An Alternative Model of Amino Acid Replacement

Gavin E. Crooks* and Steven E. Brenner
Dept. of Plant and Microbial Biology, 111 Koshland Hall #3102,
University of California,
Berkeley, CA 94720-3102, USA

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Abstract

The observed correlations between pairs of homologous protein sequences are typically explained in terms of a Markovian dynamic of amino acid substitution. This model assumes that every location on the protein sequence has the same background distribution of amino acids, an assumption that is incompatible with the observed heterogeneity of protein amino acid profiles and with the success of profile multiple sequence alignment. We propose an alternative model of amino acid replacement during protein evolution based upon the assumption that the variation of the amino acid background distribution from one residue to the next is sufficient to explain the observed sequence correlations of homologs. The resulting dynamical model of independent replacements drawn from heterogeneous backgrounds is simple and consistent, and provides a unified homology match score for sequence-sequence, sequence-profile and profile-profile alignment.

Introduction

During evolution, a protein’s amino acid sequence is altered by the insertion and deletion of residues and by the replacement of one residue by another. In principle, the alignment of proteins and the subsequent detection of protein homologs requires a dynamical model of this sequence evolution. The most common and widely used residue replacement dynamics is the standard Dayhoff model, which assumes that the substitution probability during some time interval depends only on the identities of the initial and replacement residues and that the dynamics is otherwise homogeneous along the protein chain, and between protein families, and across evolutionary epochs. In other words, under this model the dynamical model of amino acid substitution resembles a continuous time, first order Markov chain [Dayhoff et al. 1972, 1978; Gonnet et al. 1992; Jones et al. 1992; Müller & Vingron 2000].

However, it has long been known that this widely used Markovian substitution model is fundamentally unsatisfactory. One major problem is that short and long time substitution dynamics are incompatible [Gonnet et al. 1992; Benner et al. 1994; Müller & Vingron 2000; Benner et al. 1994] suggests that this is because at short evolutionary times the patterns of substitution are influenced by single base mutations between neighboring codons, whereas for more diverged sequences the genetic code is irrelevant and the patterns of replacement are dominated by the selection of chemically and structurally compatible residues.

A more serious problem with the Dayhoff Markovian model is that it assumes that every residue in every protein has the same background distribution of amino acids and that protein sequences rapidly evolve to this uninteresting equilibrium. In actuality, the amino acid background distribution varies markedly from one residue position to the next, as can be seen, for example, in figure 1. These large site-to-site variations are stable across relatively long evolutionary time-scales, and they account for the success of protein hidden Markov models and other profile based multiple sequence alignment methods. (See, for example, Sjölander et al. 1996, Durbin et al. 1998) Profile methods can detect substantially more remote homologies than pairwise alignment [Park et al. 1998, Green and Brenner, Unpublished data].

In short, the dynamics of amino acid substitution are not Markovian, stationary, nor homogeneous, and the prediction of rapidly decaying sequence correlations is at odds with the success of profile based remote homology detection.

A natural solution to the limitations of the Markov model is to assume that residue replacement is governed by different Markov processes for each position, each process potentially possessing its own background distribution and substitution probabilities. The appropriate Markov matrix for a particular protein position is chosen based upon predictions of the protein structure, or directly from the sequence data.

Here, we propose that the observed sequence correlation between diverged homologs is principally due to the heterogeneous, stable, background distribution of each protein site and, therefore, that a Markovian amino acid replacement dynamics is overly complicated and possible unnecessary for the accurate construction of protein sequence alignments and phylogenies. As an alternative, we construct a dynamical model of amino acid replacement that explicitly assumes that each protein site has a different equilibrium distribution of the 20 canonical amino acids (which we refer to as that site’s amino acid background, \( \theta \)) and that the
A protein is often stable across large evolutionary time-scales, but varies markedly from one site to another. This figure illustrates the helix-turn-helix motif from the CAP family of homodimeric DNA binding proteins. The height of each letter corresponds to the amino acid frequency in a multiple alignment of 100 diverse homologous sequences. (For details, see Crooks et al., 2004) Schneider & Stephens, 1990). These background distributions are determined by structural, functional and evolutionary constraints. For example, positions 180, 181 and 185 are critical to the sequence specific binding of the protein to DNA, the conserved glycine at position 177 is located inside of the turn between the helices, and the buried sites 172, 176, 178, 183, 187 and 190 contain mostly hydrophobic residues. It should be noted that the correlations inherent in these background distributions are far stronger than can be explained by local structural features (such as burial and secondary structure) alone. (Crooks & Brenner, 2004).

Under our residue replacement model, the principle origin of sequence correlation between diverged homologs is the background distribution of each protein site. This is also the central idea underlying profile based multiple sequence alignment algorithms. Therefore, we are not proposing a radically different method for homolog detection or sequence alignment; rather we are proposing a concrete and consistent dynamics for the underlying evolutionary process. The implications of this dynamics can be readily extended to cover not only profile-sequence based alignment, but also profile-profile and pairwise sequence-sequence alignment. Moreover, when we consider pairwise, sequence alignment below, we find that our model is essentially equivalent to the standard pairwise alignment methods, as they are used in practice. This alternative dynamical model of amino acid replacement is biologically reasonable, conceptually straightforward and can adequately explain many of the observed patterns of homolog sequence correlation without invoking a Markovian dynamic. The correlations between pairs of homologous residues can be summarized by an amino acid substitution matrix, \( S \), whose entries represent the log probability of observing the homologous pair of amino acids \( q_{ij} \) in a properly aligned pair of homologous proteins, against the probability \( p_i p_j \) of independently observing the residues in unrelated sequences. (Altschul, 1991).

\[
S_{ij} = \frac{1}{\lambda} \log \frac{q_{ij}}{p_i p_j} \tag{1}
\]

Units of one third bits are traditional for substitution matrices (base 2 logarithm, \( \lambda = 1/3, \approx 1.16 \) digits), although the scaling
Figure 3: This substitution matrix (Eq. 1, a conventional description of amino acid replacement propensities) has been directly constructed from the dist.20comp Dirichlet mixture model of amino acid background probabilities (Fig. 2; Karplus, 1995), using the conditionally independent substitution model of amino acid replacement (Eqs. 3-10). As a consequence of the heterogeneity and stability of amino acid background probabilities – illustrated in figs. I and 2 – the amino acid identity of a pair of alignable, homologous residues is non-trivially correlated over long evolutionary time scales, simply because both residues are drawn from the same background. Scores are in units of \( \frac{1}{3} \) bits, rounded to the nearest integer.

is arbitrary. Assuming conditionally independent replacements, we can directly construct the large time limit substitution matrix from the background probability distribution, \( P(\theta) \). Fortunately, this large distribution has previously been investigated and parameterized by fitting many columns from multiple alignments of homologous protein sequences to a mixture of Dirichlet distributions (Karplus, 1995; Durbin et al., 1998). Fig. 2 displays a projection of the dist.20comp parameterization (Karplus, 1995) and Fig. 3 displays the corresponding substitution matrix. The mathematical details of matrix construction are given below.

At shorter evolutionary times there is a significant chance that no mutation has occurred at all, resulting in an enhanced probability of amino acid conservation. Let \( c \) be the probability of zero mutation events, then the substitution matrix, adjusted for the possibility of zero mutations, is

\[
S_{ij}(c) = \log \frac{c p_i \delta_{ij} + (1 - c) q_{ij}}{p_i p_j},
\]

(2)

where \( \delta_{ij} \) is the Kronecker delta function. A reasonable default model for the conservation probability \( c \) would be to assume that substitutions are Poissonian. Then \( c = \exp(-t/\tau) \), where \( \tau \) is the mean time between replacements. Note that although replacement is Markovian (albeit zeroth order), the dynamic decay of residue correlations at a position is not, due to the heterogeneity of the amino acid background at that position (an unobserved hidden variable).
alignment and remote homology detection. These three matrices have been created using very different evolutionary models; the dist.20comp matrix is based upon our heterogeneous background/independent substitution model, and the dist.20comp background distribution is, in turn, derived from the columns of many multiple alignments of homologous protein sequences; the popular BLOSUM matrices are empirically derived from the BLOCKS database of reliable protein sequence alignments (Henikoff & Henikoff, 1992; Henikoff et al., 2000); and the classic PAM (Dayhoff et al., 1978) and modern VTML (Müller et al., 2002) matrix families are explicitly based upon the Markovian model of amino acid replacement. In a recent evaluation of pairwise remote homology detection, the VTML160 matrix was found to be more effective than any other VTML, PAM or BLOSUM matrix. (Green & Brenner, 2002) However, as can be seen in figure 6, the difference in remote homology detection ability of the three matrices is relatively small.

In summary, the important sequence correlations can be adequately explained by assuming conditionally independent replacements drawn from background distributions that vary from site-to-site, but are stable over evolutionary time-scales. The standard, Markovian model of amino acid replacement is unnecessary, overly complicated and inconsistent with observed substitution patterns.

This alternative, heterogeneous background, independent substitution model may be particularly useful for simultaneous sequence alignment and phylogenetic tree reconstruction, since it is necessary to align pairs of close homologs at the leaves, and multiply align many remote homologs at the interior nodes of the tree. Therefore, a simple (yet realistic) evolutionary dynamic that is consistent across a wide range of divergence times, and that leads naturally to sequence-sequence, sequence-profile and profile-profile alignment algorithms, may be advantageous.

Mathematical Details

A collection of homologous residues can be represented by a 20 component canonical amino acid count vector, \( n = \{n_1, \ldots, n_{20}\} \). The total number of counts can be 1, if the observation is taken from a single sequence, or many if the collection represents an entire column of a multiple sequence alignment or some other related set of residues.

In general, we wish to estimate whether two collections of homologous residues are related, given that detectably homologous residues are drawn from the same background amino acid distribution. The appropriate test statistic is the log odds of sampling the two amino acid count vectors from the same, but unobserved, background distribution, against the probability of independently sampling the two count vectors from different distributions.

\[
S(n^1, n^2) = \log \frac{P(n^1, n^2)}{P(n^1)P(n^2)}
\]  

(3)

The probability of independently sampling a particular collection of homologous residues \( n \) from the background amino acid profile from which those residues are drawn, \( \theta = \{\theta_1, \ldots, \theta_{20}\} \), follows the multinomial distribution;

\[
P(n|\theta) = M(n|\theta) = \frac{1}{M(n)} \prod_{i=1}^{20} \theta_i^{n_i}, \quad M(n) = \prod_{i=1}^{20} \frac{n_i!}{\sum n_i!}
\]  

(4)

This is the multivariate generalization of the common binomial distribution.

The probability distribution of background distributions \( P(\theta) \) has been studied and measured by collating columns from many multiple protein sequence alignments. Since this is a very large, multi-modal probability it is necessary to parameterize the distribution into a convenient representation. Typically, a mixture of Dirichlet distributions is used (Karplus, 1995; Sjölander et al., 1998; Durbin et al., 1998).

\[
P(\theta) = \sum_{k=1}^{m} \rho_k D(\theta|\alpha^k)
\]  

(5)
distribution is smooth, but lumpy. (See Fig. 2)

The probability of independently sampling two count vectors, \( n^1 \) and \( n^2 \), from the same undetermined background is

\[
P(n^1, n^2) = \int d\theta P(n^1|\theta)P(n^2|\theta)
\]

\[
= \int d\theta \mathcal{M}(n^1|\theta)\mathcal{M}(n^2|\theta) \sum_k \rho_k D(\theta|\alpha^k)
\]

\[
= \sum_k \rho_k \frac{1}{Z(\alpha^k)M(n^1)M(n^2)} \int d\theta \prod_{i=1}^{20} \theta_i^{n_i^1+n_i^2+\alpha_i^k-1}
\]

\[
= \sum_k \rho_k \frac{Z(n^1+n^2+\alpha^k)}{Z(\alpha^k)M(n^1)M(n^2)}
\]

The last line follows because the product in the previous line is an unnormalized Dirichlet with parameters \( n + \alpha^k \). Therefore, the integral over \( \theta \) must be equal to the corresponding Dirichlet normalization constant, \( Z(n + \alpha^k) \). The final result is a mixture of multivariate negative hypergeometric distributions. The negative hypergeometric is an under-appreciated distribution (e.g., Eq. 11.23, Durbin et al. 1998) which bears the same relation to the hypergeometric as the negative binomial does to the binomial distribution. The multivariate generalization appears in this case as the combination of a Dirichlet and a multinomial. Confusingly, the negative hypergeometric distribution is sometimes called the inverse hypergeometric, an entirely different distribution, and vice versa.

The probability of independently sampling two count vectors, \( n^1 \) and \( n^2 \), from the same undetermined background is

\[
P(n^1, n^2) = \int d\theta P(n^1|\theta)P(n^2|\theta)P(\theta)
\]

\[
= \int d\theta \mathcal{M}(n^1|\theta)\mathcal{M}(n^2|\theta) \sum_k \rho_k D(\theta|\alpha^k)
\]

\[
= \sum_k \rho_k \frac{1}{Z(\alpha^k)M(n^1)M(n^2)} \int d\theta \prod_{i=1}^{20} \theta_i^{n_i^1+n_i^2+\alpha_i^k-1}
\]

\[
= \sum_k \rho_k \frac{Z(n^1+n^2+\alpha^k)}{Z(\alpha^k)M(n^1)M(n^2)}
\]

Combining Eqs. 7 and 8 with the log likelihood ratio, Eq. 3 generates a generic profile-profile sequence alignment score that is valid whether the number of counts is small or large.

\[
S(n^1, n^2) = \log \frac{\sum_k \rho_k \frac{Z(n^1+n^2+\alpha^k)}{Z(\alpha^k)M(n^1)M(n^2)}}{\sum_k \rho_k Z(\alpha^k)M(n^1)M(n^2)}
\]

For the particular case that one of the count vectors contains only a single observation this score reduces to the standard

\[
S(n^1, n^2) = \log \frac{\sum_k \rho_k \frac{Z(n^1+n^2+\alpha^k)}{Z(\alpha^k)M(n^1)M(n^2)}}{\sum_k \rho_k Z(\alpha^k)M(n^1)M(n^2)}
\]
sequence-profile score frequently used by hidden Markov model 
protein sequence alignment. This is inevitable, since the underlying mathematics is the same.

If both count vectors contain only a single observation, then this profile-profile score reduces to a pairwise substitution matrix. Note, given that \( n_{x}^{1} = \delta_{x1} \) and \( n_{x}^{2} = \delta_{x2} \) (where \( \delta_{xj} \) is a Kronecker delta function), then all but the \( j \)th element of the product \( Z(\delta_{xj} + \alpha^{k})/Z(\alpha^{k}) \) cancels. Thus,

\[
S_{ij} = \log \frac{q_{ij}}{p_{i}p_{j}}
\]

\[
p_{i} = \sum_{k} \rho_{k} \frac{\alpha_{i}^{k} \alpha_{j}^{k}}{A_{k}}
\]

\[
q_{ij} = \begin{cases} 
\sum_{k} \rho_{k} \frac{\alpha_{i}^{k} \alpha_{j}^{k}}{A_{k}(A_{k} + 1)} & i \neq j \\
\sum_{k} \rho_{k} \frac{\alpha_{i}^{k} (\alpha_{i}^{k} + 1)}{A_{k}(A_{k} + 1)} & i = j
\end{cases}
\]

Applying Eq. (10) to the 20 component Dirichlet mixture \( \text{dist.20comp} \) generates the pairwise substitution matrix illustrated in Fig. 3.

An interesting feature of this model is that it provides a unified homology match score for sequence-sequence, sequence-profile and profile-profile alignment (Eq. 9). As far as we are aware, this profile-profile score has not been evaluated in a profile-profile alignment algorithm, although it is a natural generalization of the established hidden Markov model profile-sequence score. However, in the large sample limit Eq. 9 reduces to the Jensen-Shannon divergence between the two empirical amino acid distributions, a measure that has shown some promise in profile-profile alignment (Yona & Levitt, 2002).

Edgar & Sjölander, 2004; Marti-Renom et al., 2004.

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