

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 the relationship between genotype, phenotype, and fitness and uses a model of allele substitution from population genetics to calculate the fixation probability of a newly arisen mutant. 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 multilocus 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, in mechanistic model the substitution matrices 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: DNA, codon and amino acid (AA) level. DNA-based models use the most data and are often the most powerful in terms of their ability to distinguish between closely related sequences. [NO, CODON BASED MODELS HAVE THE MOST DATA: ALL THE INFO IN DNA, PLUS KNOWING WHAT AA WILL BE MADE] 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. 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 [EXCEPTIONS?]. 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 to 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}' . 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 \bar{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]. 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 amino acid and gene specific parameterizations of a continuous time Markov models to describe the amino acid substitution process in a lineage. Our approach, therefore, is applicable to any homologous protein-coding DNA sequence dataset where any gaps have been removed. In our model we assume that for any given site there is an optimal amino acid for that site. The strength of natural selection for the optimal amino acid can vary between sites and genes. The strength of selection is modeled to be 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. As a result, our approach uses 20 different substitution rate matrices, one for each amino acid. The states of the substitution matrices are these same 20 amino acids. Currently, each substitution matrix consists of a 20×20 rate matrix $\mathbf{Q}^k(\vec{\Lambda}) = \{Q_{i,j}^k(\vec{\Lambda})\}$ where $Q_{i,j}^k(\vec{\Lambda})$ represents the instantaneous rate that amino acid i will be substituted by amino acid j given a set of parameters $\vec{\Lambda}$. Our model could be formulated more generally, such as at the codon level or to include nonnatural amino acids.

Conceptually, we subdivide the parameter set $\vec{\Lambda}$ into two different groups $\vec{\Lambda}_1$ and $\vec{\Lambda}_2$. $\vec{\Lambda}_1$ contains the mutation rates between the different codons while $\vec{\Lambda}_2$ contains terms related to genotype fitness and fixation probability. More specifically, $\vec{\Lambda}_1$ includes parameters describing the sensitivity of the protein’s function to the differences in the physiochemical properties of the optimal and observed amino acid, the average expression level of the gene, specifically protein production rate ϕ , and effective population size N_e . The separation of the parameters in $\vec{\Lambda}$ reflects the two step manner in which we chose to calculate the terms of a given rate matrix Q^k . The first step is calculating the rate at

which a given amino acid i mutates into amino acid j , $M(\vec{\Lambda}_1) = \{M_{i,j}(\vec{\Lambda}_1)\}$, given the mutation rates between nucleotides and the structure of the genetic code. The second step is calculating the probability that the newly introduced allele goes to fixation $F(\vec{\Lambda}_2) = F_{i,j}^k(\vec{\Lambda}_2)$ given the wild type amino acid j , the mutant amino acid i , and the optimal amino acid k for the site, and $\vec{\Lambda}_2$. Unlike most other work in the field of phylogenetics, the fixation probability $F_{i,j}$ is based on models in the field of population genetics and includes the effects of both natural selection for the optimal amino acid and genetic drift. Unlike most other work in the field of phylogenetics, the fixation probability $F_{i,j}$ is based on models in the field of population genetics and includes the effects of both natural selection for the optimal amino acid and genetic drift. The elements of the substitution matrices are, therefore, defined as $Q_{i,j}^k = M_{i,j}(\vec{\Lambda}_1)F_{i,j}^k(\vec{\Lambda}_2)$ for $i \neq j$. The diagonal elements of Q are equal to the opposite of their off diagonal row sums. As a result, as usual, the row sums of Q^k equal 0. Furthermore, the matrix describing the substitution probability of amino acid i to amino acid j as a function of time is $P^k(t|\vec{\Lambda}) = \exp \left[Q^k(\vec{\Lambda})t \right]$. [IS MY PHRASING CORRECT?] ===== llllllll .r376

Conceptually, we subdivide the parameter set $\vec{\Lambda}$ in two: $\vec{\Lambda}_M$ and $\vec{\Lambda}_F$. The subset of parameters $\vec{\Lambda}_M$ contains the mutation rates between the different amino acids while the subset $\vec{\Lambda}_F$ contains parameters related to genotype fitness and fixation probability. More specifically, $\vec{\Lambda}_F$ includes parameters describing the sensitivity of the protein's function to the differences in the physiochemical properties of the optimal and observed amino acid, the average expression level of the gene, specifically protein production rate ϕ , and the lineages effective population size N_e . The separation of the parameters in $\vec{\Lambda}$ reflects the two step manner in which we chose to calculate the terms of a given rate matrix \mathbf{Q}^k . The first step is calculating the rate at which a given amino acid i mutates into amino

acid j , $M(\vec{\Lambda}_M) = \{M_{i,j}(\vec{\Lambda}_M)\}$, given the mutation rates between nucleotides and the structure of the genetic code. The second step is calculating the probability that the newly introduced allele goes to fixation $F(\vec{\Lambda}_F) = F_{i,j}^k(\vec{\Lambda}_F)$ given the resident amino acid j , the mutant amino acid i , and the optimal amino acid k for the site, and $\vec{\Lambda}_F$. Unlike most other phylogenetic studies, here the fixation probability $F_{i,j}^k$ is based on the result of a model from the field of population genetics and, consequently, includes the effects of both natural selection for the optimal amino acid and genetic drift. The elements of the substitution matrices are, therefore, defined as $Q_{i,j}^k = M_{i,j}(\vec{\Lambda}_M)F_{i,j}^k(\vec{\Lambda}_F)$ for $i \neq j$. The diagonal elements of \mathbf{Q}^k are equal to the opposite of the sum of the off diagonal elements in a given row such that the row sums of \mathbf{Q}^k equal 0. Furthermore, the matrix describing the substitution probability of amino acid i to amino acid j as a function of time is $P^k(t|\vec{\Lambda}) = \exp[\mathbf{Q}^k(\vec{\Lambda})t]$. [IS MY PHRASING CORRECT?]

Calculating the Mutation Rate Matrix M

For simplicity, in this study we assume an underlying time reversible model of mutation. [IS THIS TRUE?] Our current model formulation is at the amino acid level, but in order to properly construct M we need to consider the structure of the genetic code so we begin with a 61×61 sense codon mutation rate matrix. 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.]

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.] The mutation process is time reversible, i.e. $\pi_I \mu_{IJ} = \pi_J \mu_{JI}$ is satisfied for all $1 \leq I, J \leq 20$.

[THIS SECTION STILL NEEDS WORK]

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 probability any such mutation will go to fixation. Unlike the vast majority models in phylogenetics (e.g [CITATIONS], but see [CITATIONS] for exceptions [OR MENTION EARLIER IN INTRODUCTION]), we calculate F using a population genetics model which explicitly include the effects of natural selection and genetic drift.

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 positive or negative effect of an amino acid on a protein's function depends largely on its physiochemical properties. As a result, we assume that for each site i in the protein there is an optimal amino acid k_i and that the substitution of non-optimal amino acids at a given site reduces the protein's functionality. While the biological role of a protein varies between genes, we define a protein as having 100% functionality if it consists solely of the optimal amino acid at every site. Non-optimal amino acids reduce functionality below 100% 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, as well as 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: In our model we avoid having to calculate the absolute functionality of a given protein and, instead, define it relative to some optimal amino acid sequence. More specifically, the relative functionality of a given amino acid sequence $\vec{a} = (a_1, a_2, \dots, a_n)$ is a function of the differences between its physiochemical properties and the optimal amino acid sequence $\vec{a}' = (a'_1, a'_2, \dots, a'_n)$ where n is protein length. ? developed a set of scaled physiochemical distance metrics 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 non-carbon elements in end groups or rings to carbons in the side chain. The last two properties are from published data (add ref). Numerous studies [CITATIONS] have since shown that there is a strong, negative correlation between the substitution rates between amino acids and the differences in their physiochemical properties as identified by Grantham. Following ?, we calculate the overall physicochemical difference between any two amino acids i and j , d_{ij} as a function of their weighted distances in the c , p , and v physiochemical space, i.e. $d_{ij} = [\alpha(c_i - c_j)^2 + \beta(p_i - p_j)^2 + \gamma(v_i - v_j)^2]^{1/2}$ where α, β, γ are the corresponding weights for the 3 components. Clearly other metrics and scalings could be used. ===== Composition is defined as the atomic weight ratio of noncarbon elements in end groups or rings to carbons in the side chain while side chain polarity and molecular volume are well established. Numerous studies [CITATIONS] have since shown that there is a strong, negative correlation between the substitution rates between amino acids and the differences in the physiochemical properties c , p , and v . Following ?, we define the physiochemical Grantham distance between amino acids a_i and a_j as $d(a_i, a_j)$ and assume it is a function of their weighted distances in c , p , and v physiochemical space. More precisely, we assume $d(a_i, a_j) = [\alpha(c(a_i) - c(a_j))^2 + \beta(p(a_i) - p(a_j))^2 + \gamma(v(a_i) - v(a_j))^2]$

where α, β, γ are the corresponding weights for the 3 components. Clearly, other properties, distance measures, and scalings could be used. .r376

In Grantham (1974) the physiochemical weighting factors α, β , and γ were calculated such that the mean distance between all possible amino acids pairs in any given axis of physiochemical space, i.e. c, p , or v , is 1. For example, given the values for this property $c(a_i)$'s for 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 weightings have thus been shown to be very useful, there is no aprior biological reason to adopt this weighting. Additionally, one might expect these weightings 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. [SHOULD MOVE THIS DISCUSSION TO THE INTRO AND RE-VISIT IN THE DISCUSSION SECTION] Instead of assuming that the mean weighted pairwise distance within a physiochemical property sums to one as in Grantham's original study, we assume that the mean weighted pairwise distance across all physiochemical properties sums to 1, i.e. $(\alpha^2 + \beta^2 + \gamma^2)^{1/2} = 1$. Because of this constraint on the of sum of the weighting terms, we fix the weight α to be 1.833 as in Grantham's weights, and estimate the free variables β and γ .

Thus for any given amino acid sequence we can calculate a distance vector $\vec{d} = (d(a_1, a'_1), d(a_2, a'_2), \dots, d(a_n, a'_n))$ describing the physiochemical differences between the given and optimal amino acids at each position. In order to

ensure that protein functionality declines with its deviation from the optimal sequence and with a lower bound of zero, we define the relative functionality of the protein \vec{a} as,

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

where g is a gene specific Grantham sensitivity coefficient which quantifies the sensitivity of a given gene's function to deviation from the physiochemical properties of the optimal sequence. Note that the optimal amino sequence has a relative functionality of 1. In order to simplify our notation, we will drop the \vec{a}' and g when convenient and there is no potential ambiguity.

Defining Relative Protein Functionality Based on an Amino Acid Sequence: Following our previous work relating protein production cost, relative functionality, and energy expenditure to fitness ??, we define a cost-benefit function $\eta(\vec{a}|\vec{a}')$ which represents the cost of producing the protein sequence \vec{a} over its expected functionality. Here we assume that the cost of protein translation does not vary between \vec{a} of a given protein, but instead is simply proportional to the length of the protein produced. Thus,

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

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 $a_1 = 4\text{ATP}$ is the cost in ATPs of ribosome assembly on an mRNA transcript and $a_2 = 4\text{ATP}$ 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 genotype i as,

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

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 alleles 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,

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

where $\alpha = 1$ for a diploid population and 2 for a haploid population. Here we focus on diploid populations. This formulat for the fixation of a mutant allele is valid under weak mutation assumptions, i.e. $\mu N_e \ll 1$. [SELLA AND HIRSH NEVER MENTION WEAK SELECTION IN MAIN TEXT. ONLY WEAK MUTATION. SHOULD CHECK SUPPORTING MATERIALS] Alternative fixation calculations, such as the canonical forms derived by ?? or ? could also be used. [FISHER AND WRIGHT CITATIONS TAKEN FROM KIMURA PAPER. SHOULD CHECK.]

The fixation probability described by Equation 3 depends on the ratio of the mutant and resident alleles fitnesses, f_i/f_j . Using the definitions of protein

translation cost $C(n)$ and functionality $F(\vec{a}|\vec{a}')$ in Equation 1, we get

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

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

$$\frac{f(\vec{a}_i)}{f(\vec{a}_j)} = \left(\frac{f(\vec{a}_i^k)}{f(\vec{a}_j^k)} \right)^{\frac{1}{n}} \quad (5)$$

$$= \exp \left[-q\phi \left(\frac{C(n)}{F(\vec{a}_i)} - \frac{C(n)}{F(\vec{a}_j)} \right) \right] \quad (6)$$

$$= \exp \left[-q\phi \frac{C(n)}{n} \left(d_k^{(i)} - d_k^{(j)} \right) g \right] \quad (7)$$

where, again, a_2 is the cost of each elongation step during protein translation. Substituting in the definition of f_i/f_j in Equation (??) into Equation (3) gives,

$$\pi_{ij} = \frac{1 - \exp \left[-q\phi a_2 \left(d_k^{(i)} - d_k^{(j)} \right) g \right]}{1 - \exp \left[-q\phi a_2 \left(d_k^{(i)} - d_k^{(j)} \right) g 2N_e \right]}. \quad (8)$$

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 different distances increases the value of an ATP, q , the cost of an elongation step a_2 , the gene's expression level ϕ , and the sensitivity of protein function to its deviation from the optimal sequence g . Equation (8) 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, 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, i.e. 2 times the product of effective population size N_e and the mutation rate μ_{ij} , times fixation probability of the mutant π_{ij} . That is,

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

Given the values for $(M_\nu, g, \alpha, \beta, \gamma, C, \Phi, q, N_e)$, the frequencies of different amino acids and the optimal amino acid at a site, we can calculate the 20×20 instantaneous substitution rate matrix \mathbf{Q}^k for the Markov process. \mathbf{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 $P(t) = \exp(\mathbf{Q}t)$ 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.

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

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
u_{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

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 parame-

ters. 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: $g, \beta/\alpha, \gamma/\alpha$, 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 chromosomes 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 phylogeny, 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 op-

timal amino acids. The loglikelihood and AIC values are also compared with those under empirical amino acid based models from ProtTest (reference).

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.)

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 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

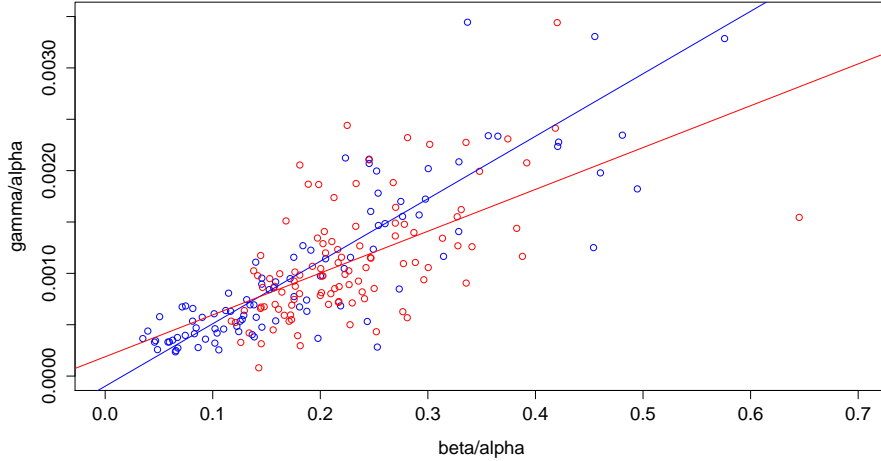
each position in the sequence, the number of parameters is much greater since we add 1 parameter at each site. However, the improvement of log likelihood is so big that this model still performs much better than the best empirical model, with AIC value 40,000 units smaller. With the weighted approach, 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

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 $R^2 = 0.3258$



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.

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.

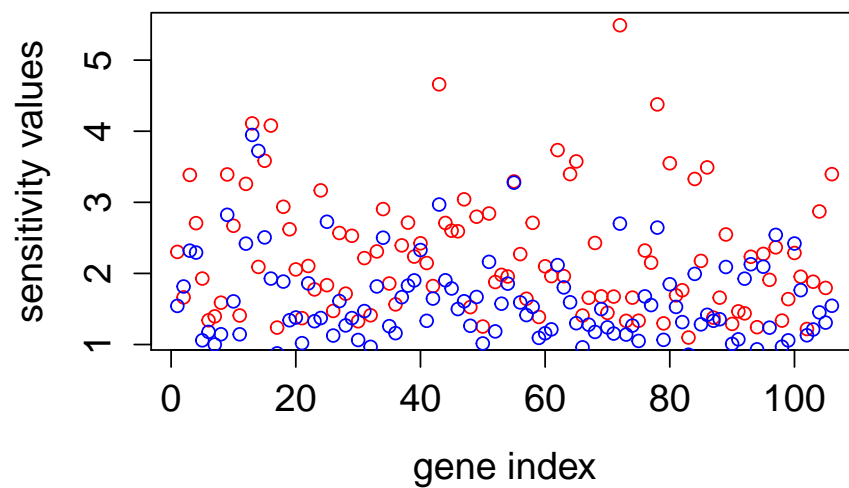
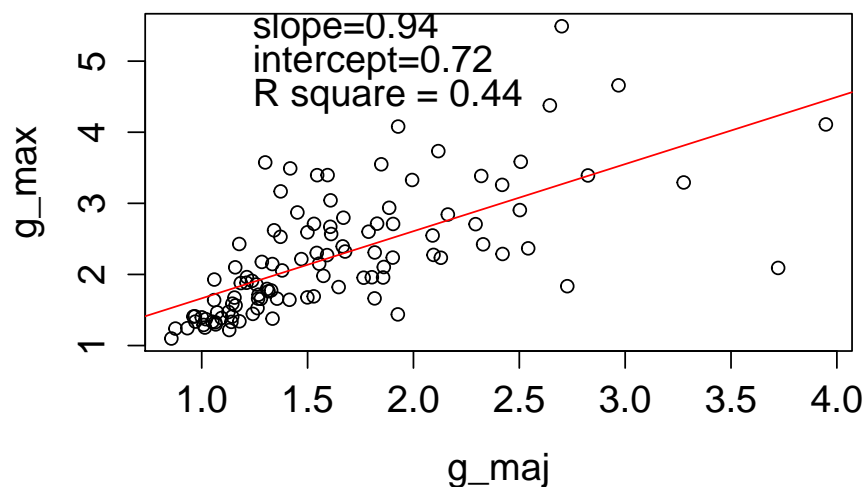


Figure 3: Plots of Grantham sensitivities across all the genes. Values under max rule are plotted against under maj rule and the linear regression line is shown in red.

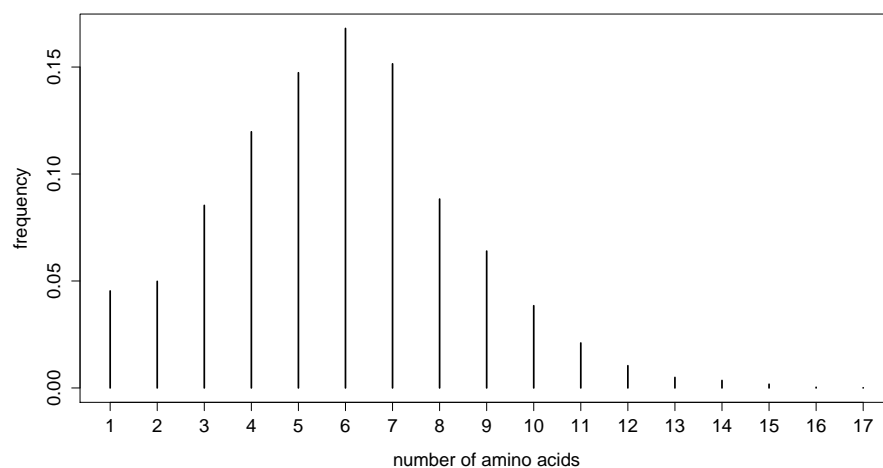


4.1.3 Confidence of estimates of optimal amino acids

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 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??)

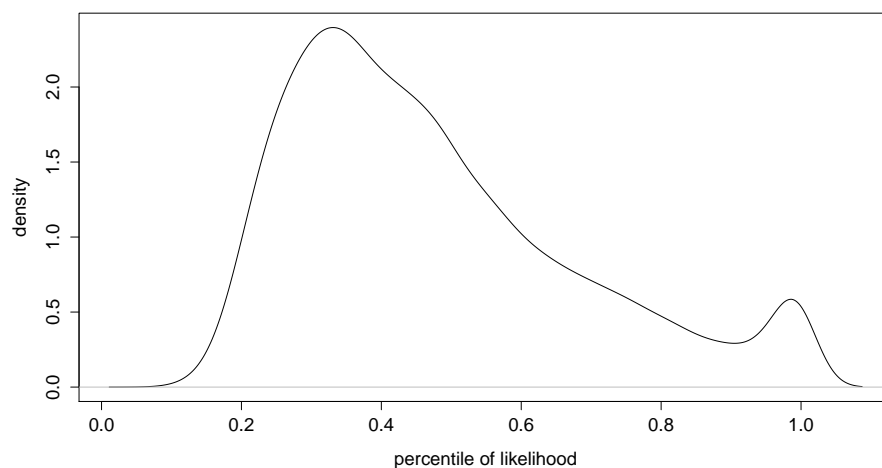
Figure 4: Histogram of the number of optimal amino acids together to cover at least 95% of the total likelihood attained by all possible optimal amino acids.



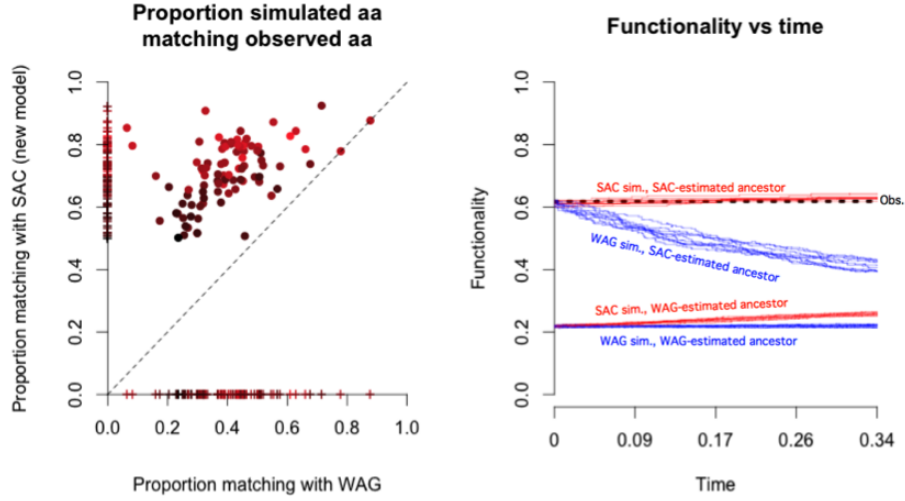
4.2 Simulated data vs. observed data

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

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



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 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]