Generalized Mixture Models for Molecular Phylogenetic Estimation

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Abstract.—The rapidly growing availability of multigene sequence data during the past decade has enabled phylogeny estimation at phylogenomic scales. However, dealing with evolutionary process heterogeneity across the genome becomes increasingly challenging. Here we develop a mixture model approach that uses reversible jump Markov chain Monte Carlo (MCMC) estimation to permit as many distinct models as the data require. Each additional model considered may be a fully parametrized general time-reversible model or any of its special cases. Furthermore, we expand the usual proposal mechanisms for topology changes to permit hard polytomies (i.e., zero-length internal branches). This new approach is implemented in the Crux software toolkit. We demonstrate the feasibility of using reversible jump MCMC on mixture models by reexamining a well-known 44-taxon mammalian data set comprising 22 concatenated genes. We are able to reproduce the results of the original analysis (with respect to bipartition support) when we make identical assumptions, but when we allow for polytomies and/or use data-driven mixture model estimation, we infer much lower bipartition support values for several key bipartitions. [Bayesian phylogenetic inference; mixture models; model selection; polytomous trees; reversible jump Markov chain Monte-Carlo.]

Recent high-throughput genetic sequencing technology advances have increasingly enabled researchers to pursue multilocus phylogenetic analyses (e.g., Murphy et al. 2001; Rokas et al. 2003; Kjer and Honeycutt 2007; Prasad et al. 2008). However, this presents the need to account for heterogeneity in the processes of molecular evolution. It is well known that violating model assumptions can introduce systematic error into phylogeny inference even for single loci (e.g., Sullivan and Swofford 1997). This problem obviously extends to multilocus data sets (e.g., Mossel and Vigoda 2005), which has lead to the common practice of partitioning multilocus data sets (Kjer and Honeycutt 2007; Frajman et al. 2009) wherein a separate model of evolution is applied to each gene, or even a separate model for each codon position within each gene. Unfortunately, uncritical data partitioning can conflict with the goal of increasing inferential informativeness because it increases the number of parameters that must be estimated. One automated approach to the partitioning problem is to apply Dirichlet process priors (e.g., Huelsenbeck and Suchard 2007). In this paper, we instead accommodate process heterogeneity by focusing on mixture models in conjunction with reversible jump Markov chain Monte Carlo (MCMC) methods (Metropolis et al. 1953; Hastings 1970; Green 2003).

The general time-reversible (GTR) model (Yang 1994a) is the basis for most commonly used models of molecular evolution. As applied to nucleotide sequences, the GTR model includes four base frequency parameters (π_A, π_C, π_G, π_T), and six relative mutation rate parameters (α, β, γ, δ, ε, η), which are used to compose a stochastic Q matrix of the form

\[
\begin{pmatrix}
A & C & G & T \\
A & π_A & π_{CA} & π_{GA} & π_{TA} \\
C & π_{AC} & π_C & π_{GC} & π_{TC} \\
G & π_{AG} & π_{CG} & π_G & π_{TG} \\
T & π_{AT} & π_{GT} & π_{TG} & π_T
\end{pmatrix}
\]

The diagonal terms are set such that each row sums to zero, and the entire matrix is scaled such that branch lengths represent mean substitutions per site. The parameter richness of the GTR model can be reduced by constraining subsets of the relative mutation rates to equal each other or by applying fixed base frequencies; this results in 203 special cases (Huelsenbeck et al. 2004). The GTR model is also commonly extended to account for relative mutation rate variation among sites using two separate but complementary methods. The more flexible method, GTR+Γ, uses a mixture of (commonly) four evenly weighted rate categories with Q matrices that are identical except for relative mutation rate multipliers. These multipliers are chosen to conform to a discrete Γ distribution with empirically estimated shape that is normalized to a mean value of 1 so that branch lengths are still scaled to represent mean substitutions per site (Yang 1994b). The computationally simpler method, GTR+I, uses an empirically weighted mixture of model components in which one Q matrix is specific to invariant sites, that is, all the relative mutation rates are zero. Models that incorporate both of these methods are referred to as GTR+1+Γ.

Recently, researchers have started applying less constrained mixture models to phylogenetic inference (Pagel and Meade 2004; Lartillot and Philippe 2004, Venditti et al. 2008). In the general case, these mixtures consist of empirically weighted independent Q matrices, whereas the GTR+1+Γ models are constrained to...
use closely related Q matrices. Pagel and Meade (2004) utilized this relationship to compare the effectiveness of GTR+Γ models versus mixture models with independent relative mutation rates among Q matrices, but one set of base frequency parameters. They used their BayesPhylogenies program to show that mixture models are effective for analyzing concatenated multi-gene data sets.

In the remainder of this paper, we provide a brief introduction to Bayesian MCMC methods, then return to describing our generalized mixture model methods and our approach to treating the number of Q matrices in a mixture as a random variable. In addition, we provide a generalization of the extending tree bicorrelation and reconnection (eTBR) tree transformation that allows for polytomous trees. We apply each of these approaches separately and in combination to reanalyze the 44-taxan mammalian data set originally analyzed by Murphy et al. (2001), and later reanalyzed by Pagel and Meade (2005) using mixture models. We also provide insight into the inferential effectiveness of mixture models via secondary experiments that (i) vary the Bayesian prior for mixture model reversible jumps, and (ii) fix the number of Q matrices.

**Bayesian MCMC**

We make extensive use of Bayesian MCMC methods (Metropolis et al. 1953; Hastings 1970), and in particular the reversible jump methods of Green (2003); a brief introduction is included here in order to make concepts, terminology, and notation clear before applying them to novel MCMC methods. For MCMC-based molecular phylogenetic inference, each sample in a Markov chain is a super parameter τ that includes tree topology, branch lengths, relative mutation rates, and so forth. Each proposed state τ′ is based on τ, and is accepted with probability α_m(τ, τ′) according to the proposal ratio

\[ \alpha_m(\tau, \tau') = \min \left\{ 1, \frac{L(\tau')\pi(\tau')}{L(\tau)\pi(\tau)} \cdot \frac{j_m(\tau')}{j_m(\tau)} \cdot \frac{g_m(\tau')}{g_m(\tau)} \cdot \left| \frac{\partial(\tau', \tau)}{\partial(\tau, \tau)} \right| \right\} \quad (1) \]

where \(L(\tau)\pi(\tau)\) is the likelihood of state τ times its prior probability, \(j_m(\tau)\) is the probability of choosing move \(m\) when in state \(\tau\), \(g_m(\tau)\) is the density transformation for the vector \(u\) of random variables, and \(\left| \frac{\partial(\tau', \tau)}{\partial(\tau, \tau)} \right|\) is the absolute value of the Jacobian that accounts for change of variables from \((\tau, u)\) to \((\tau', u')\). If the proposed state change is rejected, then the current τ is preserved, which results in sequential chain samples that are identical. In the limit, the Markov chain converges on the stationary distribution.

Note that in the context of Metropolis coupling (Altekar et al. 2004), some terms in the proposal ratio for heated chains are exponentiated

\[ \alpha_m(\tau, \tau') = \min \left\{ 1, \left[ \frac{L(\tau')\pi(\tau')}{L(\tau)\pi(\tau)} \right]^{\text{heat}} \cdot \frac{j_m(\tau')}{j_m(\tau)} \cdot \frac{g_m(\tau')}{g_m(\tau)} \cdot \left| \frac{\partial(\tau', \tau)}{\partial(\tau, \tau)} \right| \right\} \quad (2) \]

The following derivations include intermediate factored expressions that can be adapted in a straightforward fashion for use with Metropolis coupling. However, even though Crux (Evans 2009), our implementation, employs Metropolis coupling, we omit those details from the derivations in order to simplify the exposition.

It is possible for one proposal type to explicitly enable/disable other proposal types (e.g., state frequency proposals are irrelevant if all Q matrices use fixed state frequencies), and although Crux accounts for these proposal interactions, we omit the interactions in the following proposal descriptions because they are particular to the combination of proposals in Crux.

**Modifying Exponentially Distributed Parameters**

As described by Lakner et al. (2008), for each exponentially distributed parameter change \(\rho' = \rho x\), we generate a multiplier \(x = e^{\lambda(u-0.5)}\), where \(\lambda\) is a tuning parameter and \(u\) is a uniform \([0, 1]\) random variable. This leads to the density transformation \(g_m(x) = 1/(\lambda x)\). To reverse a proposal, the inverse multiplier, \(x' = 1/x\) must be randomly drawn. Therefore, the Jacobian is

\[ J = \begin{vmatrix} \frac{\partial \rho'}{\partial x} & \frac{\partial \rho}{\partial x} \\ \frac{\partial \rho'}{\partial \xi} & \frac{\partial \rho}{\partial \xi} \end{vmatrix} = \begin{vmatrix} \frac{\partial \rho}{\partial x} & \frac{\partial \rho}{\partial \xi} \\ \frac{\partial \rho}{\partial \xi} & \frac{\partial \rho}{\partial x} \end{vmatrix} = \left| \begin{array}{cc} x & \rho \\ -1 & -\frac{1}{\lambda^2} \end{array} \right| = \frac{-x}{\lambda^2} \quad (3) \]

so \(|J| = 1/x\). The resulting proposal contribution of \(\rho'\) is

\[ \pi(\rho') \cdot j_m(\rho') \cdot g_m(x) \cdot \left| \frac{\partial(\rho', x')}{\partial(\rho, x)} \right| = \frac{e^{-\lambda\rho'}}{e^{-\lambda\rho}} \cdot 1 \cdot \frac{1}{\lambda^2} \cdot \frac{1}{x} = e^{-\lambda\rho(x-1)} x. \quad (4) \]

**Generating Exponentially Distributed Parameters**

All parameters that are added/removed by the model jump proposals described below are exponentially distributed, and the same procedure can be used for independently computing the contribution of each parameter to the proposal ratio. To draw a parameter \(\rho' = 1/\theta\rho\), we use an auxiliary variable to draw random numbers from \(x \sim \text{Exp}(1)\), where \(u \sim \text{Unif}(0, 1)\) is easily computer generated, \(x = -\ln(1-u)\). The density transformation is \(g_m(x) = e^{-x}\). The prior density for \(\rho'\) is

\[ \pi(\rho') = \theta \rho e^{-\theta \rho} = \theta \rho e^{-\theta \rho(x)} = \theta \rho e^{-x}. \quad (5) \]

The Jacobian that accounts for change of variables from \(x\) to \(\rho'\) is

\[ \frac{\partial \rho'}{\partial x} = \frac{\partial}{\partial x} \left( \frac{1}{\theta \rho} \right) = \frac{1}{\theta \rho^2}. \quad (6) \]
The resulting proposal ratio contribution of $\rho'$ is

$$\frac{\pi(\rho')}{\pi(\rho)} \cdot \frac{j_m(\rho')}{j_m(\rho)} \cdot \frac{g_m'(x')}{g_m(x)} \left| \frac{\partial x'}{\partial \rho} \right| = \theta_p e^{-x} \cdot 1 \cdot \frac{1}{\theta_p} = 1. \quad (7)$$

To remove a parameter $\rho$, we must reverse the process in a way consistent with how $\rho$ was introduced. Therefore, $x' = \theta_p \rho$, and the density transformation on $x'$ is $g_m(x') = e^{-x} \theta_p$. The Jacobian that accounts for change of variables from $\rho$ to $x'$ is

$$\frac{\partial x'}{\partial \rho} = \frac{\partial}{\partial \rho} \theta_p \rho = \theta_p. \quad (8)$$

The resulting proposal ratio contribution of $x'$ is

$$\frac{\pi(\rho')}{\pi(\rho)} \cdot \frac{j_m(\rho')}{j_m(\rho)} \cdot \frac{g_m'(x')}{g_m(x)} \left| \frac{\partial x'}{\partial \rho} \right| = \frac{1}{\theta_p} e^{-\theta_p \rho} \cdot 1 \cdot \frac{1}{\theta_p} = 1. \quad (9)$$

**Mixture Models**

Mixture models are compelling for multigene analyses because a priori site partitioning is unnecessary; all mixture components apply to all sites according to the mixture component weights, and the weight parameters can be estimated to fit the data. This approach is conceptually different than site partitioning for which a separate model is applied to each partition, but it tends to work well because mixture components typically contribute very little to per site likelihood except when there is a reasonable fit between site history and component parameterization. Consequently, it is quite possible for a mixture model to adequately account for the variability of a multigene data set using fewer parameters than are in a correspondingly adequate partitioned model.

Our mixture models differ from those used in BayesPhylogenies by Pagel and Meade (2004) in two key ways. First, each Q matrix has its own set of base frequencies, rather than sharing a single set across all Q matrices. Second, each Q matrix incorporates a relative rate multiplier, $s_Q$, which can be thought of as a fixed scalar that affects the mutation rate for a Q matrix as a whole. The scalar $s_Q$ is needed because in the context of MCMC, the relative rate parameters have exponentially distributed priors, all with the same expected value. In the absence of $s_Q$ parameters, the relative rates prior effectively posits that all models in the mixture have correlated mutation rates (i.e., there are no slow or fast models within the mixture), and we want the prior to allow for a mixture of fast and slow models. By applying an exponentially distributed prior to $s_Q$, each Q matrix effectively has rates that are independent of all other Q matrices. The mixture as a whole is scaled such that branch lengths represent mean substitutions per site, so the only effect of $s_Q$ is to allow Q matrices to vary independently of each other.

The problem of choosing in advance how many mixture model components to use (i.e., mixture degree, denoted as $d(M)$) is similarly vexing to the site partitioning problem, but the simpler structure of mixture models allows for an automated solution. It is possible to use reversible jump MCMC methods (Green 1995) to sample among models of differing dimensionality, such as mixture models with differing numbers of components. Venditti et al. (2008) made use of reversible jump MCMC for mixture models, but their algorithm for jumping between mixture models of varying degree is specific to unconstrained relative mutation rate parameters (personal communication). The Crux software toolkit, which we used for our experiments, also samples from the 203 relative mutation rate parameter constraint cases of the GTR model (Huelsenbeck et al. 2004), so we developed novel reversible jump proposals for mixture models, such that the mixture degree is treated as a random variable.

**Mixture Model Jumps**

We refer to proposals for adding/removing a Q matrix to/from mixture M of degree $d(M)$ as $M+$ and $M−$ proposals, respectively. The $M+$ proposal is the primary challenge because it involves the addition of up to 12 parameters in a single step: the mixture weight $w_Q$, one to six rate class rates $r_Q$, $i \in \{1, 2, \ldots, 6\}$, the rate multiplier $s_Q$, and zero or four base frequencies \{\pi_A, \pi_C, \pi_G, \pi_T\}. $w_Q \sim \text{Exp}(1)$, $r_Q \sim \text{Exp}(1)$, $s_Q \sim \text{Exp}(1)$, and $\pi_{(A,C,G,T)} \sim \text{Dirichlet}(1,1,1,1)$, but we must also determine the number of rate classes according to the rate class resolution prior $C_R$. The rate class jump proposals (Huelsenbeck et al. 2004) only increment/decrement the number of rate classes within a Q matrix, and because MCMC allows for arbitrary starting points (not necessarily drawn from the prior), it would be acceptable to always start an MCMC run with a single Q matrix and a fully resolved rate class. However, adding a Q matrix during an MCMC run requires that all possible Q matrices be drawn with some probability in order to make $M+$ proposals balance with $M−$ proposals that can remove any possible Q matrix from the mixture. To simplify the math, we choose among all possible rate classes in a manner consistent with $C_R$. The prior probability of resolution class $R$ is defined as

$$\pi(R = x) = \frac{1}{C_R} \left[ \frac{1}{C_R} \right]^6, \quad (10)$$

where $x \sim \text{Uniform}(1,2,\ldots,6)$. Because we had to solve the problem of drawing Q matrices from the prior distribution for the $M+$ proposal, we modified Crux to start each MCMC run by drawing $d(M)$ and all Q matrices from the prior.

The prior, $\pi(d(M))$ is assumed to be geometrically distributed, therefore $P(d(M) = k) = (1 - p_M)^k p_M$, where $p_M$ is a fixed prior probability. This leads to the prior ratios for $d(M)$:

$$\frac{\pi_{M+}}{\pi_M} = \frac{(1 - p_M)^{k+1} p_M}{(1 - p_M)^k p_M} = 1 - p_M, \quad (11)$$
There is no absolute upper limit on the number of $Q$ matrices, but there must always be at least one $Q$ matrix. The following special cases for $j_m(M')/j_m(M)$ result

$$M + : \frac{j_m(M')}{j_m(M)} = \left\{ \begin{array}{ll} \frac{1}{2} & \text{if } d(M) = 1 \\ 1 & \text{if } d(M) > 1. \end{array} \right. \tag{13}$$

$$M - : \frac{j_m(M')}{j_m(M)} = \left\{ \begin{array}{ll} \frac{1}{2} & \text{if } d(M) = 2 \\ 1 & \text{if } d(M) > 2. \end{array} \right. \tag{14}$$

The $M-$ proposal randomly chooses which $Q$ matrix to remove, and one might expect there to be a factor that accounts for that random choice. However, we discovered when testing with no data (to verify that our implementation was sampling from the prior distribution) that no such factor is needed because $M$ is conceptually unordered. Consider that even if $M$ were ordered and the $M+$ and $M-$ proposals always extended/truncated $M$, it would be possible to interject a rearrangement proposal between any pair of forward/reverse $M+/M-$ proposals to move an arbitrary $Q$ matrix to the end of $M$. This rearrangement proposal would have a proposal ratio of 1 (and therefore would always be accepted) because the rearrangement would have no impact on the likelihood.

When $d(M) > 1$, other proposal types that modify individual $Q$ matrices must choose which $Q$ matrix to modify. Again, one might expect the requirement for a factor that accounts for this choice, but a similar argument to the one above applies. Consider that if $M$ were ordered, it would be possible to preface any forward/reverse proposal pair with an $M$ rearrangement proposal that would always succeed, and the forward/reverse proposal pair could always operate on the last $Q$ matrix within $M$. Therefore, for proposal types that operate on individual $Q$ matrices we can choose a $Q$ matrix with uniform probability, and the presence of multiple $Q$ matrices can be ignored when computing the proposal ratios.

### Polytomies

Systematists commonly focus on bipartition support values (i.e., split frequencies) to test hypotheses regarding the relationships among taxa. Furthermore, branch lengths are commonly considered nuisance parameters for such analyses. There is now a growing awareness among researchers that misleading posterior bipartition support values can result from Bayesian MCMC analyses if the data are forced to conform to fully resolved tree topologies that contain little or no signal for full resolution (Suzuki et al. 2002; Cummings et al. 2003). Lewis et al. (2005) developed reversible jump MCMC proposals that sample among trees with zero or more polytomies, and they showed that this solves the “star tree paradox.” We adopted their method, but found existing polytomous tree topology proposals inadequate for analyzing large data sets. We solved the problem by generalizing the eTBR proposal (Lakner et al. 2008).

#### Generalized eTBR Topology Proposals

In principle, the polytomy proposals developed by Lewis et al. (2005) are sufficient for sampling among all polytomous and resolved trees but only one branch is modified per step in the Markov chain, which slows convergence, especially for trees with many taxa. Therefore, we used an eTBR proposal (Lakner et al. 2008) that we generalized to apply to terminal branches and polytomous trees. The generalizations allow branch-count-preserving topology transformations to any (non-star) tree, including polytomous trees and resolved four-taxon trees. Even though eTBR does not modify the number of branches, and therefore cannot change the topology of star trees, its branch length changes are applicable even to star trees. Figure 1 depicts the eTBR transformation.

In the following derivations, we assume a flat topology prior within each resolution class. For a given set of taxa, each resolution class contains all tree topologies that are composed of the same number of branches. We decompose the proposal ratio computation for eTBR into three independent components:

1. Random selection of $B_A$. The branch $B_A$ is selected with uniform probability $1/|B|$, where $|B|$ is the number of branches, so $j_m(B_A) = 1/|B|$. Where branches are arbitrarily enumerated $[0, |B|]$ and $u$ is a uniform $[0, 1)$ random variable, $g_m(B_A) = [u]$. The priors cancel because the choice of $B_A$ is symmetrical for the forward/reverse moves. The Jacobian is 1 because no dimension change occurs. The proposal ratio for $B_A$ selection (ignoring the likelihood ratio) is

$$\frac{\pi(\tau') j_m(\tau') g_m(u') \mid h(\tau', u')}{\pi(\tau) j_m(\tau) g_m(u) \mid h(\tau, u)} \mid = \frac{1}{|B|} \frac{1}{|B|} |u| = 1.$$

In general, as long as the same set of independent random number transformations is used to generate the forward/reverse proposals, we can avoid tracking them when computing proposal ratios, as this example demonstrates. The following derivations take advantage of this observation to avoid tedious notation for factors that cancel anyway.

2. Extension in one or both directions from $B_A$, as depicted by Figure 1, depending on whether $B_A$ is a
terminal branch. Extension in each direction contributes independently (though distinctly) to the eTBR proposal ratio; the following derivation is for extension in a single direction. For each extension step \( i \), extension proceeds with probability \( p_{\text{ext}} \) with uniform probability \( 1/(d(n_i) - 1) \), where \( d(n_i) \) is the degree of node \( i \). Given that the path \( B_A - B_R \) includes \( \nu \) internal nodes, if extension is constrained (\( B_S \) is a terminal branch), then

\[
j_m(\tau) = \prod_{i=1}^{\nu} \left( \frac{1}{d(n_i) - 1} \right),
\]

whereas in the unconstrained case

\[
j_m(\tau) = (1 - p_{\text{ext}}) \prod_{i=1}^{\nu} \left( \frac{1}{d(n_i) - 1} \right).
\]

The reverse move requires that the same path \( B'_A - B'_R = B_R - B_A \) be selected. Path reversal has no impact on the probabilities except at the end points, and therefore the proposal ratio is determined solely by \( j_m(\tau')/j_m(\tau) \). Whether the path selection is constrained/unconstrained can differ between \( B_R \) and \( B'_R \), which results in four cases. The proposal ratio for extension in the constrained/unconstrained case is

\[
\frac{j_m(\tau')}{j_m(\tau)} = \frac{\prod_{i=1}^{\nu} \left( \frac{1}{d(n_i) - 1} \right)}{(1 - p_{\text{ext}}) \prod_{i=1}^{\nu} \left( \frac{1}{d(n_i) - 1} \right)} = \frac{1}{(1 - p_{\text{ext}})}.
\]

In the constrained/unconstrained case, the ratio is \( 1 - p_{\text{ext}} \) and in the other two cases, the ratio is 1. Note that although Lakner et al. (2008) derived \( j_m(\tau')/j_m(\tau) \) for all four cases, only two cases actually applied, because they required that \( B_A \) be an internal branch.

3. Two or three branch length changes, depending on whether extension in both directions from \( B_A \) is possible (i.e., whether \( B_A \) is an external or internal branch, respectively). We incorporate the branch length multiplier proposal ratio, as described earlier, for each branch that changes length.

**Case Study Methods**

We converted the 44-taxon mammalian data set of Murphy et al. (2001) consisting of 22 concatenated genes (19 nuclear and 3 mitochondrial) from Nexus format (Maddison et al. 1997) to FASTA format (Pearson and Lipman 1988) using custom scripts that also removed excluded characters (see Supplementary material online). The resulting alignment contained 16,397 characters (10,349 unique).

We used the “redpoint” program from Crux (Evans 2009) to perform four Bayesian MCMC analyses of the data using all combinations of models with/without mixture models of estimated degree (+eQ), and with/without polytomy support (+P). This allowed us to measure the impacts of the two model enhancements separately and together and compare the results to the simpler GTR+I+I model, which was used by Murphy et al. (2001).

Each Q matrix in Crux consists of four normalized base frequency parameters \( \{\pi_A, \pi_C, \pi_G, \pi_T\} \sim \text{Dirichlet}(1,1,1,1) \), a rate scaler \( s_Q \sim \text{Exp}(1) \), and six normalized relative mutation rate parameters \( \{\alpha, \beta, \gamma, \delta, \epsilon, \eta\} \sim \text{Dirichlet}(1,1,1,1,1,1) \).
The $s_Q$ scaler allows the relative mutation rates among independent $Q$ matrices within a mixture to vary more freely than if all rate parameters were constrained to the same exponential prior distribution. We used Crux’s default set of MCMC proposals, which means that in addition to estimating $s_Q$ and jumping among the 203 relative mutation rate parameter constraint cases, our analyses jumped between equal/estimated base frequency parameters.

For the +eQ analyses, we used Crux’s default mixture model prior of 1/3 for the geometrically distributed mixture degree, which effected a prior expectation of 3 $Q$ matrices.

We ran two independent sets of four Metropolis-coupled chains for a minimum of $1 \times 10^6$ steps, sampled the latter halves of the cold chains every 1000 steps, and terminated once the $R_{\text{coverage}}$ online convergence diagnostic (Brooks and Gelman 1998, p. 441) indicated convergence of log-likelihood distributions, using a coverage of 95%, ±1%. We visually inspected the log-likelihood plots after termination to check for prestationarity trends in the log likelihoods because the $R_{\text{coverage}}$ diagnostic does not detect such trends if the independent runs follow similar trajectories.

**CASE STUDY RESULTS AND ANALYSIS**

The four models differ in their fit to the data, as evidenced by the log-harmonic means of the posterior likelihoods shown in Table 1. Polytomy capability has little impact on the mean posterior likelihoods, but mixture models allow dramatically higher likelihoods, using approximately 50 unconstrained $Q$ matrices, compared with 5 constrained $Q$ matrices used by the simpler models.

The GTR+I+4Γ results closely matched those of Murphy et al. (2001), thus providing assurance that our analyses are directly comparable with the originally published results. The GTR+I+4Γ+P results did not differ substantially, but the GTR+eQ and GTR+eQ+P models inferred dramatically lower support for eight bipartitions, as shown in Figure 2. Furthermore, the bipartitions labeled A, B, and H had weaker support than the (also weakly supported) alternatives labeled I, J, and K in Figure 3. Figure 4 depicts the lettered bipartition support differences between the GTR+I+4Γ analysis and the other three analyses. The large discrepancies for bipartitions A, C, D, E, F, and G indicate fundamentally distinct phylogenetic interpretations.

The low posterior support values for eight bipartitions that were formerly reported to be well resolved strongly indicate that the mixture model methods detect and fit data patterns that are ignored in traditional single-model analyses. However, this raises the question of whether the mixture model analyses overfit the data. We used Crux’s mixture model prior parameter, $p_M$ (mixtureJumpPrior), to vary the probability of successful $M+$ and $M−$ proposals and thus control the mean number of parameters. The prior expectation of the mixture degree is $\pi(d(M)) = 1/p_M$, and we ran a series of eight analyses with exponentially spaced priors. All other settings were the same as for the primary GTR+eQ+P analysis. The results are shown in Table 2. Bipartition support was reasonably stable regardless of the prior.

Despite exerting extreme pressure on mean $d(M)$ with the $p_M$ prior, the mean mixture degree did not drop below 21. Attempts to further reduce $\pi(d(M))$ were stymied by convergence problems, though exploratory analyses with 16 Metropolis-coupled chains per run indicated that this could be overcome, given adequate computational resources. In general, as $\pi(d(M))$ is decreased, stationarity becomes more difficult to achieve. We note here that although we are reasonably confident that the primary analyses converged, the $d(M)$ results reported in Figure 2 may trend low as $\pi(d(M))$ decreases. We would ideally conduct more thorough MCMC analyses with longer chains and more Metropolis-coupled chains, but doing so would require many thousands of hours of computer time, which is currently beyond our means.

In order to better understand the effect of mixture degree on bipartition support values, we ran a series of 12 analyses with fixed mixture degree. All other settings were the same as for the primary GTR+eQ+P analysis. The results are shown in Table 3. The analyses with $d(M) \leq 4$ are largely consistent with the GTR+I+4Γ analysis. However, bipartitions B, I, J, and K have blatantly nonmonotonic support trends, thus indicating that care should be taken to ensure adequate mixture degree when performing analyses with fixed $d(M)$.

None of our analyses directly reproduced those performed by Pagel and Meade (2005) because they used Γ-distributed rates in conjunction with mixture models of fixed degree for all of their analyses. Nonetheless, we can compare the bipartition support values reported by Pagel and Meade (2005) for a GTR+4Q+4Γ analysis: C: 0.55, D: 0.87, E: 0.96, F: 1.0, G: 0.98, J: 0.65, and K: 0.80. In the context of Table 3, only K stands out as being in stark contrast to our results. None of our analyses ascribed strong support to any resolution of the elephant/hyrax/sirenian clade, and this was particularly evident in our analyses that allowed for polytomies.
**FIGURE 2.** Consensus mammal phylogeny reported by Murphy et al. (2001), with bipartition support values computed via four different models, including GTR+I+4Γ as used in the original study. The +eQ methods infer very weak support for most of the eight lettered bipartitions. Inclusion of polytomous candidate trees does not by itself have a large impact on results (GTR+I+4Γ+P vs. GTR+I+4Γ), but in combination with mixture models results in much lower bipartition support values.

**DISCUSSION**

Our mixture model reanalyses produced strikingly more conservative results than those published by Murphy et al. (2001), clearly as a result of fitting numerous heterogeneous patterns in the data. However, Table 3 suggests that even with mixture models, misleading results are quite possible if \( d(M) \) is insufficiently large. Our reversible jump MCMC method for estimating \( d(M) \) appears to have circumvented that problem, and the results appear to be rather insensitive to the \( p_M \) prior parameter.
Figure 3. Consensus mammal phylogeny inferred by mixture model methods, with bipartition support values computed via four different models. As compared with Figure 2, the three-lettered bipartitions differ, but none of them are strongly supported.

Given the apparent insensitivity to the \( p_M \) prior, one might be tempted to choose an extreme prior that substantially depresses \( d(M) \), in order to reduce computation. However, we had considerable trouble with convergence for the runs with the lowest \( p_M \) values. It appears that frequent \( M+ \) and \( M- \) jumps reduced the chances of getting stuck in local optima; with very low \( p_M \) we saw independent runs get stuck with mixture degrees that differed by several \( Q \) matrices. Although mixture models appear to excel at fitting heterogeneous patterns, extracting useful information about pattern structure is extremely difficult for nontrivial data sets. This is because the \( Q \) matrices within a mixture model compose an unordered set.
we are to extract structural information from a stationary posterior distribution, we must somehow canonize and classify the samples, then perform second-order analysis of the classes. This appears to us as a very hard problem, made even harder by estimating $d(M)$. However, if practical solutions to the classification problem can be found, this may become a valuable tool for inferring varied constraints among sites.

Table 2. Bipartition support for varied $d(M)$ prior

<table>
<thead>
<tr>
<th>$\pi(d(M))$</th>
<th>$d(M)$</th>
<th>$\ln H(M)$</th>
<th>Bipartition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>A</td>
</tr>
<tr>
<td>$1 + 2^{-11}$</td>
<td>21.70</td>
<td>-207862.19</td>
<td>0.050</td>
</tr>
<tr>
<td>$1 + 2^{-9}$</td>
<td>22.61</td>
<td>-207852.20</td>
<td>0.043</td>
</tr>
<tr>
<td>$1 + 2^{-7}$</td>
<td>24.69</td>
<td>-207838.67</td>
<td>0.053</td>
</tr>
<tr>
<td>$1 + 2^{-5}$</td>
<td>28.58</td>
<td>-207786.01</td>
<td>0.038</td>
</tr>
<tr>
<td>$1 + 2^{-3}$</td>
<td>32.60</td>
<td>-207792.70</td>
<td>0.048</td>
</tr>
<tr>
<td>$1 + 2^{-1}$</td>
<td>39.33</td>
<td>-207727.73</td>
<td>0.016</td>
</tr>
<tr>
<td>$1 + 2^{+1}$</td>
<td>52.69</td>
<td>-207748.31</td>
<td>0.049</td>
</tr>
<tr>
<td>$1 + 2^{+3}$</td>
<td>59.01</td>
<td>-207766.14</td>
<td>0.049</td>
</tr>
</tbody>
</table>

Notes: The second to bottom row shows results for the primary GTR+eQ+P analysis. There are only minor fluctuations in posterior bipartition support values depending on $p_M$, and all the runs qualitatively agree that the bipartitions are poorly supported.
Table 3. Bipartition support for various fixed $d(M)$

<table>
<thead>
<tr>
<th>$d(M)$</th>
<th>In $HM(L)$</th>
<th>Bipartition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-226299.90</td>
<td>1.00 0.00 1.00 1.00 1.00 1.00 1.00 1.00 0.995 1.00 0.00 0.00 0.00 0.00 0.00 0.00 0.00 0.00</td>
</tr>
<tr>
<td>2</td>
<td>-212750.80</td>
<td>0.990 0.040 0.998 1.00 0.987 1.00 1.00 0.944 0.385 0.001 0.00 0.00 0.00 0.00 0.00 0.00 0.00 0.00</td>
</tr>
<tr>
<td>3</td>
<td>-210667.42</td>
<td>0.839 0.780 0.996 1.00 0.955 0.996 0.984 0.528 0.058 0.129 0.424</td>
</tr>
<tr>
<td>4</td>
<td>-209721.52</td>
<td>0.013 0.008 0.993 0.943 0.984 0.959 0.932 0.331 0.332 0.977 0.266</td>
</tr>
<tr>
<td>6</td>
<td>-208816.11</td>
<td>0.108 0.022 0.742 0.612 0.680 0.650 0.753 0.309 0.161 0.828 0.258</td>
</tr>
<tr>
<td>8</td>
<td>-208465.86</td>
<td>0.047 0.006 0.534 0.473 0.272 0.580 0.617 0.235 0.083 0.834 0.251</td>
</tr>
<tr>
<td>12</td>
<td>-208120.06</td>
<td>0.059 0.024 0.339 0.458 0.172 0.194 0.515 0.196 0.093 0.666 0.251</td>
</tr>
<tr>
<td>16</td>
<td>-207942.20</td>
<td>0.037 0.024 0.245 0.280 0.074 0.232 0.501 0.171 0.118 0.665 0.203</td>
</tr>
<tr>
<td>24</td>
<td>-207817.92</td>
<td>0.047 0.015 0.257 0.308 0.047 0.180 0.328 0.143 0.118 0.556 0.207</td>
</tr>
<tr>
<td>32</td>
<td>-207766.66</td>
<td>0.058 0.010 0.203 0.293 0.040 0.231 0.349 0.127 0.095 0.567 0.184</td>
</tr>
<tr>
<td>48</td>
<td>-207769.76</td>
<td>0.035 0.018 0.217 0.192 0.035 0.161 0.362 0.096 0.101 0.577 0.194</td>
</tr>
<tr>
<td>64</td>
<td>-207767.70</td>
<td>0.033 0.017 0.167 0.218 0.035 0.176 0.314 0.121 0.063 0.538 0.180</td>
</tr>
</tbody>
</table>

Note: In general, bipartition support values decrease as $d(M)$ increases, but support trends are blatantly nonmonotonic for several bipartitions (B, I, J, and K).

Supplementary Material

Supplementary material, including data files and/or online-only appendices, can be found at http://www.sysbio.oxfordjournals.org/.

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References


