealing with phylogenetic uncertainty in comparative analyses is important for at least two reasons: (i) estimates of phylogeny are subject to both systematic and stochastic errors (*5*, *6*) and (ii) evolutionary biologists often have only a peripheral interest in phylogeny and are more interested in testing evolutionary hypotheses.

Recently developed methods for phylogenetic analysis with molecular data make it possible to perform comparative analyses in such a way that inferences are averaged over all possible trees (*7*, *8*); the character analysis is performed on each tree, and the result is weighted by the posterior probability (*9*) that the tree is correct. For even moderate numbers of species, it is usually not feasible to perform a comparative analysis by considering all possible trees. This ideal can be approximated, however, by sampling trees according to their (posterior) probability given the existing data. We illustrate this idea by asking how many times the horned soldier caste evolved and was lost in aphids (*10*). This question is typical of the type of question asked by many evolutionary biologists. There are two sources of uncertainty in studies of the evolution of a particular character: (i) uncertainty about the phylogenetic tree of the species under study and (ii) uncertainty about the character transformations on that tree. We illustrate two ways in which such uncertainties can be considered. The first approach accommodates uncertainty in the phylogenetic tree by averaging over possible trees weighted according to their posterior probabilities but ignores the inherent

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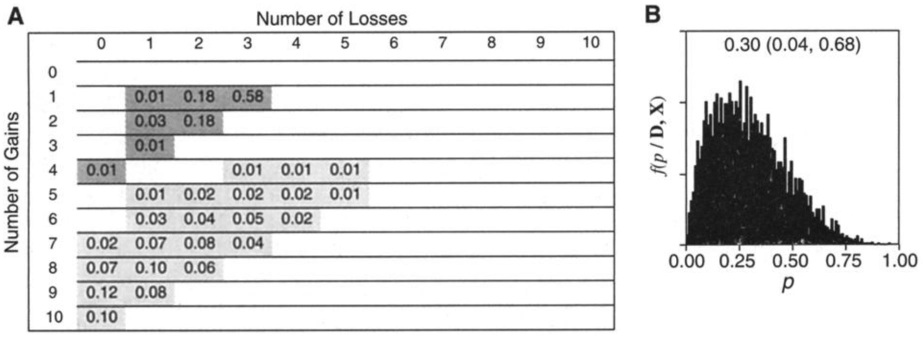
uncertainty of parsimony-based character reconstructions. The second approach uses a simple stochastic model of character evolution to accommodate uncertainties about the number of gains and losses by instead estimating the probability density of the bias (direction) of change (gain or loss).

In the first approach, we performed a Bayesian analysis of 34 aphid mitochondrial DNA sequences using Markov chain Monte Carlo (MCMC) (*11–13*) to approximate the posterior probabilities of trees. We then reconstructed the number of gains and losses of the horned soldiers on each tree using the parsimony criterion. The parsimony reconstruction was weighted by the probability of the tree on which the character was reconstructed. The dark-shaded region of Fig. 1A shows the probability of various reconstructions of the number of gains and losses of the character. The data are mostly consistent with one gain and two losses, one gain and three losses, or two gains and two losses. However, even a reconstruction that has two gains and one loss has a reasonable probability of being correct (0.03). The maximum parsimony tree suggests that a reconstruction with one gain and two losses is best (*10*). However, the sum of the posterior probabilities of trees consistent with this reconstruction is only 0.18. The light-shaded region of Fig. 1A shows the posterior probabilities of different reconstructions if each tree is equally probable. This would be the distribution of character reconstructions in the absence of any information on the taxonomy or biology of the aphids. The posterior probability is spread over many different reconstructions, many of which involve a large number of gains or losses of the trait.

There are disadvantages to the use of parsimony to reconstruct the number of gains and losses of a character: It does not account for multiple unobserved changes, providing at best an estimate of the minimum number of character changes; and the uncertainty of inferred character transformations is not considered. Our second approach avoids these problems by assuming instead that the evolution of the horned soldier trait can be described by a simple stochastic model of the process of character gain and loss. The probability that a gain occurs during some interval dt is $p\mu dt$ and the probability of a loss is $(1 - p)\mu dt$. The direction of change is determined by the bias parameter p and the rate of change by the rate parameter μ. This model is the well-known Markov-Bernoulli process. We approximated the posterior probability of p by using MCMC (*14*). Figure 1B shows the posterior probability of a gain versus a loss of the horned caste trait. The posterior probability of the bias parameter has more weight for $p < 0.5$; this is consistent with the idea that a loss of the horned soldier caste occurs more often than a gain. However, the spread of the posterior density also suggests that our information about p is quite uncertain (the range of the 95% credible set of values for p goes from 0.04 to 0.68).

Most evolutionary biologists now accept that correlations among characters as a result of their shared phylogenetic history should be accommodated in comparative studies. However, the results of a comparative study based on a particular phylogeny may be overturned if a reexamination of the group results in a different tree. The Bayesian approach to comparative analysis outlined here reduces the probability of such a radical outcome because it considers all potential trees, weighted according to the probability that each is correct, in testing an evolutionary hypothesis. Such an approach is likely to be more robust because it takes better account of the phylogenetic status quo. Bayesian methods using MCMC are arguably among the most significant new developments in phylogenetics over the last decade. The methods described here can be extended to many other questions involving comparative analysis. Promising applications include analyses of covariation among two or more characters, biogeographical comparisons, and studies of rates of molecular evolution and of the origin and evolution of gene families. All these examples involve evolutionary processes that depend on a phylogeny that is imperfectly known. Bayesian inference provides a set of formal tools for accommodating the inherent uncertainty of phylogenetic trees.



	0	1	2	3	4	5	6	7	8	9	10
0											
1		0.01	0.18	0.58							
2		0.03	0.18								
3		0.01									
4	0.01			0.01	0.01	0.01					
5		0.01	0.02	0.02	0.02	0.01					
6		0.03	0.04	0.05	0.02						
7	0.02	0.07	0.08	0.04							
8	0.07	0.10	0.06								
9	0.12	0.08									
10	0.10										

Fig. 1. **(A)** The sum of the posterior probabilities of phylogenetic trees having different numbers of reconstructed gains and losses of the horned soldier caste character. For the dark-shaded numbers, the posterior probabilities of trees were approximated with the program BAMBE (*13*) under the HKY85 (*15*) model of DNA substitution with site-specific rate variation. The light-shaded numbers are the posterior probabilities for different reconstructions when all trees are taken to be equally likely. **(B)** The posterior probability distribution of the parameter p expressing the bias in the rate of gain of the horned soldier caste character. The numbers represent the mean of the posterior distribution with the 95% credibility interval for p.

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$$f(\tau_i|\mathbf{X}) = \frac{f(\mathbf{X}|\tau_i)\,f(\tau_i)}{\sum_{j=1}^{B(s)} f(\mathbf{X}|\tau_j)\,f(\tau_j)}$$

where $f(\mathbf{X}|\tau_i)$ is the likelihood and $f(\tau_i)$ is the prior probability of the *i*th tree [here, $f(\tau_i) = 1/B(s)$]. The summation is over all $B(s)$ trees possible for s species. The likelihood is an integral over all possible branch lengths (v_i) and parameters of the substitution model (θ)

$$f(\mathbf{X}|\tau_i) = \int_\theta \int_{v_i} f(\mathbf{X}|\tau_i,v_i,\theta)\,f(v_i)\,f(\theta)\,dv_i\,d\theta$$

The posterior probability is interpreted as the probability that τ_i is the true phylogeny under the specified model of DNA substitution.
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$$f(\mathrm{p},\mu|\mathbf{D},\mathbf{X})) = \frac{f(\mathbf{D}|\mu,p,\mathbf{X})f(\mu)\,f(p)}{C}$$

where $\mathbf{D}$ is the aphid caste data and C is a normalizing constant. The posterior probability distribution was approximated with MCMC (*11*, *12*). An exponential prior was placed on the rate of change (μ) and a uniform prior on p. The results for an exponential prior with mean 1.0 are shown. However, the posterior probability of p was not affected by the prior placed on μ, suggesting that the inferences of the direction of change (p) are robust to assumptions about the rate of evolution of the aphid caste character (μ).
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