A phylogenetic and population genetic model of amino acid substitution

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1 Abstract

We introduce a new, mechanistic Markov model for studying the evolution of amino acid sequences within a phylogenetic context. Our model links together genotype, phenotype, and fitness of proteins, by calculating the fixation probability of a newly arisen mutant using a model of allele substitution from population genetics. As a result, our model explicitly includes the effects of mutation bias, genetic drift, and natural selection favoring an optimal amino acid sequence for a given protein. We assume that the strength of purifying selection is a function of the physiochemical differences between a given amino acid and the optimal amino acid for the site, how a protein's functionality declines with this distance, and the target expression level of the gene. Analysis of a multi-locus yeast data set using AIC shows that our new model provides a substantially better fit to data than the standard empirical models and allows researchers to estimate biologically meaningful parameters such as the sensitivity of a protein's function to an amino acid substitution and the optimal amino acids at a given site. Further, because our model is based on explicit models of

various biological processes, unlike empirical models it can easily be modified in the future to include other important biological phenomena such as selection on codon usage or test alternative hypotheses about the relationship between amino acid sequence and protein functionality.

2 Introduction

Importance of building accurate model for protein evolution.

Known models of amino acid substitution fall into two categories: empirical models and mechanistic models. In empirical models, the substitution rates are based solely on the amino acid frequencies observed in given database. Empirical models are commonly used in phylogenetic studies and include Dayhoff, JTT, WAG, LG, etc. [CAN ONE FORMULATE A NON-MECHANISTIC CODON LEVEL MODEL? AMINO ACID MODEL? -YES] In contrast, the substitution matrices in mechanistic model are based on the actual biological processes thought to drive sequence evolution, such as natural selection and mutation bias. Although less commonly used, there are a number of notable models in this area including [CITATIONS].

Mechanistic models are usually formulated at one of three different levels: mono-nucleotide level in DNA, codon level, and amino acid (AA) level. The nucleotides, codons, or amino acids are assumed to evolve independently. DNA- and codon-based models use more data and are often the most powerful in terms of their ability to distinguish between closely related sequences. Of these two, codon-based models use all the information in DNA and know the product of amino acid additionally. On the other hand, AA-based models ignore synonymous differences between sequences by focusing not on the codons themselves

but the amino acids they code for.

Nucleotide level models use the most data and are often the most powerful in terms of their ability to distinguish between closely related sequences [CUT →?] such as those caused by synonymous substitutions which are invisible at amino acid level [WOULDN"T THIS APPLY TO CODON-LEVEL MODELS AS WELL? YES]. [DISCUSSION OF CODON LEVEL MODELS– HOW DO THEY DIFFER FROM DNA MODELS?] A popular example of a mechanistic, codon level model is **?**. This implemented a few mechanistic models at the level of codons and explicitly modeled the biological processes involved, including different mutation rates between nucleotides, translation of the codon triplet into an amino acid, and the acceptance or rejection of the amino acid due to selective pressure on the protein. In contrast to codon level models, amino acid level models ignore synonymous differences between sequences by focusing not on the codons themselves but the amino acids they code for. =======

A popular example of a mechanistic, codon-based model is **?**. This implemented a few mechanistic models at the level of codons and explicitly modeled the biological processes involved, including different mutation rates between nucleotides, and the translation of the codon triplet into an amino acid. In contrast to codon-based models, AA-based models ignore synonymous differences between sequences by focusing not on the codons themselves but the amino acids they code for. ¿¿¿¿¿¿¿ .r374 Since synonymous codon usage is largely driven by mutation bias in low expression genes and selection on translational efficiency for high expression genes [CITATIONS], ignoring this aspect of the data has the advantage of reducing the noise in sequence data for low expression genes but at the cost of losing potentially useful information held in the high expression genes. Such an approach makes sense for most phylogenetic models since they ignore the effect of gene expression on sequence evolution [EXCEP-

TIONS?]. However, as we show here, our models provide a natural framework for including the effect of gene expression on sequence evolution.

One of the most commonly used codon-based model is that of Goldman and Yang (GY) (1994, MBE). This uses the nucleotide-level information in DNA sequences and the amino-acid level information of synonymous and non-synonymous nucleotide substitutions simultaneously [DON'T ALL CODON-BASED MODELS DO THIS?] Their model incorporated transition vs. transversion bias, synonymous vs. nonsynonymous variation in a gene, and the physiochemical differences between amino acids. The selective restraints at the amino acid level was accounted for by multiplying the substitution rate by a factor $\exp(d_{aa_i,aa_j}/V)$ where d_{aa_i,aa_j} is the distance between amino acids aa_i and aa_j given by Grantham (1974) (i.e. Grantham Distances) and V is a parameter representing the variability of the gene or its tendency to undergo non-synonymous substitution.

It is important to note that whether empirical or mechanistic, most phylogenetic models, including the GY model, are time-reversible. [GTR IS A PARTICULAR MODEL, NOT THE TERM FOR ALL TIME REVERSIBLE MODELS] In time-reversible models, the substitution rate matrix used is symmetric such that the exchange rate of any given site going from state i to state j is equal to the rate of that site going from state j to state i. While time reversibility provides substantial mathematical and computational advantages, the substitution rate matrices are difficult to interpret biologically if one assumes natural selection is acting consistently on a given site. Surprisingly this lack of inconsistency has gone largely unrecognized. By definition, if the substitution of state i to j is favored by natural selection, in the absence of mutation bias it must occur at a faster rate than the reverse substitution of j to i. While mutation bias can alter this requirement when selection is weak, it can only do

so when the assumption of time reversibility is violated. For example, consider a time-reversible codon-level substitution models where synonymous substitutions occur at a faster rate than non-synonymous substitutions. For any given state of the system, such a model implies that the current amino acid is optimal since synonymous substitutions occur at a faster rate than non-synonymous ones. However, once a non-synonymous substitution has occurred (and as time goes to infinity it will), the time reversible aspects of the model now imply that the new state is the optimal state and the old state is sub-optimal. Thus, the only reasonable way to interpret such a time-reversible model is that the substitution matrix is actually describing the rate at which the optimal state switches at a given site and that once such a switch has occurred the system instantaneously shifts to the new state. If, in contrast, one were to assume the converse, that non-synonymous substitution occur at a faster rate than synonymous, then the interpretation of time-reversible models becomes even more problematic from a biological perspective. In such a scenario, not only is the optimal state constantly changing, the current state of any given site is always sub-optimal.

While time-reversible models have played an important role in the development of the field of phylogenetics, in order be able to model natural selection and mutation bias in a realistic manner the assumption of time reversibility must be relaxed. In this study we develop an amino-acid based model in which we assume that for each individual site there's a corresponding optimal amino acid $\vec{a}'$. The optimal state can be assigned or, as we demonstrate, estimated from the data itself. As with the GY model, we assume that the substitution rate between amino acids at a given site is a function of their Grantham distances $d_{i,j}$ from optimal amino acids and, in a similar manner, assume genes can vary in their sensitivities to such changes. Here the sensitivity to amino acid changes

is calculated using a cost-benefit framework we have developed previously for studying the evolution codon usage bias **???**. More specifically, the cost represents the cost of protein synthesis while the benefit represents the functionality provided by the protein encoded by the sequence. For simplicity, we assume that the synthesis cost is proportional to the length of the coding sequence, and that utility of the protein produced is an inverse function of its distances from the optimal amino acid sequence. Unlike the GY model, we also incorporate the effects of gene expression on the substitution rate, with higher expression genes being under stronger selection to be at the optimal state $\vec{a}'$. ¡¡¡¡¡¡¡ .mine Furthermore, unlike most models in phylogenetics, we define the fitness of a given genotype explicitly. We then use a model from population genetics to calculate the substitution rate between any two genotypes explicitly taking into account the fitness differences between genotypes as well as the effects of mutation bias and genetic drift. We illustrate our approach by fitting our model's likelihood function to the **?** data set of 103[?] genes sequenced from 8 different species of yeast. When fitting our model, we can estimate the phylogenies of the yeast species as well as additional model parameters such as the a Grantham sensitivity of a gene (roughly comparable to $1/V$ in the GY model), as well as state optimal amino acid $\hat{a}$ for any given site in a coding sequence. Using AIC we compare our model's fit to the Rokas data to other commonly used GTR amino acid level models [LIST MODELS – IF WE STICK TO AA MODELS, WHY DO WE SPEND SO MUCH TIME DISCUSSING THE GY MODEL WHICH IS A CODON LEVEL MODEL?]. ======= Furthermore, unlike most models in phylogenetics, we define the fitness of a given protein explicitly. We then use a model from population genetics to calculate the substitution rate between any two genotypes by explicitly taking into account the fitness differences between genotypes as well as the effects of mutation bias and genetic drift. We illustrate

our approach by fitting our model's likelihood function to the **?** data set of 106 genes sequenced from 8 different species of yeast. When fitting our model, we can estimate the phylogenies of the yeast species, the Grantham sensitivity g of a gene (roughly comparable to $1/V$ in the GY model), as well as state optimal amino acid $\vec{a}'$ for any given site in a coding sequence. Using AIC we compare our model's fit to the Rokas data to other commonly used GTR AA-based models [LIST MODELS – IF WE STICK TO AA MODELS, WHY DO WE SPEND SO MUCH TIME DISCUSSING THE GY MODEL WHICH IS A CODON LEVEL MODEL? –ANSWER: WE DON'T HAVE A CODON MODEL, WE HAVE AN AA MODEL WHERE THE MUTATION RATES ARE BASED ON DNA DATA. SO WE TRY TO EXPLAIN THE AA DATA AND COMPARE IT TO AA MODELS]. ¿¿¿¿¿¿¿ .r374 These results show that even with our most parameter rich model in which we estimate the optimal amino acid at every site, thereby introducing tens of thousands of additional parameters, our model does a substantially better job fitting the Rokas dataset. So although the computational cost of our model is greater than most GTR models, our ability to fit the phylogenetic data and extract biologically meaningful information is substantially greater than other models. Furthermore, because our approach explicitly links genotype to phenotype, phenotype to fitness, and fitness to fixation rate, the biological assumptions underlying our model are clearly stated and our ability to incorporate additional biological factors, such as selection on codon usage bias, are greatly enhanced.

ADDITIONAL POINTS?

- We also investigate the evolution process of protein with different parameters.

- We use simulations and information from empirical data to find cases

where populations of intermediate size may evolve faster than populations of large size.

3 Methods & Materials

In this study we use a series of continuous time Markov models with amino acid and gene specific parameterizations to describe the process of amino acid substitution for a given protein in a lineage.

Our approach, is applicable to any homologous protein-coding DNA sequence dataset where any gaps have been removed. In our model we assume that there is an optimal amino acid for any given site. The strength of natural selection for the optimal amino acid is a function of the gene's expression level, the physiochemical properties of a given amino acid, and the sensitivity of the gene's functionality to changes in these properties. It can vary between genes and different sites in a gene. (IN CURRENT IMPLEMENTATION SENSITIVITY DOES NOT VARY WITHIN A GENE) As a result, for each of 20 natural amino acids as optimal we have a 20×20 substation rate matrix with the same 20 amino acids as its states. Additional, non-natural amino acids can be easily incorporated into the framework.

Conceptually the substitution process consists of two steps. First, a given amino acid i mutates to amino acid j, the rate of which depends on the mutation rates between nucleotides and the structure of the genetic codes. Second, the newly arisen allele gets fixed in the population with certain probability, which is based on the models from population genetics and includes the effects of natural selection and genetic drift. The is new comparing with the vast majority of models in phylogenetics (EMPIRICAL MODELS AND YANG'S CODON MODEL). Therefore, when the optimal amino acid is k the substitution rate

matrix can be written as $Q_{i,j}^{(k)} = M_{i,j}F_{i,j}^{(k)}$ for $i \neq j$, where M is the mutation rate matrix between amino acids and F is the matrix of fixation probabilities. Note that only F depends on the choice of optimal amino acid k. As usual the row sums of Q equal 0 and the matrix of substitution probabilities after time t is $P^{(k)} = \exp[Q^{(k)}t]$

Calculating the Mutation Rate Matrix M

We use a time reversible model for mutation between amino acids, i.e. $\pi_i M_{i,j} = \pi_j M_{j,i}$ where π_i is the equilibrium frequency of the state i. For all time reversible models, the substitution rate matrix can be written as $M = S\Pi$ where S is a symmetric matrix called the exchange rate matrix and Π is the diagonal matrix of base frequencies π_i's of the states. The model is formulated at the amino acid level, so the calculation of M involves 2 steps.

We begin with finding the mutation rate matrix between 61 sense codons. For simplicity we assume that the mutations occur independently between nucleotides within a codon, and denote the mutation rate matrix for nucleotides by M_ν. For codons that differ only by one nucleotide, the rate between codons is equal to the rate between the said pair of nucleotides. For all other codons, since the changes involving two or more nucleotides during time Δt have probabilities on the order of Δt^2, their mutation rates are set to 0. Therefore the 61×61 mutation rate matrix will have many 0 entries.

Second, from this somewhat sparse codon level mutation rate matrix we can obtain 20×20 amino acid exchange rate matrix S_{aa} by grouping together the synonymous codons for each amino acid. For simplicity, the synonymous codon frequencies for any given amino acid are assumed to be the same. Following the approach developed by Yang (MBE 1998), S_{aa} for a reversible Markov process

of amino acid mutation has entries:

$$(S_{aa})_{ij} = \frac{\sum_{u \in I} \sum_{v \in J} \pi_u \pi_v s_{uv}}{\pi_I \pi_J}$$

where i and j are two different amino acids, I and J are the corresponding sets of synonymous codons for i and j correspondingly, i.e. $c_u = i$ for $u \in I$ and $c_v = j$ for $v \in J$; $\pi_J = \sum_{v \in J} \pi_v$ is the equilibrium frequency of amino acid j by combining the frequencies of synonymous odors for it; and s_{uv} is the exchange rate between codons u and v. The matrix S_{aa} obtained in this way is symmetric, and we can find the mutation rate matrix by $(M)_{ij} = (S_{aa})_{ij}\pi_J$.

——————MIKE (start)——————-

For simplicity, in this study we assume an underlying time reversible model of mutation. [IS THIS TRUE? THIS IS TRUE] In order to calculate the terms of this matrix, we first assume that mutations occur independently between nucleotides within a codon. For codons that differ by only one nucleotide, the mutation rate between codon i and j is equal to the mutation rate between these nucleotide. For all other codons, because changes involving two or more nucleotides during time Δt will have probabilities on the order of Δt^2, we set their mutation rates to 0.

For simplicity, we also assume that synonymous codon frequency for any given amino acid is uniform. Therefore, the equilibrium mutation rates between amino acids only depend on the frequencies of amino acids. This assumption of uniform codon usage could be altered by using a codon, rather than an amino acid, level model.

For all time reversible models, the substitution rate matrix is a product of a symmetric matrix S and the the base frequencies of different states: $Q = S\Pi$, where S is called the exchange rate matrix and Π is a diagonal matrix of the

base frequencies. The mutation rate matrix for 20 amino acids are derived from the 4×4 exchange rate matrix for nucleotides. This reduces the number of rate parameters from 190 to 6 comparing to treating all the amino acid exchange rates as parameters.[THE FACT THAT WE ARE DEFINING A NEW Q IS CONFUSING.-CHANGED IT TO M TO AVOID NO CONFUSION]

From this somewhat sparse 61×61 codon level mutation rate matrix we can obtain 20×20 amino acid exchange rate matrix M by grouping together the synonymous codons for each amino acid. For example, suppose the sets of synonymous codons for amino acids i and j are I and J correspondingly, i.e. $c_u = i$ for $u \in I$, and $c_v = j$ for $v \in J$.[I GOT LOST AFTER THIS. NOTE THAT π_i IS NEVER CLEARLY DEFINED AND SEEMS TO DIFFER FROM LATER USAGE.] Combining synonymous codons J into one state, we have $\pi_J = \sum_{v \in J} \pi_v$ as the equilibrium frequency of amino acid j. The exchange rate matrix for a reversible Markov process of amino acid mutation S has entries:

$$\mu_{IJ} = \sum_{u \in I} \sum_{v \in J} \pi_u \pi_v s_{uv} / (\pi_I \pi_J)$$

And $q_{IJ} = s_{IJ}\pi_J$ with $s_{IJ} = s_{JI}$ constitutes the mutation rate matrix M. For detailed derivation see Yang(MBE 1998). [CAN'T YOU JUST SAY WE FOLLOW THE APPROACH DEVELOPED BY YANG 1998? NO MATTER WHAT, WE SHOULD MENTION THIS PAPER EARLIER. -YES, I AGREE.] The mutation process is time reversible, i.e. $\pi_I \mu_{IJ} = \pi_J M_{JI}$ is satisfied for all $1 \leq I, J \leq 20$. [THIS SECTION STILL NEEDS WORK]

————————MIKE (end)————————-

Calculating the Fixation Probability Matrix F

While the mutation rate matrix M accounts for the effects of the structure of the genetic code and variation in mutation rates on the generation of new alleles,

F describes the probabilities of any such mutation going to fixation.

Modeling the relationship between amino acid sequence and protein function is a complex and challenging problem [CITATION]. No general techniques that accurately and reliably predict a protein's structure, much less function, currently exist. However, empirical data indicates that the effect of an amino acid on a protein's function depends largely on its physiochemical properties. Therefore, we assume that for each site i of the protein there is an optimal amino acid k_i. If a protein consists solely of the optimal amino acids at all sites then it is defined to have 100% functionality, non-optimal amino acids reduce functionality and are, therefore subjected to purifying selection. In our model, the strength of purifying selection is a function of the physiochemical differences between the non-optimal amino acid and the optimal amino acid, a functional sensitivity term, and the expression level of the gene, specifically its average protein production rate ϕ.

Linking Amino Acid Sequence to Protein Functionality: The relative functionality of a given amino acid sequence $\vec{a} = (a_1, a_2, \cdots, a_n)$ is a function of the differences between its physiochemical properties and those of the optimal amino acid sequence $\vec{a}' = (a'_1, a'_2, \cdots a'_n)$ where n is the protein length. Grantham [1974, Science] developed a physiochemical distance metric based on the composition (c), polarity (p) and molecular volume (v) of an amino acid's side chain. Composition is defined as the atomic weight ratio of the on-carbon elements in ending group or rings to carbons in the side chain while side chain polarity and molecular volume are well established (CITATION). Numerous studies (CITATION) have since shown that there is a strong negative correlation between the substitution rates between amino acids and the differences in the three physiochemical properties. Following Grantham (1974) we define

the Grantham distance $d(a_i, a_j)$ between amino acids a_i and a_j as a function of their weighted distances in c, p and v physiochemical space. More precisely, $d_{ij} = [\alpha(c(a_i) - c(a_j))^2 + \beta(p(a_i) - p(a_j))^2 + \gamma(v(a_i) - v(a_j))^2]^{1/2}$ where α, β, γ are the corresponding weights for the 3 components. Other properties, distance measures and scalings could also be used.

Grantham weighted each property by dividing them by the mean distance found with it alone in the formula, afterwards the distances are scaled so that the mean distance between all possible amino acid pairs is 100. For example, given the values for property c of 20 amino acids, its weight α is defined as $(1/\bar{D}_c)^2 = 1.833$ where $\bar{D}_c = \sum_{i=1}^{20} \sum_{j=1}^{20} [(c\,(a_i) - c\,(a_j))^2]^{1/2} / \binom{20}{2}$. Correspondingly, the weights for polarity and molecular volume are $\beta = 0.1018$ and $\gamma = 0.000399$. Subsequent studies using these Grantham distances have used these same weights across different genes and taxa. Although these weights have thus been shown to be useful, there is no prior biological reason to adopt this weighting. On the other hand, one might expect these weights to vary between different proteins. For example, changes in polarity might have a bigger effect on the functionality of a transmembrane protein than changes in composition, while in cytosolic enzyme, the opposite could hold. Consequently, in our model, the weights α, β, γ are treated as estimable parameters rather than being fixed.

With the distance between amino acids defined as above, we define the relative functionality of a protein $\vec{a}$ with optimal sequence $\vec{a}'$ as follows:

$$F(\vec{a}|\vec{a}', g) = \frac{n}{\sum_{k=1}^{n} (1 + d_k g)} \tag{1}$$

where g is a gene specific Grantham sensitivity coefficient which quantifies the sensitivity of a given protein's function to the deviation of physiochemical properties from the optimal sequence, and d_k is the distance between the given and optimal amino acids at the k^{th}position. Note that the optimal amino acid se-

quence has a relative functionality of 1. In order to simplify the notation we will drop $\vec{a}'$ and g when there is no potential ambiguity.

Defining Protein Fitness: Following our previous work relating protein production cost, relative functionality, and energy expenditure to fitness [CITATION] **??**, we define a cost-benefit function $\eta(\vec{a}|\vec{a}')$ as the cost of producing the protein sequence $\vec{a}$ over its expected functionality. Here we assume that the cost of protein translation is simply proportional to the length of the protein produced. Thus,

$$\eta\left(\vec{a}|\right) = \frac{C(n)}{F\left(\vec{a}|\vec{a}', \vec{g}\right)}, \tag{2}$$

where $C(n)$ is the cost of producing a protein of length n. Based on the basic biology of protein translation, we define $C(n) = a_1 + a_2 n$ where $a_1 = 4$ ATPs is the cost in ATPs of ribosome assembly on an mRNA transcript and $a_2 = 4$ ATPs is the cost in ATPs of tRNA charging and the moving the ribosome forward one codon.

For a given gene, we assume that there is a mean target functionality production rate, i.e. the average rate at which the organism requires the production of the functionality encoded by that gene. Thus, if $\eta(\vec{a})$ represents the cost of producing one unit of functionality and the organism, on average, needs to produce that functionality at rate ϕ, then $\eta(\vec{a}) \times \phi$ represents the rate at which the organism must spend energy to meet its functionality requirement for a given gene. Letting q represent the proportional gain in fitness for each ATP saved per unit time, we can define the relative fitness of a protein $\vec{a}_i$ as

$$f(\vec{a}_i) = f_i \propto \exp\{-\frac{\Phi q C(n)}{F\left(\vec{a}_i|\vec{a}'\right)}\}.$$

Clearly, fitness f_i is an increasing function of functionality F and the strength of selection on F increases with protein length n and expression level ϕ. Note

that n, ϕ, and $\vec{a}'$ vary between loci, $\vec{a}$ varies between allels at a given locus, but q is the same for all genes.

Following the model of allele fixation presented by **?**, the fixation probability of a newly introduced mutant allele $\vec{a}_j$ in a Fisher-Wright population with the resident allele $\vec{a}_i$ and an effective size of N_e is,

$$\pi_{ij} = \pi\left(\vec{a}_i \rightarrow \vec{a}_j\right) = \frac{1 - \left(f\left(\vec{a}_i\right)/f\left(\vec{a}_j\right)\right)^{\alpha}}{1 - \left(f\left(\vec{a}_i\right)/f\left(\vec{a}_j\right)\right)^{2N_e}}. \tag{3}$$

where $\alpha = 1$ for a diploid population and 2 for a haploid population. Here we focus on diploid populations. This formula for the fixation of a mutant allele is valid under weak mutation assumptions, i.e. $s, \frac{1}{N_e}, Ns^2 \ll 1$ where $s = 1 - \frac{f(\vec{a}_i)}{f(\vec{a}_j)}$.[SELLA AND HIRSH NEVER MENTION WEAK SELECTION IN MAIN TEXT. ONLY WEAK MUTATION. SHOULD CHECK SUPPORTING MATERIALS -CHECKED, SINGLE MUTATION IN A LARGE POPULATION, AND WEAK SELECTION, THIS IS ALSO THE CONDITION FOR THE CANONICAL FORMULA] Alternative fixation calculations, such as the canonical forms derived by Fisher, Moran(1960, 1961) **??** or **?** could also be used. [FISHER AND WRIGHT CITATIONS TAKEN FROM KIMURA PAPER. SHOULD CHECK. - ALSO MORAN]

The fixation probability described by Equation 3 depends on the ratio of the resident and mutant alleles fitnesses, f_i/f_j. Using the definitions of protein translation cost $C(n)$ and functionality $F\left(\vec{a}|\vec{a}'\right)$ in Equation 1, we get

$$\frac{f\left(\vec{a}_i\right)}{f\left(\vec{a}_j\right)} = \prod_{k=1}^{n}\left(\frac{f\left(\vec{a}_i^{\,k}\right)}{f\left(\vec{a}_j^{\,k}\right)}\right)^{\frac{1}{n}}. \tag{4}$$

Thus under the assumptions of our model, the fitness ratio of the resident and mutant genotypes $\vec{a}_i$ and $\vec{a}_j$ is the geometric mean of the fitness ratios between the two amino acids at all sites. When $\vec{a}_i$ and $\vec{a}_j$ only differ at a single amino

acid position k, the fitness ratio f_i/f_j simplifies to

$$\begin{aligned}
\frac{f\left(\vec{a}_i\right)}{f\left(\vec{a}_j\right)} &= \left(\frac{f\left(\vec{a}_i^k\right)}{f\left(\vec{a}_j^k\right)}\right)^{\frac{1}{n}} && (5)\\
&= \exp\left[-q\phi\left(\frac{C(n)}{F\left(\vec{a}_i\right)}-\frac{C(n)}{F\left(\vec{a}_j\right)}\right)\right]\\
&= \exp\left[-q\phi\frac{C(n)}{n}\left(d_k^{(i)}-d_k^{(j)}\right)g\right] && (6)\\
&= \exp\left[-q\phi a_2\left(d_k^{(i)}-d_k^{(j)}\right)g\right] && (7)
\end{aligned}$$

where, again, a_2 is the cost of each elongation step during protein translation. Equation (7) shows that in our model even though the $F(\vec{a})$ is a non-linear function of the entire sequence, the fitness ratio of two alleles depends only on the site that differs. Thus, within a given gene each amino acid site evolves independently of the other. This equation also indicates that the strength of selection on these distance differences increases with the value q, the cost of an elongation step a_2, gene's expression level ϕ, and the sensitivity of protein function to its deviation from the optimal sequence.

Substituting Equation (7) into Equation (3) gives,

$$\pi_{ij}=\frac{1-\exp\left[-q\phi a_2\left(d_k^{(i)}-d_k^{(j)}\right)g\right]}{1-\exp\left[-q\phi a_2\left(d_k^{(i)}-d_k^{(j)}\right)g2N_e\right]}. \qquad (8)$$

Equation (3) shows that the fixation probability of an allele is a function of the fitness ratio f_i/f_j as well as effective population size N_e.

Therefore, connecting mutation and fixation steps together, the instantaneous substitution rate q_{ij} from $\vec{a}_i$ to $\vec{a}_j$ is equal to the rate at which a mutant is introduced, times fixation probability of the mutant π_{ij}:

$$q_{ij}=2N_e\mu_{ij}\pi_{ij}. \qquad (9)$$

Given the values for $(M_\nu, g, \alpha, \beta, \gamma, C, \Phi, q, N_e)$, the frequencies of different amino acids Π and the optimal amino acid k at a site, we can calculate the

20×20 instantaneous substitution rate matrix $Q^{(k)}$ for the Markov process. Q is scaled by the frequencies of amino acids to satisfy $\sum_{i=1}^{20} \pi_i q_{ii} = -1$. Under this restraint, the length of a branch represents the expected number of substitutions along the branch. With the probabilities $P(t) = \exp(Qt)$ the likelihood for a given tree topology can be calculated following Felsenstein (1981). Since all sites are independent, we can calculate the likelihood of observing the sequence data at the tips of a phylogenetic tree T with given topology and branch lengths by multiplying the likelihood values at all sites.

Table 1: parameters in the model

s_{ij}	exchange rates between nucleotides i and j
μ_{ij}	mutation rates from nucleotides i to j
π_{ij}	fixation probability of single mutant j from i
q_{ij}	substitution rate from amino acid i to amino acid j
g	sensitivity coefficient of functionality to physicochemical distance
(α, β, γ)	weights for the 3 physicochemical properties in amino acid distance formula
C	cost of producing a protein
ϕ	expression level
q	scaling factor
N_e	effective population size

3.1 Identification of optimal amino acids

To calculate the likelihood values, the optimal amino acids need to be identified. We implement 3 approaches to identify the optimal amino acid at a certain site. First one is called "max rule". We calculate the likelihood values when each

of 20 amino acids is optimal with all other parameters given and choose the one that maximizes the likelihood as optimal. This method treats the optimal amino acids as estimable parameters in the maximum likelihood computation. The number of parameters increases with the number of distinct sequence patterns at the tips, which often is a big number.

Second approach uses the "majority rule", i.e. the most frequent amino acid in the sequence is chosen as the optimal amino acid. If more than 1 amino acid has the same highest frequency, then one of them is picked randomly as optimal. If the sequences have evolved long enough to reach equilibrium, the optimal amino acid has the highest probability to be observed. If the evolving time is short, or there are not enough substitutions during evolution process, the optimal amino acids estimated this way can be inaccurate.

The third method is "weighted rule". 20 amino acids are assigned weights of being optimal. If the same weights are used for all sites, then the number of parameters added is 19 compared to hundreds or more in the first approach. The weights are expected to vary with the environment, function of proteins and other factors. Therefore an alternative is to use different weights for different genes or gene groups in a protein sequence.

Apparently the first method gives the best likelihood value but uses the most parameters. On the other hand, the third method uses much fewer parameters. However, if the optimal amino acids vary a lot between different sites, the likelihood values will decrease significantly. We'll compare the performance of different approaches in the Results section.

3.2 Identifiability of parameters

Since C, Φ, q and g are multiplied together as a composite parameter, we fix the values of C, Φ, q and search for MLE for g. As mentioned earlier, for the weights used in the Grantham distance formula, α is fixed and β, γ are estimated. In addition, the effective population size is assumed to be fixed in this paper. Suppose the phylogenetic topology is given, we are estimating the following parameters: $g, \beta/\alpha$, γ/α, frequencies of amino acids, branch lengths, and the exchange rate matrix for nucleotides.

4 Results

4.1 Results on Rokas et al.'s data on yeast

We analyzed data previously studied by Rokas (2003 Nature). This genome sequence data have been obtained for 7 *Saccharomyces* species (*S. cerevisiae, S. paradoxus, S. mikatae, S. kudriavzevii, S. bayanus, S.castellii* and *S. kluyveri*) as well as for the outgroup fungus *Candida albicans*. It includes 106 genes that are distributed throughout the *S. cerevisiae* genome on all 16 chromomosomes and comprises a total length of 42,342 amino acids. Rokas et.al analyzed this data set to investigate the conflict of gene trees. We use the tree topology that is supported by the concatenated genome sequence, which is also supported by the majority of the genes as found in Rokas et al.'s paper. Since the new model is not time reversible the tree is rooted at the out group *C.alb*.

4.1.1 maximum likelihood estimation

First, the 106 gene sequences are concatenated as 1 whole sequence with 42,342 amino acids. We use ProtTest to find maximum log likelihood values under

empirical models and compare their AIC values. We also find the maximum log likelihood values under our new model, with all 3 approaches to treat the optimal amino acids. In all the analyses, tree branch lengths are optimized while the topology is not.

The log likelihood values and the AIC values are compared in Table 2. Under the empirical models, the substitution rates are fixed instead of being optimized. I denotes that proportion of invariable sites is estimated in the model, G means that Gamma distributed rate variation across all sites is included in the model. In models with F, amino acid frequencies are treated as free parameters and estimated by the observed frequencies in the sequence data. Otherwise, the equilibrium frequencies under the substitution rate matrix are used.

Under the new model, amino acid frequencies are treated as 19 free parameters and estimated from the observed frequencies in the sequence data. In addition, there are 5 free parameters for exchange rates between nucleotides with the rate between G and T fixed as 1, 14 branch lengths for the 8-species rooted phylogeney, Grantham sensitivity g, 2 free parameters for the weights in the physicochemical distance formula β and γ. These 41 parameters are optimized in the maximum likelihood analyses as well as the optimal amino acids.

All the parameters are treated the same across all the sites except the optimal amino acids. The loglikelihood and AIC values are also compared with those under empirical amino acid based models from ProtTest (reference).

If the optimal amino acids are not counted as free parameters being estimated, the majority approach gives the best AIC value. ΔAIC value for the

Table 2: Log-likelihood values and parameter estimates under empirical models and new model for the sequence with 42,342 amino acids

Model	ΔAIC	l	Tree length	Parameters
New+maj	0.00	-257790.10	10.43	41
New+max	48576.60	-239736.40	11.75	42,383
New+weights	123709.40	-319625.80	2.74	60
LG+I+G+F	81803.98	-298699.09		34
LG+G+F	81801.98	-298699.09		33

Note: The last two models in the table are the best models picked out by ProtTest.(What happens when the weights of amino acids being optimal are gene specific and other parameters are fixed across genes? Total number of parameters is 40,369. Better case scenario, we estimate different optimal weights and other parameters genewise, this should give a better likelihood value in total compared to only optimal weights are gene specific. In this better case, the total loglikelihood value is -311186. Even with this loglikelihood value and number of parameters 40,369, the ΔAIC value is 187447.8, which means it performs worse than the third model in the table. The real loglikelihood value under this model is -317214.68; it gives a larger AIC value.)

best empirical model LG+I+G+F is more than 8,0000 units, which indicates a substantially better fit under the new model. One thing that needs to point out is that under the maximizing approach for identifying optimal amino acids at each position in the sequence, the number of parameters is much greater since we add 1 parameter at each site. However, the improvement of log likelihood is so big that this model still performs much better than the best empirical model, with AIC value 40,000 units smaller. With the weighted approach, i.e. across all sites, every amino acid has the same probability of being optimal, the decrease

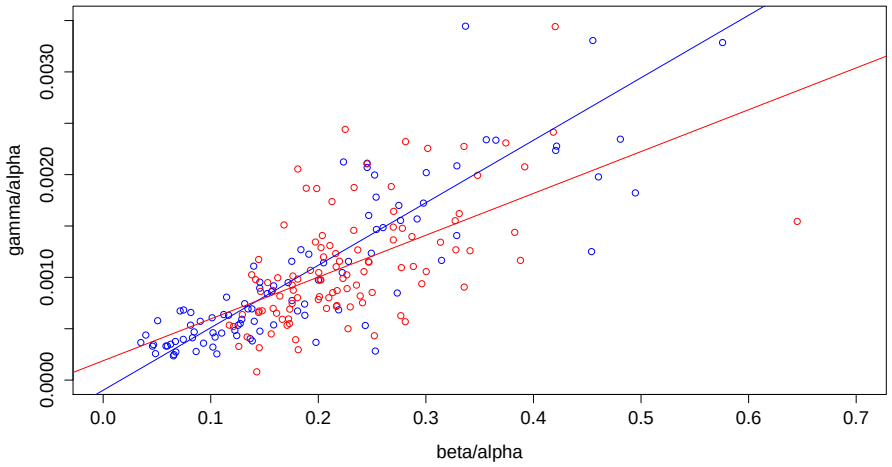
in the log likelihood outweighs the reduction in the number of parameters. This also indicates that the optimal amino acids vary a lot across the sites.

4.1.2 Parameter variation between genes

We also analyzed Rokas et.al.'s data gene by gene. Under both approaches (max and maj) of obtaining the optimal amino acids, the estimates for Grantham sensitivity and weights are on the similar scale. As expected, the weights for physicochemical properties are also similar to the ones that Grantham proposed. Figure 1 showed the correlation between β and γ. Linear regression suggests strong linear relationship between the 2 parameters, especially under the maximizing rule where R^2 is very close to 1. Note that α value is fixed for all sites as 1.833, the results indicate that the ratios between weights for the 3 components in the distance formula do not vary a lot.

Since the variation of Grantham weights across genes is small, we set β and γ across all genes to be the same and optimized g for each gene to get the maximum likelihood. Other parameters values are retained from the maximum likelihood estimates in the max approach. We then did optimization on the common physicochemical weights β and γ. The ML estimates are $\beta = 0.1182$ and $\beta = 0.000574$, comparing to Grantham's weights $\beta = 0.1018$ and $\gamma = 0.000399$; and the log likelihood value is -236935.65. The log likelihood value increased by 2800 units comparing to the max approach when g is the same across all genes. If other parameters including tree branch lengths and exchange rates between nucleotides are also optimized the likelihood will be increased further. Since the number of genes is 106, allowing each gene to have different Grantham sensitivities increases the AIC value.

Figure 1: Correlation between β and γ. Blue line is the maximizing rule, with $R^2 = 0.9899$, Red line is the majority rule with $R^2 = 0.3258$

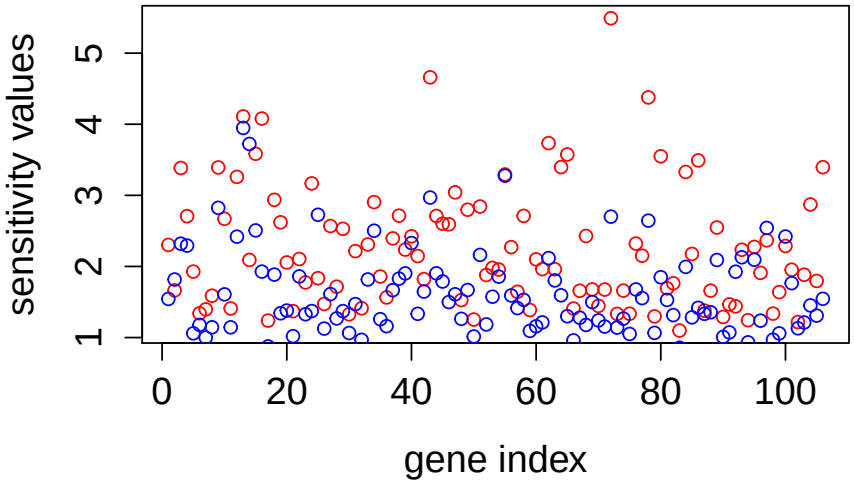


There are several reasons to explain the variation of g values between genes. One, these genes have different structures, which caused the different degrees of sensitivity to the difference from the optimal amino acids. For example, hydrophobic cores of proteins can be efficiently repacked with different hydrophobic sequences. All polar amino acids can form hydrogen bonds whose thermodynamic energy varies sharply with distance and angle, providing a rationale for the greater variability of the fitness of polar amino acids. Two, we use the same tree topology for all genes. However, sequences in some gene might better support a different tree topology, therefore causes other parameter estimates to be inaccurate.

Next we examine the g values across all 106 genes in the data by estimating all the parameters in the model separately for each gene. From figure 2, we

can see that the estimates of g values under max and maj rule are consistent with max rule having a slight bigger variation. Figure 3 confirms the linear correlation between the estimates under the 2 approaches for finding optimal amino acids.

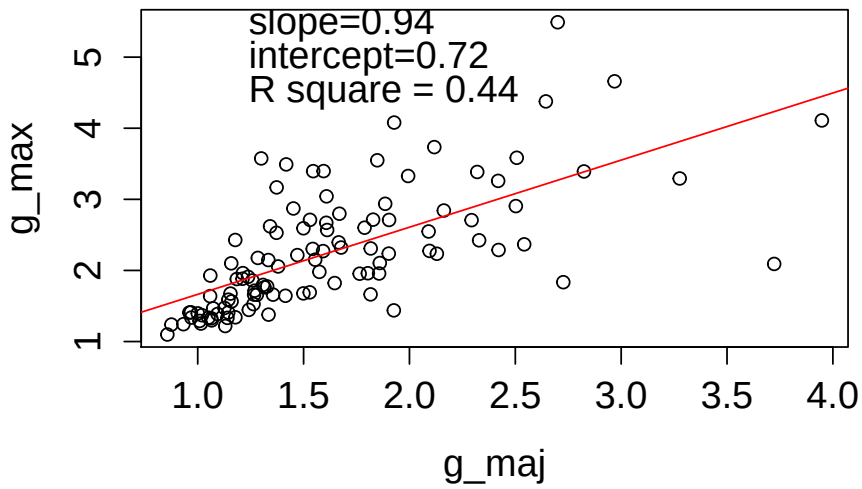
Figure 2: Plots of Grantham sensitivities across all the genes. Red are the values under max rule, and blue are under maj rule.



4.1.3 Confidence of estimates of optimal amino acids

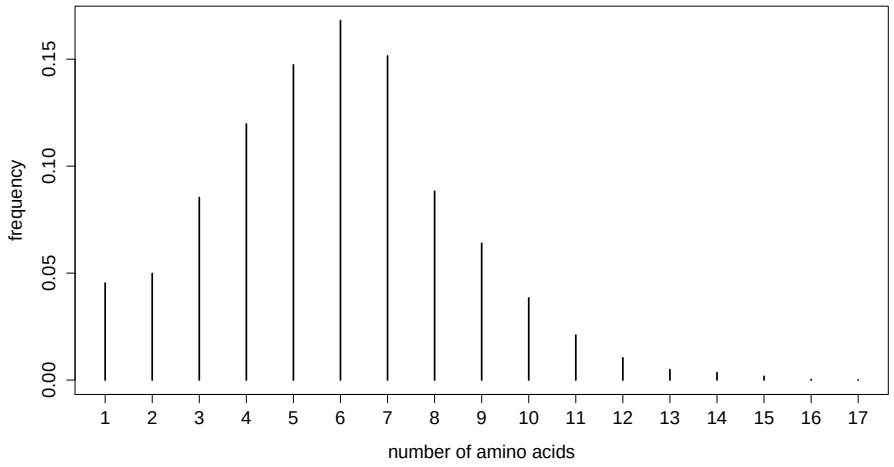
To get the confidence level of the estimates for optimal amino acid at each site with the maximizing approach, we found the smallest set of amino acids being optimal that cover more than 95% of the total likelihood. In Rokas's data there are about 9000 different state patterns at the 8 species. For each of the 9000+ sites, the likelihood values achieved by assuming each amino acid as optimal

Figure 3: Plots of Grantham sensitivities across all the genes. Values under max rule are plotted against under maj rule and the linear regression line is shown in red.



is ordered decreasingly, therefore the likelihood under the max optimal amino acid is ranked the first. Then the next amino acid is included in the optimal set of amino acids until the total likelihood exceeds 95% of the total likelihood. Figure 4 shows the histogram of numbers of optimal amino acids in the set. The mean value for all 9000+ patterns is 5.855, and mode is 6. The case where there are more than 10 amino acids in the set rarely happened. Figure 5 showed the density of percentages of total likelihood value covered by the optimal amino acid found with max rule only. Mean percentage is 0.4749 and the peak of the density distribution is between 0.3 and 0.4. (How confident are we now??)

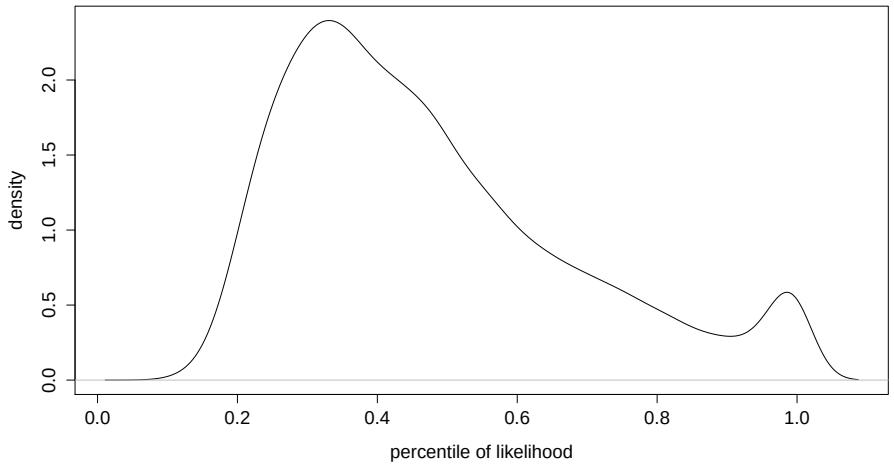
Figure 4: Histogram of the number of optimal amino acids together to cover at least 95% of the total likelihood attained by all possible optimal amino acids.



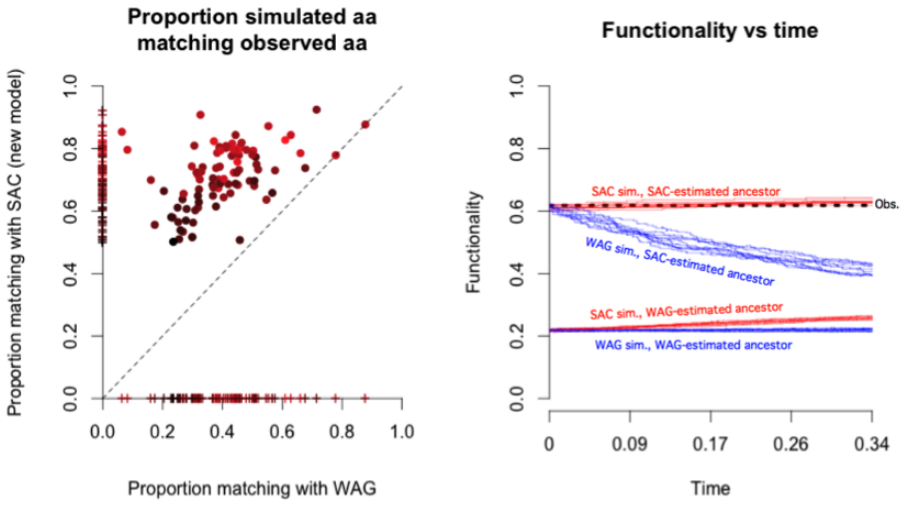
4.2 Simulated data vs. observed data

We also evaluate the models by simulating data under models and comparing them with the observed data. First, a single taxon (Scas) is deleted from the Rokas's yeast gene tree, model parameters are estimated from the data on the remaining taxa. Then the ancestral sequence where the missing taxon is attached is estimated, where the probabilities of observing all 20 amino acids at each site are calculated. Then the deleted data is put back and the length of the truncated branch earlier is estimated using the parameters on the pruned tree. With the parameter values, the length of the missing branch, and the sequence at the start of the branch, we simulate the evolution of a gene's coding sequence from the reconstructed taxon to the deleted taxon from our analysis. We then compare the simulated sequence at the deleted taxon with the real data. The results are shown in Figure 4.2. On the left, each dot represents the

Figure 5: Density plot of percentages of the likelihood achieved by the optimal amino acid found by max rule.



proportion of amino acids that differ between the simulated and the observed sequence for a given gene. Our new model performed much better than the standard WAG model in matching sequences, especially for genes under high selection that are shown in brighter dots. On the right repeated simulations are plotted under our new model (red) and WAG model (blue) starting from the ancestral sequence estimated under the estimated sequences under the new model (upper lines) and WAG model (lower lines) for a single gene. The dotted line represents the functionality for the observed sequence. When the ancestral sequences are estimated from WAG model, they have much lower fitnesses. If evolved under WAG model, the fitness does not improve much at the end of the branch. On the other hand there is directional selection leading to an increase in fitness if the sequences evolve under the new model. When the ancestral sequences are estimated from the new model the fitness is significantly



higher. And WAG simulation leads to a decrease in fitness while the fitness is maintained under simulation with the new model. No matter how the ancestral sequences are obtained, the new model presents a better match to the observed data. This realistic behavior shows that the new model is more adequate than the WAG model for Rokas's data. [I THINK WE SHOULD USE THE ACTUAL BEST MODEL UNDER PROTTEST, PLUS AA BASED ON GY94 AND GTR CODON/NUCLEOTIDE MODELS]