Modelling heterotachy in phylogenetic inference by reversible-jump Markov chain Monte Carlo

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The rate at which a given site in a gene sequence alignment evolves over time may vary. This phenomenon—known as heterotachy—can bias or distort phylogenetic trees inferred from models of sequence evolution that assume rates of evolution are constant. Here, we describe a phylogenetic mixture model designed to accommodate heterotachy. The method sums the likelihood of the data at each site over more than one set of branch lengths on the same tree topology. A branch-length set that is best for one site may differ from the branch-length set that is best for some other site, thereby allowing different sites to have different rates of change throughout the tree. Because rate variation may not be present in all branches, we use a reversible-jump Markov chain Monte Carlo algorithm to identify those branches in which reliable amounts of heterotachy occur. We implement the method in combination with our ‘pattern-heterogeneity’ mixture model, applying it to simulated data and five published datasets. We find that complex evolutionary signals of heterotachy are routinely present over and above variation in the rate or pattern of evolution across sites, that the reversible-jump method requires far fewer parameters than conventional mixture models to describe it, and serves to identify the regions of the tree in which heterotachy is most pronounced. The reversible-jump procedure also removes the need for a posteriori tests of ‘significance’ such as the Akaike or Bayesian information criterion tests, or Bayes factors. Heterotachy has important consequences for the correct reconstruction of phylogenies as well as for tests of hypotheses that rely on accurate branch-length information. These include molecular clocks, analyses of tempo and mode of evolution, comparative studies and ancestral state reconstruction. The model is available from the authors’ website, and can be used for the analysis of both nucleotide and morphological data.

Keywords: heterotachy; covarion; Markov chain Monte Carlo; mixture model; phylogeny

1. INTRODUCTION

These are times of plenty for molecular phylogenetics. By the spring of 2008 GenBank (www.ncbi.nlm.nih.gov) could boast over 80 million distinct gene sequences in its database. After a slow start in the early 1980s GenBank’s growth was catalysed by the discovery of the polymerase chain reaction and has been growing at an exponential or nearly exponential pace ever since, currently with a period doubling time of approximately 2–4 years. Many of these sequences are used in establishing the phylogenetic relationships among species and this is reflected in the growing use of phylogenies in biological research. The Web of Science database catalogued by the end of 2007 over 25 000 articles using or describing molecular phylogenies and this number is currently growing quadratically, increasing by over 3000 published articles per annum (figure 1; cf. Pagel 1999). Another growth phenomenon has, over this same time period, increasingly enabled computational biologists to exploit gene sequences on acceptable time scales. What has come to be known as Moore’s Law was the playful but shrewd suggestion by one of the co-founders of the Intel Corporation that computational power would raise exponentially throughout the decades of 1970s, 1980s, 1990s and now into the twenty-first century. Moore was proved right and the trend continues with no end currently in sight.

The confluence of these three trends means that investigators can, as never before, attempt to infer phylogenetic relationships among ever-greater numbers of species and using ever-greater numbers of genes. The increasing size and taxonomic range of gene-sequence alignments also means that the models of sequence evolution used to characterize these data must be up to the task of identifying a varied and potentially complex range of signals. Different sites may show different patterns and rates of evolution and these patterns and rates may vary among genes, regions of genes, between ribosomal and protein coding genes, spacers and introns or even repetitive DNA. One source of variation in gene-sequence alignments that has received relatively little attention in phylogenetic models is the phenomenon of heterotachy, despite being pointed out by Walter Fitch and colleagues (e.g. Fitch & Markowitz 1970; Fitch 1971) close to 40 years ago. Heterotachy refers to a site in a gene-sequence alignment having a different rate of evolution in different parts of the tree. Fitch supposed that
heterotachy could arise if evolutionary changes to one region of a gene made it more likely that other regions of the gene became less constrained. He called this the covarion hypothesis for ‘concomitantly variable codons’. Heterotachy, whether of the specific sort that Fitch envisioned or arising from other evolutionary considerations such as varying selective pressures, appears to be common in gene-sequence alignments (Lopez & Philippe 2002; Ane et al. 2005; Philippe et al. 2005; Taylor et al. 2006).

Tuffley & Steel (1998) proposed a simple and elegant model of covarion-like behaviour applicable to gene-sequence data. Their model supposes that a given site stochastically switches between being ‘on’ or ‘off’ throughout the tree. When ‘on’ a conventional Markov substitution process among nucleotides describes its evolution. When ‘off’ the site’s rate of evolution goes to zero. Switches are also described by a Markovian process and therefore can occur anywhere in the tree, including within a branch, giving rise to a variety of possible rates of evolution throughout the phylogeny. The model achieves this complexity with just two extra parameters over that of a conventional model of sequence evolution, those describing the rate at which sites switch from on to off and back again. Modifications to the original Tuffley and Steel model include allowing more than one category of on rates (Galtier 2001), variable on rates and an off state (Wang et al. 2007), a parametric distribution of rates in the on state (Huelsenbeck 2002), and allowing the covarion model itself to vary throughout the tree (Penny et al. 2001).

An alternative approach to accounting for heterotachy exchanges the simple parametric elegance of the covarion model for a model requiring more parameters, but not linked to parametric assumptions about the degree or distribution of heterotachy throughout the tree. Consider two sites, one of which evolves at a more or less constant rate throughout the tree and another whose rate of evolution accelerates sporadically in one or more regions. Given a common underlying model of sequence evolution, the differing behaviour of these two sites can be captured by assigning them different branch lengths on a shared topology.

Generalizing this approach across all sites, a model emerges in which some number of extra branch-length sets is sufficient to describe the heterotachy in the data. It is unlikely that the information about which branch-length sets correspond to which site or sites would be known in advance, but an approach to statistical inference known as mixture models removes the need to know this.

Mixture models, as applied to phylogenetic inference, sum the likelihood at each site of the alignment over more than one model of evolution (e.g. Koshi & Goldstein 1998; Huelsenbeck & Nielsen 1999; Lartillot & Philippe 2004; Pagel & Meade 2004; Blackburne et al. 2008). For example, we (Pagel & Meade 2004, 2005) reported a mixture model for characterizing what we called ‘pattern heterogeneity’ in the evolution of nucleotide sequences. This model fits more than one model of gene-sequence evolution to an alignment, without specifying in advance how many models there will be or to which sites they best correspond. Instead, the method finds the optimal number of models and sums the likelihood at each site over all of them, weighted by their prior probabilities. At the same time, Lartillot & Philippe (2004) reported a similar mixture model for protein sequence data. Both models routinely return large improvements in likelihood, and reduce long-branch attraction. They also avoid the problem of assigning sites to partitions and have been shown to improve on partitioned likelihoods, despite requiring no advance information from the user, as well as reduce the so-called ‘node-density’ artefacts (Venditti et al. 2008).

Applied to the problem of heterotachy, a branch-length set mixture model will seek to find some optimal number of extra branch-length sets, summing the likelihood at each site over all of them. Kolaczkowski & Thornton (2004) presented a model with two branch-length sets but calculated the likelihood incorrectly (Spencer et al. 2005) and their model was only applicable to data in which it was known in advance which branch-length set best characterized a given site. In more recent papers, these same authors, others and we report more fully developed branch-length sets mixture models for heterotachy applicable to phylogenetic inference (Zhou et al. 2007; Meade & Pagel 2008; Kolaczkowski & Thornton 2008).

An unwanted feature of the multiple branch-length set approaches is the potentially large number of extra parameters required to describe the heterotachy. Each extra branch-length set requires $2s - 3$ additional parameters (for an unrooted tree) where $s$ is the number of species or taxa in the alignment, plus an empirical estimated weighting component. Even for a modest tree of 40 species this means estimating $77 + 1$ additional parameters just to accommodate one extra branch-length set. Zhou et al. (2007) suggested that, given the burden of these extra parameters, the mixture model may, despite its better absolute likelihood, not be favoured over the simpler covarion model. These authors report that the relatively lenient Akaike information criterion (AIC) may prefer the branch lengths mixture model to the covarion (Akaie 1974; Felsenstein 2004) but that the more stringent Bayesian information criterion (BIC; Schwarz 1978;
Felsenstein 2004) prefers the covarion. We have found similar results in five published nucleotide alignments—the mixture model with extra branch-length sets is often favoured under the AIC but is far less likely to be supported using the BIC (Meade & Pagel 2008).

Our goal here is to describe and evaluate a mixture-model approach to accounting for heterotachy that potentially reduces the number of extra parameters required to explain the data. Our approach is motivated by the belief that heterotachy is a common source of variation in gene-sequence data, but that it may be confined to only a small number of sites or to small regions of the tree. If either of these situations is true, then many of the branches in the tree will not require an additional branch length. Our approach considers an extra branch-length set but then tests pairs of branches corresponding to the same edge of the phylogenetic tree to see whether they can be collapsed into a single branch, or if two additional branch-length sets are considered, to see whether three branches can be collapsed to two, and so on. At the same time, the model continually proposes adding a branch length if one is found inadequate to explain the data, and performs these actions while exploring different tree topologies.

We implement our model using Markov chain Monte Carlo (MCMC; Gilks et al. 1996; Gelman 2003) methods. MCMC methods employ a Markov chain to explore the potential universe of models of evolution that might describe the data. Most implementations of MCMC methods explore models of a fixed number of dimensions, such as the parameters of a model of gene-sequence evolution. However, a variant of MCMC known as reversible-jump MCMC (RJMCMC; Green 1995) allows the Markov chain to move among models of different dimensionality. Boys & Henderson (2001) and Helsenbeck et al. (2004) used the reversible-jump approach to explore models of sequence evolution, Suchard et al. (2001) used it to choose among alternative phylogenetic topologies, Pagel & Meade (2006) used it in a model of correlated evolution of binary traits, and we also use the RJMCMC approach in our model of pattern heterogeneity to determine the number of extra models of gene-sequence evolution (available in BayesPhylogenies package, www.evolution.reading.ac.uk). Here, we adopt the reversible-jump approach in a multiple branch-length sets mixture model for heterotachy to identify how many extra parameters—corresponding to distinct additional branch lengths—are needed to explain the data. Our hope is that this RJMCMC algorithm can return substantial improvements to the likelihood but often with far fewer parameters than simply force-fitting an entire extra branch-length set. We implement this reversible-jump heterotachy model in combination with our earlier model of pattern heterogeneity (Pagel & Meade 2004). This means that the heterotachy that our model identifies is that which exists over and above any tendency for different sites to adopt different patterns or rates of evolution.

In what follows we describe the model in more detail, then apply it to simulated and real datasets. It is not our goal to provide a general evaluation of branch-length mixture models for heterotachy as we and others have already reported results from simulated and real data. Our primary interests are to evaluate the reversible-jump procedure as a way of overcoming the burden of extra parameters, and as a method for identifying where in the tree and for which sites or genes in the alignment rates of evolution have been accelerated or slowed.

2. REVERSIBLE-JUMP BRANCH-LENGTH SETS MIXTURE MODEL

We define the likelihood of a model of gene-sequence evolution as an amount proportional to the probability of the data given the model of sequence evolution and a phylogenetic tree

\[ L(Q) \propto P(D|Q, T), \tag{2.1} \]

where \( D \) will normally be an aligned set of sequence data; \( Q \) is the familiar substitution rate matrix that defines the model of evolution; and \( T \) is the phylogenetic tree. In the case of nucleotide data, \( Q \) is a 4 x 4 matrix of transition rates among A, C, G and T (Swofford et al. 1996). For protein data \( Q \) is a 20 x 20 matrix representing the transition rates among all pairs of amino acids.

Given an aligned set of gene-sequence or other character-state data, the probability of the data in \( D \) is found as the product over all of the sites of the individual probabilities of each site.

\[ P(D|Q, T) = \prod_i P(D_i|Q, T). \tag{2.2} \]

A mixture model for additional branch-length sets modifies this basic framework by including more than one set of branches for any given tree topology \( T \). The branching structures (topology) of the trees are fixed while independent branch lengths, represented as a vector \( t \), are free to vary. The probability of the data is now calculated by summing the likelihood at each site over all of the different \( t \) for a given tree. Thus, defining the branch-length sets as \( t_{12}, t_{23}, \ldots, t_{\text{Jlength}} \), the probability of the data under the mixture model is

\[ P(D|t_1, t_2, \ldots, t_J, Q, T) = \prod_i \sum_j w_j P(D_i|t_j, Q, T), \tag{2.3} \]

where the summation over \( j \) now specifies that the likelihood of the data at each site is summed over \( J \) separate branch-length sets, the summation being weighted by the \( w_j \) where \( w_1 + w_2 + \cdots + w_J = 1.0 \) and represents our prior beliefs about the suitability of a set of branches for a given site. The number of branch-length sets, \( J \), can be determined either by prior knowledge of how many different patterns are expected in the data, or they can be empirically estimated, along with the prior weights, from the data. Equation (2.3) describes the model that Zhou et al. (2007), Meade & Pagel (2008) and Kolaczkowski & Thornton (2008) use.

Although our primary interest is in heterotachy we need to digress from equation (2.3) for a moment to describe the wider context of the heterotachy mixture model. Nothing in equation (2.3) describes the variation that one might expect among sites in the alignment, such as the well-known gamma rate heterogeneity
(Yang 1994) or the pattern heterogeneity that we
describe elsewhere (Pagel & Meade 2004). But there
is every reason to expect that these across-site sources of
variation will exist alongside the within-site variation in
rates that models of heterotachy are designed to
describe, and so a complete model should account for
both. Failure to do this will not just mis-characterize the
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It is straightforward to rewrite equation (2.3) to
accommodate both pattern heterogeneity and hetero-
tachy. We now consider a model in which we sum the
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lengths into one. Whenever a split or merge move is proposed the resulting change to the likelihood is either accepted or rejected following the acceptance rule described above. Over large numbers of iterations these moves will settle on a stationary distribution of the number of extra branch lengths that are required to explain the data.

To incorporate the split and merge moves into the RJMCMC algorithm requires carefully constructed proposal mechanisms and calculation of the Jacobian term. We present details of these mechanisms, the proposal ratio and the Jacobian terms in the electronic supplementary materials.

3. PRIORS AND RUNNING THE MARKOV CHAIN
The Markov chain requires specification of the prior distributions of the parameters of the model. We used uniform priors on the interval 0–100 for all parameters of the models of sequence evolution, uniform (0–1) priors on the empirical weights of the mixture models, and exponential (10) priors on the branch lengths for changes that are proposed independently of splitting or merging. Trees are given a uniform prior. We normally ran at least four independent chains for each dataset to check that they converged to the same region of the parameter space. Chains were allowed to reach an apparent steady state (this being operationally defined as a chain whose mean likelihood remains unchanged over many iterations) and then they were run at least 10 million further iterations. We sampled from ‘converged’ chains every 10 000 iterations retrieving at least 1000 trees for purposes of drawing inferences.

Log-likelihoods reported below are the logarithms of the harmonic means of the posterior distribution of likelihoods.

4. APPLICATION TO SIMULATED DATA
We generated a random topology of 70 taxa, and then drew two random sets of branch lengths from a uniform distribution ranging between 0 and 0.2 (figure 2, inset shows the two sets of branch lengths are uncorrelated). We generated 1500 simulated nucleotides for each
branch-length set, using *Seqgen* (Rambaut & Grassly 1997) and a common general-time-reversible (GTR) model of sequence evolution (Swoford et al. 1996). We combined the alignments from the two randomly varying branch-length sets into a single alignment of 3000 simulated nucleotides.

We analysed the simulated alignment using a conventional GTR likelihood model with one set of branches, and also with the heterotachy mixture model fitting two full branch-length sets, and finally using the reversible-jump algorithm.

5. APPLICATION TO PUBLISHED DATA

We applied the model to the five published datasets described in table 1, including algae, plants, nematode worms and salamanders. The alignments include nuclear and mitochondrial genes of both ribosomal and protein coding functions. Each alignment contains at least two genes, and no fewer than 3600 nucleotides, but the number of taxa ranges from just 14 to 119 (all datasets available from TreeBASE, Piel et al. 2002). In our previous study of heterotachy (Meade & Pagel 2008), we found that each of these datasets could be described by two branch-length sets, save for the Plathodontids for which the AIC supported three branch-length sets, but a BIC supports two. Accordingly, we study the behaviour of the reversible-jump mixture model for two branch-length sets for each alignment.

6. RESULTS

(a) Simulated data

Table 2 shows the mean log-likelihoods for the conventional model with one branch-length set, and the improvement in likelihood from the two branch-length set mixture models and the reversible-jump mixture model. The addition of a second branch-length set, as expected, accounts for a large improvement in the log likelihood (significant by both AIC and BIC). The mixture model is able accurately to estimate the additional branches: the correlation between the additional branches: the correlation between the improvement in the log likelihood (significant by both AIC and BIC).

(b) Real datasets

In real datasets, we might expect a range of degrees of heterotachy, from it being confined to a small number of branches up to including nearly every branch in the tree. Especially when it is confined to a small number of branches, the reversible-jump mixture model should be more sensitive to detecting these effects.

Table 3 reports the mean log likelihoods for the conventional one branch-length set model as applied to the five published datasets, and the improvement in likelihood from the two branch-length sets and reversible-jump mixture models. The results from the reversible-jump model explain why the AIC often supports two full branch-length sets in the conventional mixture model but the BIC does not. The last column of table 3 shows that the proportion of branches showing evidence of heterotachy ranges from just 5 to approximately 60%. For the Chlorophyceae the full branch-length sets mixture model is forced to fit 74 branches as opposed to just 4 ± 3 for the reversible-jump model. The message here is that real datasets can be expected to vary greatly in the amount of heterotachy they show and in how many branches in the tree are affected. It is not necessary to test the
reversible-jump results further: the mean of the posterior distributions of extra branches directly estimates how much heterotachy is in the tree. Nevertheless, each of the reversible-jump results would be judged significant using the more stringent BIC as applied to their likelihood improvement and numbers of additional branches required.

The reversible-jump algorithm usefully pinpoints the positions in the tree most affected by heterotachy. Figure 5 shows the consensus tree derived from the posterior distribution of trees for the 119 species in the Gnetales alignment (table 1), pruned to make it easier to view. The Gnetales are a small group of seed plants that may form a sister group to the angiosperms. The branch lengths of the consensus tree are, in the case of edges with more than one length, the weighted averages of the two branches found for that edge. The reversible-jump algorithm settles on an average of 44.5 extra branches (table 3) and those with a posterior probability greater than 0.5 of having more than one length are coloured in red. This reveals a concentration of heterotachy in the clade in the lower portion of the tree, corresponding to lycopods, ferns and equisetum. The inset shows the two very different sets of branch lengths the reversible-jump algorithm finds for this clade. Meade & Pagel (2008) show that this heterotachy is associated principally with acceleration in the evolution of the rbcl and atpB protein coding genes in this clade (lower sub-clade), genes associated with ATP synthesis and photosynthesis. By comparison, the two ribosomal genes evolve slowly in this clade (upper sub-clade) and in general have a more uniform rate throughout the tree.

7. DISCUSSION
Our results show that a reversible-jump mixture model can identify heterotachy in gene-sequence alignments and often with far fewer parameters than conventional mixture-models that rely on additional complete branch-length sets (Zhou et al. 2007; Meade & Pagel 2008; Kolaczkowski & Thornton 2008). It is worth bearing in mind that our results with real datasets record the amount of heterotachy over and above any improvements in likelihood that could be attributed to pattern heterogeneity (Pagel & Meade 2004) or rate variation among sites. To our knowledge, ours is the first demonstration of the effects of heterotachy independently of these other sources of variation. The results in table 3, therefore, reinforce earlier suggestions that heterotachy is an important source of variation in real data.

Table 3. Results for real datasets. (BLS, branch-length set; RJ, reversible jump.)
The strength of the mixture-model approach to heterotachy, of using multiple branch-length sets, is that it makes no a priori assumptions about the distribution or occurrence of heterotachy throughout the tree. Rather, the mixture model acts like a non-parametric covarion, the individual branch-length sets each providing a unique and potentially idiosyncratic description of the heterotachy in the tree. But as the reversible-jump algorithm exposes, potentially many of the parameters in the additional branch-length sets are redundant: they may correspond to edges in the tree for which no heterotachy is present. The reversible-jump algorithm solves the problem of redundancy by assigning multiple lengths only to those edges for which the data justify more than one length. In just the few datasets we investigated, the proportion of edges requiring more than one length fell as low as 5 per cent and was never more than 60 per cent.

The figure of 60 per cent, if representative of the upper values we might routinely expect of real datasets, makes the clear statement that, on average, the majority of the parameters that the conventional multiple branch-length sets mixture models estimate are not needed! Zhou et al. (2007), for example, reported correlations of approximately 0.60 between the branch lengths in the two branch-length sets they fitted to one of their datasets, indicative of much redundant information between the two sets. By comparison for the Gnetales data of figure 5, the correlation between the edges that the reversible-jump algorithm identifies as requiring two distinct lengths is −0.11. In the datasets, we examined the average number of extra branches required was just 24 per cent of those used in a complete additional set of branches, a reduction of approximately 75 per cent in the amount of estimation required. We do not think this low figure arises from a lack of power to detect additional branches, but rather points up just how much redundancy there is in the full branch-length set models. Even in our simulated data, we were able to account for 92 per cent of the likelihood supplied by an additional branch-length set, but using roughly half the parameters that the additional set required. These data further suggested that the reversible-jump algorithm was able to assign two lengths to an edge when the true simulated lengths differed by approximately 0.04 units of expected nucleotide substitutions.

The reversible-jump approach may help to manage some of the awkward problems of inference that arise when quasi-frequentist ideas of hypothesis testing are applied to Bayesian problems. Zhou et al. (2007) highlighted the very different outcomes that can arise when the relatively lenient Akaike and more stringent Bayesian information criterion tests are used to select among various mixture models of heterotachy. The reversible-jump algorithm produces a direct estimate of the posterior distribution of extra branches required in the mixture model. The mean of that distribution is a measure of one’s posterior confidence in the existence of heterotachy in the data: if the mean is zero one can, other things being equal, be reasonably confident that heterotachy is not affecting the data and as it moves away from zero this provides evidence of more and more heterotachy. In this light, it is interesting that in our real data examples, the AIC always supported a complete additional branch-length set but the BIC does only for one of them (the Caenorhabditis). With no good way to choose between these two criteria, it is difficult to draw any conclusions. However, the BIC always supports the request for the smaller number of extra branches derived from the reversible-jump algorithm. The message is not that one should apply the BIC to test the results derived from the reversible-jump algorithm, rather, that the reversible-jump algorithm on its own is doing its job.

Apart from methodological considerations, an attractive feature of the reversible-jump approach is that it directly highlights the regions of the tree—and thus the taxa and their ancestors—whose evolution has been characterized by changes to rates of evolution. It does this by providing an estimate for each edge of the tree of the posterior belief in how many distinct lengths are needed to describe the evolution along those edges. Along with identifying the taxa involved, it is also straightforward to query the alignment itself to discover which sites are most directly implicated in the heterotachy. For the Gnetales data, this combination of information allows us to say directly that two protein coding genes involved in energy transfer (atpB and rbcl) have greatly accelerated their rate of evolution in the lycops, ferns and equisetum. Put together, what the model is discovering are taxa–gene interactions in the rate of evolution. This kind of information should prove valuable for understanding the selective forces acting on genes and for reconstructing the history of protein evolution in particular groups.

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