The impact of single substitutions on multiple sequence alignments

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We introduce another view of sequence evolution. Contrary to other approaches, we model the substitution process in two steps. First we assume (arbitrary) scaled branch lengths on a given phylogenetic tree. Second we allocate a Poisson distributed number of substitutions on the branches. The probability to place a mutation on a branch is proportional to its relative branch length. More importantly, the action of a single mutation on an alignment column is described by a doubly stochastic matrix, the so-called one-step mutation matrix. This matrix leads to analytical formulae for the posterior probability distribution of the number of substitutions for an alignment column.

Keywords: maximum likelihood; maximum parsimony; substitution model; posterior probability; tree reconstruction

1. INTRODUCTION

Tree reconstruction is nowadays a matter of routinely applying the available programs, comparing the resulting trees and then concluding what might be the best tree (e.g. Hillis et al. 1996; Felsenstein 2004; Schmidt & von Haeseler 2008). With the advent of Bayesian approaches, it is possible to model increasingly complex evolutionary scenarios (Huelsenbeck & Ronquist 2001). However, a detailed understanding about what can and cannot be inferred (Baake 1998; Mossel & Steel 2005) from a sequence alignment is still missing. In recent years, some theoretical insights were obtained by studying very simple models of sequence evolutions on binary sequence data (e.g. Erdős et al. 1999a,b). Although these models are not realistic in a biological sense, they have provided some profound insights in the reconstruction process per se.

Here, we will introduce another description of the evolutionary process on trees. More precisely, given a phylogenetic tree and an alignment that evolved along the tree, we now ask the following question: how does the alignment change if an additional substitution on an arbitrary branch of the tree takes place? This rather abstract question is motivated by the following biological problem. Consider a collection of morphological traits that are either in an ancestral (0) or derived (1) state. Each derived character state characterizes a monophyletic group and represents a cluster in the tree. For such a data matrix (or alignment), the tree reconstruction problem is easy. However, stochastic effects that act somewhere on the branches of the tree may disturb this signal. This noise is modeled by the assumption of throwing an arbitrary number of changes on the tree and measuring their impact on the otherwise perfect data matrix. To this end, we construct a one-step mutation (OSM) matrix. The matrix description allows a linear algebra view of evolution and comprises distance methods, maximum parsimony as well as maximum likelihood. Moreover, the description reveals a surprising connection to Hadamard matrices that were employed for phylogenetic questions (Hendy & Penny 1989; Steel et al. 1998). An immediate application of OSM matrices is the analytical computation of posterior probabilities that count the number of evolutionary changes on a tree. So far, these posterior probabilities have been estimated using Bayesian simulation (Nielsen 2002; Huelsenbeck et al. 2003) or by applying the theory of counting processes (Minin & Suchard 2008).

In the following we refrain from most technicalities but rather outline the general ideas by discussing illustrative examples. The technical details and proofs will appear elsewhere.

2. THE BINARY MODEL ON AN n-TAXON TREE

(a) Notation

We consider a set of n taxa \( S = \{1, \ldots, n\} \). With \( S \) comes some information about the common properties and differences of the taxa, typically displayed in an alignment. In the following a (sequence) alignment \( A \) is an \( n \times \ell \)-array with entries either 0 or 1, where \( \ell \) is the length of the alignment. Each of the \( \ell \) columns (sites) \( a_j \) of the alignment represents a pattern of \( n \) homologous characters, where \( a_j \in \{0,1\} \) is the state of character \( j \) in taxon \( i \). For binary character states, \( 2^n \) patterns are possible.

We are interested in the evolution of such patterns along a (rooted) tree \( T = (V,E) \) with node set \( V \) and branch set \( E \subseteq V \times V \) (Semple & Steel 2003). The node set \( V \) contains the taxon set \( S \) that forms the leaf set. Avoiding the technical details, each branch is uniquely encoded by the subsets \( X \) of \( S \) that originates from the branch. Such a set \( X \) specified by a branch will be called a cluster. A leaf is a trivial cluster.

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Finally, we introduce a function \( \lambda: E \to \mathbb{R}_+ \), such that \( \lambda(e) > 0 \) represents the length of a branch \( e \in E \). The tree-length \( A_T \) is the sum of the branch lengths. The relative branch length

\[
p_e = \frac{\lambda(e)}{A_T}
\]

(2.1)
denotes the probability that a substitution hits branch \( e \) of the rooted tree \( T \).

**The effect of substitutions on an alignment**

We now describe how a single mutation on the tree changes the current character states at the leaves. Obviously the outcome will depend on the branch where the substitution occurred. Moreover, each of the \( 2^n \) possible patterns will be affected differently by such a substitution. Therefore we introduce a \( 2^n \times 2^n \) matrix that describes the action of a substitution on the patterns for a specific branch.

Figure 1 describes the model on an example tree \( T_4 \) with four taxa. For instance, a substitution at the branch defined by cluster \{A\} changes the pattern 1011 to the pattern 1010 because only the character of taxon A is affected. Please note that order of taxa is important. For instance, a substitution at the branch corresponding to pattern 1000 results in the pattern 0110. The structure of matrix \( \sigma_A \) is displayed in figure 1c, where a black square stands for one (the patterns are connected by an edge in the substitution graph) and a white square represents zero.

The corresponding adjacency matrix \( \sigma_A \) is an example of the so-called permutation matrices with entries equal to one if the substitution converts one pattern into another (Bona 2004, p. 75).

For each branch we easily construct the corresponding permutation matrix. Without proof we state that each matrix is fix point free (a substitution changes every pattern) and has \( 2^{n-1} \) transpositions (applying a substitution twice returns the original pattern). Then it follows that this type of permutation matrix is self-inverse with respect to matrix multiplication and that they form an Abelian group if the identity matrix is included.

We point out that the permutation matrix for a non-trivial cluster is the product of the permutation matrices of its elements. In other words the action of one mutation on a branch \( e \) can be replaced by any partition of the cluster associated with \( e \), such that each set of the partition is represented by a branch in the tree. For tree \( T_4 \) we obtain six permutation matrices

\[
\sigma_A, \sigma_B, \sigma_C, \sigma_D, \sigma_{AB} = \sigma_A \cdot \sigma_B, \sigma_{CD} = \sigma_C \cdot \sigma_D.
\]

Because we study symmetric permutation matrices, it is well known that the eigenvalues are either 1 or \(-1\). Further, we observe that all those permutation matrices are diagonalized by the \( 2^n \times 2^n \)-dimensional Hadamard matrix \( H_{2^n} \) (Hendy & Penny 1989; Hendy 2005). The \( 2 \times 2 \) Hadamard matrix is defined as

\[
H_2 = \begin{pmatrix} 1 & 1 \\ 1 & -1 \end{pmatrix}
\]

The \( 2^n \times 2^n \) Hadamard matrix is then

\[
H_{2^n} = H_2 \otimes \cdots \otimes H_2,
\]

\( n \)-times

where the symbol \( \otimes \) represents the Kronecker product. Hence, for the cluster \( C(e) \) associated with branch \( e \),

\[
D_e = H_{2^{n-1}}^{-1} \sigma_{C(e)} H_{2^n}
\]

is the diagonal matrix of eigenvalues of \( \sigma_{C(e)} \).

To take the relative contribution of the branch lengths into account, we weight each permutation matrix with \( p_e \) as described in (2.1). Such matrices are a special case of the generalized permutation matrices. Then the so-called OSM matrix of the tree \( T_4 \) is simply the following convex sum:

\[
M_4 = p_A \cdot \sigma_A + p_B \cdot \sigma_B + p_C \cdot \sigma_C + p_D \cdot \sigma_D + p_{AB} \cdot \sigma_{AB} + p_{CD} \cdot \sigma_{CD}.
\]

Figure 2c shows the result of this computation. The substitution graph in figure 2b displays the effect of a substitution on all the branches of the tree on the patterns. Two patterns are connected by an edge if a substitution switches between the two patterns.

For an arbitrary phylogenetic tree \( T \) on \( n \) taxa, the OSM matrix is obtained by

\[
M_T = \sum_{e \in E} p_e \sigma_{C(e)},
\]

(2.2)

where \( C(e) \) is the cluster identified by branch \( e \in E \). Owing to its construction, \( M_T \) is also diagonalized by the Hadamard matrix.
are represented exactly once in each row and each column. The relative branch lengths matrix belongs to the class of doubly stochastic Markov transition matrices, where the relative branch lengths are represented exactly once in each row and each column. Consequently, the $k$-th power $M^k_T$ provides the probabilities to move from one pattern to another in $k$ substitutions. Thus, the repeated application of $M^k_T$ describes a random walk on the state space of the $2^n$ patterns. This random walk is very different from the standard random walk on the hypercube (Eigen et al. 1989). If $k$ is large, then $M^k_T$ will lose the phylogenetic information of the original alignment and will approximate the uniform distribution, that is each pattern occurs at the same frequency.

Figure 2. (a) Substitutions on different branches of $T_4$ give rise to branches of (b) the graph, and (c) the corresponding entries in the OSM matrix. Corresponding components are identified by common shades of grey.

3. RELATION TO TREE RECONSTRUCTION

The OSM matrix leads to a very general description of character-based phylogenetic inference techniques. Moreover, the explicit model assumptions in maximum likelihood and the implicit assumptions in maximum parsimony are directly comparable.

The OSM matrix and its powers describe the substitution process between arbitrary patterns. However, in classical phylogeny the starting point of a substitution process is ancestral states on trees. In particular, one assumes a stationary distribution $\pi=(\pi_0, \pi_1)$ of character states at the root, and the characters evolve along the tree according to a Markov transition matrix ($\mathbf{T}$avaré 1986). In our framework, this is equivalent to starting in the constant patterns $0=(0, \ldots, 0)$ or $1=(1, \ldots, 1)$ and letting it evolve according to the OSM matrix. This process has a non-stationary pattern distribution $\pi^k_T$ which starts at $\pi^0_T=(\pi_0, 0, \ldots, 0, \pi_1)$, i.e. with zero substitutions only constant patterns exist, and in each step the pattern distribution is given by $\pi^{k+1}_T = \pi^k_T \pi T$. If the number of substitutions is not weighted as in equation (2.3), then $\pi^k_T$ will approach the uniform distribution as $k$ goes to infinity. To overcome the loss of phylogenetic signal, we assume in the following that the number of substitutions on a tree is Poisson distributed with parameter $A_T$. Moreover, we assume that the substitution process is described by the symmetric Cavender–Farris–Neyman mutation model (CFN, Neyman 1971; Farris 1973; Cavender 1978). Under these assumptions, the probability of observing pattern $a$ when starting in a constant pattern is then calculated employing (2.3)

$$P[a|0,1] = \pi_0 \bar{M}_T(0,a) + \pi_1 \bar{M}_T(1,a),$$

where $\pi_0$ and $\pi_1$ are taken from the stationary distribution of character states. The resulting probability distribution for all possible patterns is then identical to the standard way of computing the probabilities of pattern (Felsenstein 2004).

(a) Distance approaches

Now, we briefly illustrate how to derive distance corrections from the OSM matrix. To this end, we consider the rooted tree with two leaves $A$ and $B$ and branch lengths $\lambda_A$ and $\lambda_B$. Then the corresponding
substitution occurred. specifies a series of branches in the tree where a paths of length \(L\) observe different character states between taxa A and alignment. Similarly we compute the probability to observe a constant pattern in an alignment column \(a_i\), the minimal number of mutations on \(T\) equals
\[
MP(a_i) = \min\{k \in \mathbb{N} | A^k(a_i, 0) > 0 \text{ or } A^k(a_i, 1) > 0\}.
\]
Thus the minimal number of mutations for an alignment \(A = (a_1, \ldots, a_l)\) equals
\[
MP(T) = \sum_{i=1}^{l} MP(a_i). \tag{3.3}
\]
This is another description of the maximum-parsimony principle.

4. Mapping Substitutions

From the computation of the powers of the OSM matrix, it is possible to derive the (posterior) probability distribution \(\text{pdf}(k|x)\) of the number of mutations that generated an observed pattern \(x\), when the process started in pattern 0 or 1. The posterior probabilities have been estimated before, employing Bayesian simulation methods (Nielsen et al. 2002; Huelsenbeck et al. 2003; Minin & Suchard 2008), but an analytic approach has not previously been attempted.

In general, the posterior probabilities \(\text{pdf}(k|a)\) for a pattern \(a\) are calculated in the following way using (3.1):
\[
\text{pdf}(k|a) = e^{-\lambda \cdot A^k(\pi_0 M_k^a(0,a) + \pi_1 M_k^a(1,a))} \quad k! \cdot \text{pdf}(a|\{0,1\})
\]
i.e. we compute for pattern \(a\) the proportion of its occurrence after \(k\) substitutions.

For the two-taxon case, we observe that
\[
\text{pdf}(0|0) = \exp(-\lambda) \cosh \lambda_A \cosh \lambda_B,
\]
\[
\text{pdf}(0|1) = \exp(-\lambda) \sinh \lambda_A \sinh \lambda_B.
\]
Thus, applying hyperbolic identities, we end up with
\[
\text{pdf}(0|0,1) = \frac{1}{2} \exp(-\lambda) \cosh \lambda.
\]
Taylor expansion of \(\cosh(x)\) around \(x=0\) then leads to the posterior probability
\[
\text{pdf}(2k|00) = \frac{\lambda^{2k}}{(2k)!} \cosh \lambda.
\]
For the remaining patterns, we obtain the following pdf:
\[
\text{pdf}(2k|11) = \text{pdf}(2k|00),
\]
\[
\text{pdf}(2k+1|01) = \text{pdf}(2k+1|10) = \frac{\lambda^{2k+1}}{(2k+1)!} \sinh \lambda.
\]

OSM matrix \(M_2\) has the following structure:
\[
M_2 = e^{-A_2} \cdot \exp \left[ \begin{array}{ccc} \lambda_A & 0 & 0 \\ 0 & \lambda_B & 0 \\ 0 & 0 & 0 \end{array} \right] + \lambda_B \begin{array}{ccc} 0 & 0 & 0 \\ 0 & 0 & 0 \\ 0 & 0 & 0 \end{array} \right].
\]

\[
= e^{-A_2} \left( \begin{array}{ccc} \cosh \lambda_A \cosh \lambda_B & \sinh \lambda_A \cosh \lambda_B & \cosh \lambda_A \sinh \lambda_B \sinh \lambda_B \\ \sinh \lambda_A \cosh \lambda_B & \cosh \lambda_A \cosh \lambda_B & \sinh \lambda_A \cosh \lambda_B \sinh \lambda_B \\ \sinh \lambda_A \sinh \lambda_B & \cosh \lambda_A \cosh \lambda_B \sinh \lambda_B \sinh \lambda_B \\ \cosh \lambda_A \sinh \lambda_B & \sinh \lambda_A \cosh \lambda_B \sinh \lambda_B \sinh \lambda_B \\ \sinh \lambda_A \sinh \lambda_B & \cosh \lambda_A \cosh \lambda_B \sinh \lambda_B \sinh \lambda_B \\ \sinh \lambda_A \sinh \lambda_B & \cosh \lambda_A \cosh \lambda_B \sinh \lambda_B \sinh \lambda_B \end{array} \right).
\]

The first and the last row of \(M_2\) yield the probabilities to observe one of the four patterns. If evolution starts with character state 0 or 1 at the root of the tree and the character states are in equilibrium \((\pi_0 = \pi_1)\), then we quickly compute
\[
P(0,1) = \frac{1}{2} (1 + \exp(-2\lambda))
\]
as the probability to observe a constant pattern in an alignment. Similarly we compute the probability to observe different character states between taxa A and B. From this it is straightforward to get the distance correction of the CFN model.

(b) Maximum likelihood

The maximum-likelihood principle for an alignment \(A\) and a tree \(T\) is easily formulated in terms of the OSM matrix. We introduce as parameter vector \(\theta\) the branch lengths of \(T\). Then the probability of \(A\) is given by
\[
L(A|T) = \prod_{i=1}^{l} \text{pdf}(a_i|\{0,1\}). \tag{3.2}
\]
where the factors on the right-hand side are defined by equation (3.1). The parameter vector \(\theta\) enters the equation via the OSM and \(L = \sum \theta\), in the obvious way. As usual, we want to find parameter assignments such that (3.2) is maximized.

(c) Maximum parsimony

We associate the adjacency matrix \(A_{OSM}\) (e.g. Cormen et al. 2001, §22.1), or simply \(A\), with the OSM matrix. \(A\) is obtained as the unweighted sum of the permutation matrices \(\sigma_{ij}\). Hence, an entry \(A_{ij}\) is equal to one when there is a branch in the tree which changes pattern \(i\) into pattern \(j\), and is zero otherwise. Finally, we note that \(A^k(i,j)\) describes the number of paths of length \(k\) between pattern \(i\) and \(j\). Each path specifies a series of branches in the tree where a substitution occurred.

Now, fix a column \(a\) in alignment \(A\), and a tree \(T\). We ask for the minimal number \(k_{min}\) such that \(A^{k_{min}}(a,0)\) or \(A^{k_{min}}(a,1)\) is greater than zero. In other words, for an alignment column \(a_i\), the minimal number of mutations on \(T\) equals
\[
MP(a_i) = \min\{k \in \mathbb{N} | A^k(a_i,0) > 0 \text{ or } A^k(a_i,1) > 0\}.
\]

This is another description of the maximum-parsimony principle.
Thus, on a two-taxon tree, an even number of substitutions leads to a constant pattern when starting in a constant pattern; whereas an odd number of substitutions will be reflected by the non-constant pattern 01 or 10.

Now, it is an easy calculation to obtain closed formulae for the posterior mean number \( \mu(a) \) of substitutions for pattern \( a \) by the following calculations:

\[
\mu(00) = \mu(11) = \frac{2kA^{2k}}{(2k)\cosh A} = A \tanh A,
\]

\[
\mu(01) = \mu(10) = \frac{(2k + 1)A^{2k+1}}{(2k+1)\sinh A} = A \coth A.
\]

Only if \( A \) is large, then the posterior mean number of substitutions will approach the expected number of substitutions per site \( A \). For a constant pattern the posterior mean is always smaller than \( A \), and for non-constant patterns the posterior mean is larger than \( A \).

Similarly we extend the calculations to a four-taxon tree. For instance, consider the four-taxon tree \( T_4 \) (figure 1a). This tree has two non-trivial clusters \{A, B\} and \{C, D\}. We want to compute the posterior probability of the number of substitutions if the constant pattern 0000 is observed. Let us assume that the two character states occur with uniform probability; then we can compute:

\[
P[0000|01] = \frac{1}{16e^A} \left( \exp(\lambda_A - \lambda_B + \lambda_C - \lambda_D - \lambda_X) + \exp(-\lambda_A + \lambda_B - \lambda_C + \lambda_D + \lambda_X) + \exp(-\lambda_A - \lambda_B + \lambda_C - \lambda_D + \lambda_X) + \exp(\lambda_A - \lambda_B - \lambda_C + \lambda_D - \lambda_X) \right),
\]

where \( \lambda_X = \lambda_{AB} + \lambda_{CD} \) is the sum of the lengths of the interior branches. Now Taylor expansion leads to the desired posterior probability distribution.

Figure 3 shows the resulting posterior probability distributions for the 16 possible patterns, assuming branch lengths \( \lambda_A = \lambda_B = \lambda_C = \lambda_D = 0.2 \) and \( \lambda_{AB} = \lambda_{CD} = 0.1 \). The symmetries in the CFN model are reflected in the symmetries of the posterior distributions. Complementary patterns (i.e., 0001 and 1111) show the same distribution. Because the tree is clocklike, the parsimonious uninformative patterns (0001, 0010, 0100, 1000) and their complements show identical distributions, as do the patterns that need at least two substitutions (0101, 0110, 1010, 1001) on \( T_4 \). Posterior probabilities may be used to compute, for instance, the number of unvaried sites (Fitch & Ayala 1994), which is exactly the proportion of the constant patterns with zero substitutions. In our example we expect approximately 42 percent constant patterns of which approximately 90 per cent are unvaried. This is only one application for posterior probabilities of the number of substitutions.

As in the two taxon case, we compute the posterior mean of substitutions for pattern \( a \) as

\[
\mu(a) = \sum_{k=0}^{\infty} k \cdot \text{pdf}(k|a),
\]

Figure 4a shows the posterior mean number of substitutions for the topology of \( T_4 \) with branch probabilities \( p_A = p_B = p_C = p_D = 0.2 \) and \( p_{AB} = p_{CD} = 0.1 \) for a constant pattern (0000), a pattern compatible with an interior branch (0011) and a pattern incompatible with the tree (0110). The difference between posterior mean and tree lengths is smaller than 0.01 if the tree lengths exceed 10 substitutions per site.

Figure 4b displays the posterior means for a tree with branch probabilities \( p_A = p_D = 0.47, p_B = p_C = 0.02 \) and \( p_{AB} = p_{CD} = 0.01 \). The proportion of \( p_A \) and \( p_D \) is so large that the incompatible pattern 0110 will be observed more often than the pattern 0011, which is compatible with a branch of the tree. Thus, this tree is
Figure 4. Posterior mean number of substitutions as a function of the tree length \( A \) for the tree topology \( T_4 \). The posterior means for patterns \( 0000 \) (dashed line), \( 0011 \) (grey solid line) and \( 0110 \) (black solid line) are shown. (a) The posterior means on the tree with relative branch lengths \( p_A = p_C = p_0 = 0.2 \) and \( p_{AB} = p_{CD} = 0.1 \). (b) The result for relative branch lengths \( p_A = p_D = 0.47 \), \( p_B = p_C = 0.02 \) and \( p_{AB} = p_{CD} = 0.01 \).

5. SUMMARY AND DISCUSSION
Here, we have presented an alternative description of how to model sequence evolution on a tree. Our approach lifts the commonly used stochastic models of sequence evolution that act on nucleotides to the set of all possible patterns for \( n \) taxa.

We have shown that available tree reconstruction principles are included in our description of the process. Moreover, the definition of the OSM matrix leads to analytical formulae to compute the posterior probability distribution of the number of substitutions for each pattern. From this distribution it is then straightforward to compute the posterior mean of the substitutions. The formulation of the substitution process as an OSM matrix leads to the introduction of the Hadamard matrix that allows an easy computation of matrix powers. Recently, Bryant (submitted) has presented a continuous version of the OSM approach and showed its connection to the Hadamard matrix (Hendy 2005).

While we have outlined only the simplest model of sequence evolution, several extensions are easily possible. The OSM approach can be augmented to the Kimura 3st model (Kimura 1981); see figure 5a for an illustration. In this framework every substitution class (transition, transversion 1 and transversion 2) uniquely generates a fix-point free \( 4^n \times 4^n \)-dimensional permutation matrix for each branch in a tree. Let \( \alpha_1 + \alpha_2 + \alpha_3 = 1 \) denote the probabilities for the three substitution classes, then the OSM matrix for the Kimura 3st model is defined as

\[
M_T = \sum_{k=1}^{3} \alpha_k \sum_{e \in E} p_e \sigma_e^k \quad (5.1)
\]

i.e. we look at the sum of generalized permutation matrices. Figure 5a shows an OSM Kimura matrix on a rooted triplet tree. Each row and each column contain 12 = (number of branches) \times (number of substitution classes) non-zero entries, where each entry is the product of a mutation class parameter \( \alpha_i \) and a branch probability \( p_e \) (equation (5.1)). All the results for binary character state models can be expanded to the Kimura 3st model. If one wants to abandon the assumption that evolution proceeds along a tree, then this is also possible within the OSM framework. Consider a set of rooted trees that give rise to a collection \( \mathcal{E} \) of possibly conflicting clusters. The associated OSM matrix is then given by

\[
M_E = \sum_{C \in \mathcal{E}} p_C \sigma_C.
\]

where \( p_C \) is the normalized sum of branch lengths of those trees in which the branch depicting \( C \) is existent. Here issues such as the meaning of the overall length of the cluster set or the meaning of a root in such sets need to be discussed. This extension bears some similarity to a maximum-likelihood reconstruction of networks (von Haeseler & Churchill 1993).

Another question concerns the Poisson weights for the number substitutions. Generally, the argument is that the process of distributing substitutions along a tree is memoryless and therefore the number of substitutions is Poisson distributed. Our framework permits a different probability distribution to be assigned to the substitution process. One possible weighting scheme could be a contagious distribution, which has been used to evaluate accident data (Kemp 1967). This approach might provide an alternative description of the evolutionary history of an alignment.

In summary, the OSM description offers a variety of potential applications in molecular systematics, which will be explored in the near future.

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