12. Phylogenetic Comparative Methods

EEOB563

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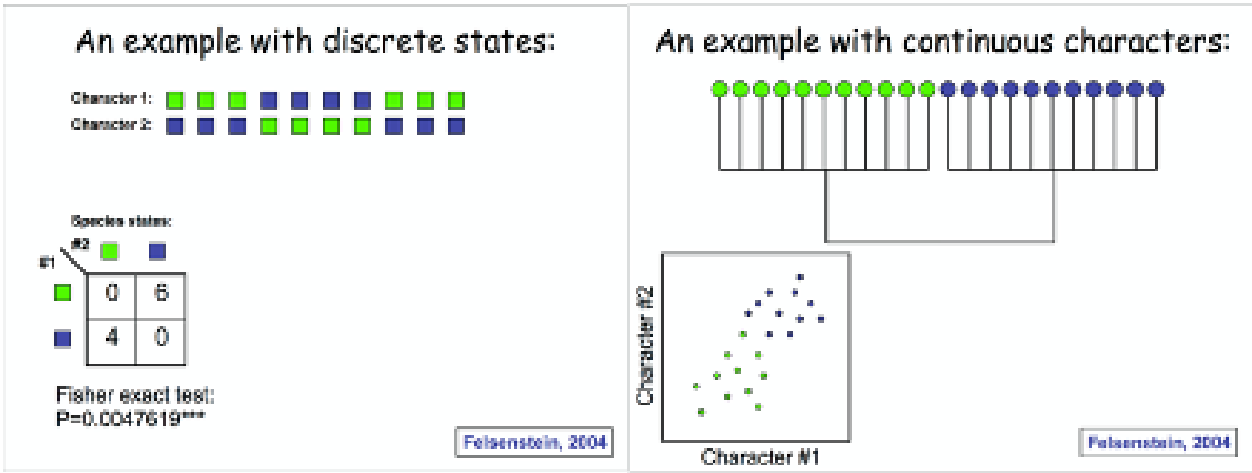
"Character state reconstructions can provide a powerful mechanism for studying many facets of the evolutionary process. However, the zeal with which these techniques are sometimes advocated belies the complexity of the problem". Swofford and Maddison, 1992.

1 Preliminary considerations

In Biology we are often interested in co-evolution of various traits (e.g., population density and body size; IQ and reproductive success; genome size and cell size; etc.). Conventional statistical methods assume that samples are independent. Such an assumption is often inappropriate in Biology, where all species share some common history. Phylogenetic comparative methods (PCMs) aim to incorporate information on the evolutionary relationships of organisms (phylogenetic trees) to compare species.

1.1 Two examples



(a) A subfigure (b) A subfigure

Figure 1: A figure with two subfigures

Four areas of molecular evolution have particularly benefited from advances in phylogenetics and will be considered in more details in this class:

- predicting adaptive evolution;
- reconstruction of ancestral characters states;

- estimation of timing of evolutionary events;
- comparative studies

1.2 Do early branching lineages signify ancestral traits? (after Crisp and Cook, 2005)

Failure to distinguish between present-day descendants and long-dead ancestors has led to incorrect interpretation of phylogenetic trees. Misinterpretation becomes evident when authors use the terms "basal" or "early diverging" to refer to extant taxa.

For example are monotremes "ancient/ancestral mammals"? Need to remember that whereas some character states can be relatively ancestral (plesiomorphic) or derived (apomorphic), these concepts should not be applied to whole organisms. Only nodes on a tree can be referred to as "basal" or "derived" relative to each other.

A common misinterpretation occurs when a species-poor/less diverse sister group is labeled as "basal" or "early-diverging" with respect to its species-rich sister. This misreading of phylogenies is encouraged by asymmetrical trees. Simplistic interpretation of phylogenies is also common in biogeography, where, for example, researchers may attempt to trace the history of a lineage back to its ancestral area.

2 Methods and models for inferring ancestral states

In reconstructing character evolution, our goal is to reconstruct the character states of hypothetical ancestors throughout the phylogenetic tree. The usual practice is to estimate the states of the hypothetical ancestors at the nodes or branch points of the tree, because these provide convenient reference points. Many methods are available (e.g., table 1 from Crisp and Cook below)

A state can be placed at the node parsimoniously if it allows the observed states in the taxa to be evolved in as few as possible evolutionary steps. If more than one state can be placed parsimoniously at a node, the node's assignment is said to be equivocal. The set of all the states that can be parsimoniously assigned to a node is called the node's most parsimonious reconstructions (MPR) set.

A reconstruction is a set of assignments to the nodes such that each node is assigned only one state. You can think of it as one particular scenario for the evolution of the trait in question. The meaning of "reconstruction" given above is a very specific one — a fully resolved (lacking ambiguity) scenario for the evolution of the character, node by node, on the tree.

2.1 Finding the most parsimonious ancestral states

Ancestral state reconstruction using parsimony is done in two or three passes up and down the tree. The details of the process may differ with the different assumptions used; the algorithm for

Table 1. Methods and models for inferring ancestral areas

Method or model	Type	Properties	Deficiencies	Examples of appropriate use	Refs
Parsimony	P[a]	Minimizes discrete-state changes over the tree; state changes can be weighted equally (Fitch) or differentially using step matrices	Cannot indicate probability of estimates; does not use branch lengths, thus underestimates change when it is frequent relative to speciation	Double fertilization, long thought to be a unique defining character of angiosperms, might have originated independently in gymnosperms, or earlier, in the first seed plants	[14,19, 41]
Dispersal vicariance analysis (DIVA)	P	Uses cost matrices to estimate ancestral areas; differentially costs vicariance and dispersal	Bias towards sympatric speciation and against early dispersal	Ancestral area reconstructions for oaks were more consistent with fossil record using DIVA than using Fitch parsimony or strict vicariance	[16,24]
Markov continuous time transition model	ML[b]	Estimates rate of discrete-state changes, allowing asymmetry	Evolutionary assumptions, e.g. that rate of change is constant throughout the tree, can be unrealistic (see punctuated models)	Overturned the parsimony estimate of origin of ruminant digestion in artiodactyls by taking rate of change into account	[21][c]
Punctuated evolution model	ML	Assumes evolutionary change occurs only at speciation	Evolutionary assumption is equivalent to having equal branch lengths and likely to be unrealistic, as in parsimony	For *Psychotria* plants in Hawaii, ancestral area inferences differ from both parsimony and ML models using differential branch lengths	[3,29]
Generalized least squares (=Squared change 'parsimony')	ML	Minimizes sum of squared changes over the tree; Brownian motion model of evolution; uses branch lengths	Does not indicate probability of estimates; non-directional version cannot reconstruct ancestral values outside range of descendant values	Size (area) of the first lower molar in mammals was reconstructed accurately, with verification from the fossil record	[18,28, 54]
Maximum likelihood with General time reversible model	ML	Based on Markov model, adapted from nucleotide modelling; allows differential rates and symmetry	Can fail if model unrealistic	Estimated ancestral areas, and rates and direction of dispersal of plants among Hawaiian islands	[3,55]
Stochastic (Bayesian inference), e.g. using Markov continuous time transition model	B[d]	Models multiple parameters including tree topology; posterior probabilities given for estimates	Can fail if model unrealistic	In contrast to parsimony, Bayesian analysis found multiple credible histories of gains and losses of horned soldiers in aphids	[26]

[a]Parsimony.
[b]Maximum likelihood.
[c]Implemented in Mesquite http://mesquiteproject.org.
[d]Bayesian inference.

unordered characters is presented below. It creates three sets of character states. The first set is called the downpass set of the node, because this is the set of states preferred by that part of the tree above the node. The second set is called uppass set and is created by uppass optimization. The uppass state set of a node is calculated from the uppass set of the node below it (its ancestor) with the downpass set of the node beside it (its sister node), using the same calculation for combining state sets as for the downpass. For the final state of each node, consider the uppass set of that node, and the downpass sets of its two descendant nodes. Choose the state that has the greatest number in all three sets. If none is in a majority, it remains ambiguous.

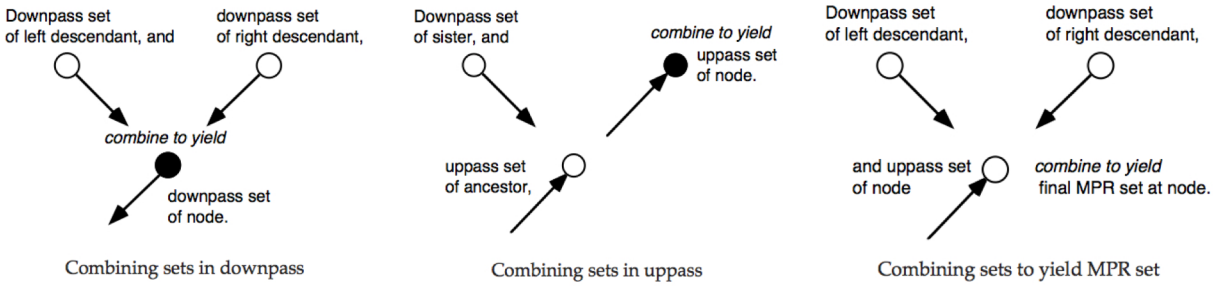


Figure 2: Parsimony reconstruction of ancestral states using Fitch algorithm

2.1.1 Reconstruction uncertainty

The first expression of uncertainty is ambiguity in the reconstruction or the existence of multiple equally parsimonious reconstructions. Ideally, when faced with multiple reconstructions, we should examine all of them. In reality, the number of possible reconstruction is often prohibitory large and only a few of them are examined/presented.

Two popular methods of choice are ACCTRAN and DELTRAN, which yield extremes of reversals versus parallelisms in the reconstruction.

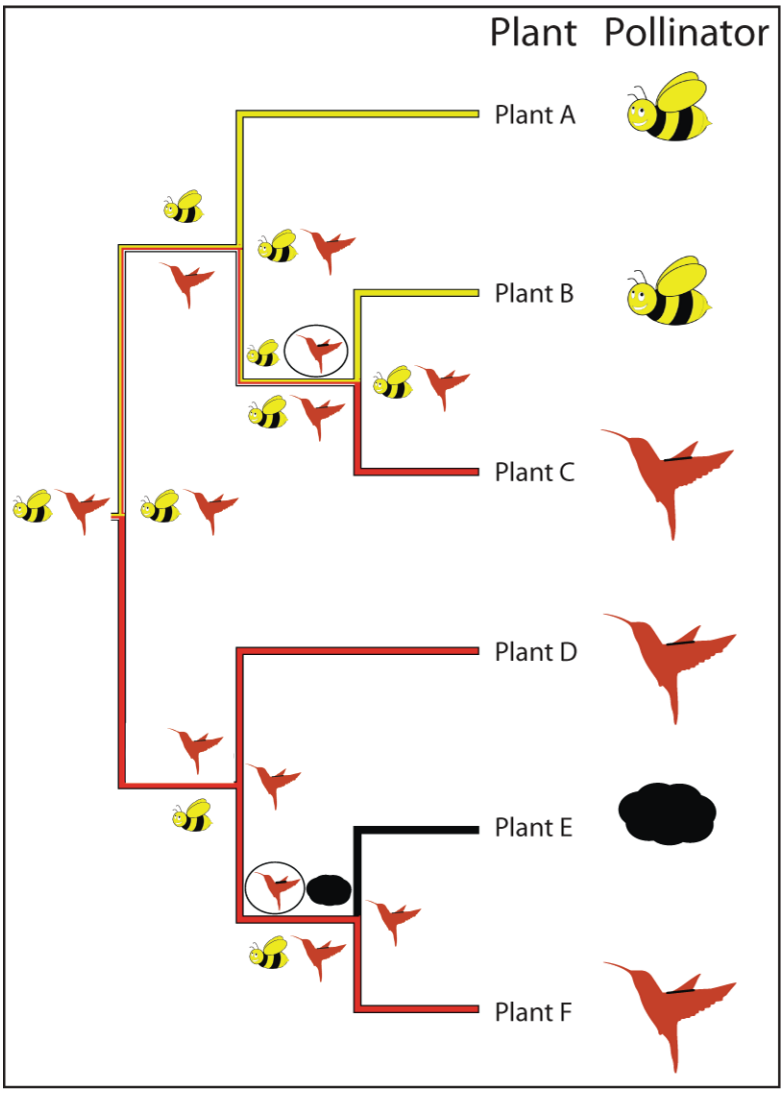


Figure 3: Parsimony reconstruction of the class example using Fitch algorithm without prior info on the root. Pictures on the left of each node show dowpass (above) and uppass(below) reconstructions. Pictures on the right of each node show final reconstruction.

2.1.2 ACCTRAN and DELTRAN

For characters of unordered and ordered type, ambiguities in character tracings can be resolved so as to choose the assignments that delay or accelerate transformations (look at the figure above and think what will happen to our reconstruction if we assign white/black color to node G). The DELTRAN option prefers most parsimonious assignments that delay changes away from the root; this maximizes parallel changes. The ACCTRAN tracing shows those assignments that accelerate changes toward the root; this procedure maximizes early gains and thus forces subsequent reversals. ACCTRAN and DELTRAN are but two of various methods to select from among the most parsimonious reconstructions.

Uncertainty does not end with ambiguity in the reconstruction. Even unequivocal assignment may be incorrect. Reconstructions of ancestral states are subject to error, as are all estimates of history. Studies of the reliability of ancestral state reconstructions have yielded mixed results, although it is clear that when rates of evolution are high over the time scale of the tree, error rates can be high. Also, the rate of change from one character state to another character state can be different (e.g., losses can be much easier than gains).

2.2 Probabilistic Methods (ML and Bayesian)

Hence the use of probabilistic methods (ML) provides several advantages: 1) Use an explicit model of character evolution 2) Consider branch length 3) Can estimate the relative probability of each character state at every node.

Many models have been developed to estimate ancestral states of discrete and continuous characters from phylogenetic data and character states of extant descendants. Such models use some well-understood random processes assume to model the evolution of a trait through time. For discrete-valued traits (such as "number of legs"), this random process is typically taken to be a Markov chain; for continuous-valued traits (such as "brain mass"), the process is frequently taken to be a Brownian motion or an Ornstein-Uhlenbeck process.

Discrete-state models If one wishes to recover the state of a given ancestral node in the phylogeny (call this node) by maximum likelihood, the procedure is: find the maximum likelihood estimate of substitution rates; then compute the likelihood of each possible state for given these rates; finally, choose the ancestral state which maximizes this. One may also use this substitution model as the basis for a Bayesian inference procedure, which would consider the posterior belief in the state of an ancestral node given some user-chosen prior. Because such models may have as many as k(k-1) parameters, overfitting may be an issue.

Binary state speciation and extinction model The binary state speciation and extinction model (BiSSE) is a discrete-space model that allows estimation of ancestral binary character states jointly with diversification rates associated with different character states; it may also be straightforwardly extended to a more general multiple discrete-state model. In its most basic form, this model involves 6 parameters: 2 speciation rates (one each for lineages in states 0 and 1); similarly, 2 extinction rates; and 2 rates of character change. This model allows for hypothesis testing on the rates of speciation/extinction/character change, at the cost of increasing the number of parameters.

Continuous-state models Although continuous traits can be split into discrete categories, it is more appropriate to model their evolution as some continuous process. In this case the likelihoods of transitions in state between adjacent nodes will be given by a continuous probability distribution such as Brownian motion. In this case, if nodes and are adjacent in the phylogeny (say is the ancestor of) and separated by a branch of length t, the likelihood of a transition from being in state x to being in state y is given by a Gaussian density with mean 0 and variance 2t. In this case, there is only one parameter (2), and the model assumes that the trait evolves freely without a bias toward increase or decrease, and that the rate of change is constant throughout the branches of the phylogenetic tree. Ornstein-Uhlenbeck process: in brief, an Ornstein-Uhlenbeck process is a continuous stochastic process that behaves like a Brownian motion, but attracted toward some central value, where the strength of the attraction increases with the distance from that value. This is useful for modelling scenarios where the trait is subject to stabilizing selection around a certain value (say 0). Under this model the above-described transition of being in state x to being in state y would have likelihood defined by the transition density of an Ornstein-Uhlenbeck process with two parameters: 2, which describes the variance of the driving Brownian motion, and , which describes the strength of its attraction to 0. As tends to 0, the process is less and less constrained by its attraction to 0 and the process becomes a Brownian motion. Because of this, the models may be nested, and log-likelihood ratio tests discerning which of the two models is appropriate may be carried out. Stable models of continuous character evolution: though Brownian motion is appealing and tractable as a model of continuous evolution, it does not permit non-neutrality in its basic form, nor does it provide for any variation in the rate of evolution over time. Instead, one may use a stable process, one whose values at fixed times are distributed as stable distributions, to model the evolution of traits. Stable processes, roughly speaking, behave as Brownian motions that also incorporate discontinuous jumps. This allows one to appropriately model scenarios in which short bursts of fast trait evolution are expected. In this setting, maximum likelihood methods are poorly suited due to a rugged likelihood surface and because the likelihood may be made arbitrarily large, so Bayesian methods are more appropriate.

Bayesian methods provide additional advantages 1) avoid potential errors in fixed parameter estimates 2) address phylogenetic uncertainty

Software: There are many software packages available, which can perform ancestral state reconstruction a selection is described below:

Within the R statistical language: APE implements a variety of ancestral state reconstruction methods for both discrete and continuous characters. Diversitree implements ancestral state reconstruction under Mk2 and BiSSE models.

HyPhy is a modular software package for hypothesis testing using phylogenies in a maximum likelihood framework. HyPhy implements a fast joint likelihood method of ancestral sequence reconstruction that can be readily adapted to reconstructing discrete ancestral character states or geographic ranges.

Mesquite implements parsimony, maximum likelihood, and Bayesian methods of ancestral state reconstruction for both discrete and continuous characters and has several display methods for resulting reconstructions.

Bayes Traits is a computer package which performs analyses on discrete or continuous characters in

a Bayesian framework and allows testing of hypotheses about models of evolution, ancestral states, and correlations among pairs of traits.

BEAST Also performs ancestral state and ancestral sequence reconstruction analyses.

Lagrange is an application which allows analyses reconstruction of geographic range evolution on phylogenetic trees available from http://www.reelab.net/home/software/.

Phylomapper implements a likelihood-based statistical framework for estimating historical patterns of gene flow and ancestral geographic locations. Available from: http://www.evotutor.org/LemmonLab/PhyloMapp