An extension of testing 6-state recoding strategies to address substitution bias and compositional heterogeneity

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LIST OF ABBREVIATIONS

JTT	Jones-Thornton-Taylor (a model for amino acid substitution)
PAM	Point accepted mutation (a model for amino acid substitution)
RFD	Robinson-foulds distance
SICB	Society for Integrative and Comparative Biology
S&R-6	Susko & Roger 6-state recoding
RAxML	Randomized Axelerated Maximum Likelihood
TOPD	TOPological Distance
FMTS	From Multiple to Single
GTR	general time-reversible model
LG	Le & Gascuel (a model for amino acid substitution)
ML	Maximum-likelihood

1 INTRODUCTION: BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

1.1 BACKGROUND INFORMATION

Dayhoff, JTT, and LG matrices are 20-state amino acid replacement models used to score amino acid substitutions in phylogenetic analyses. Recently, recoding techniques have been employed to address difficulties that these models have dealing with compositional heterogeneity and substitution saturation (Susko & Roger 2007). Dayhoff recoding (i.e., Dayhoff-6) specifically recodes amino acids from Dayhoff matrices according to 6 groups of chemically related amino acids that frequently replace one another (Hrdy et al. 2004), while JTT recoding (i.e., S&R-6) is a 6-state recoding strategy based off binning experiments on the JTT model by Susko & Roger (2007).

1.2 RATIONALE

The principle of using recoding to address substitution saturation and compositional heterogeneity is appealing from a theoretical perspective but has never been tested empirically. Evidence from our analysis presented at the SICB 2018 Conference showed that under all simulations in the study, Dayhoff-6 recoding performed worse than the PAM250 (Dayhoff) model. These preliminary results raised doubts about the benefits of using recoding approaches. However, we received feedback that our analyses did not directly address compositional heterogeneity and that since we used the same model for simulation and testing, we did not consider problems stemming from poor model fit. The proposed analyses herein aim to address these concerns.

1.3 OBJECTIVES

This is a three-part study that will serve as an extension to our project investigating 6-state recoding strategies (*https://github.com/josephryan/Hernandez_Ryan_2018_RecodingSim)*. The objectives are 1) determine how recoding performs compared to a model that was not used for simulation (i.e. does recoding improve results when the non-recoded model fit is poor?) 2) verify the performance of recoding strategies using inferred model parameters from the data under a range of saturation levels 3) determine if recoding addresses problems with compositional heterogeneity.

2 STUDY DESIGN AND ENDPOINTS

2.1 Effect of model fit on recoding vs. non-recoding using LG model

2.1.1. Reconstruct topologies in RAxML (Stamatakis 2014) using the LG model to estimate trees for the data generated in the previous analysis via simulations in Seq-Gen (Rambaut & Grassly 1997) (*https://github.com/josephryan/Hernandez_Ryan_2018_RecodingSim)*. These previous datasets were simulated over the Chang et al. (2015) phylogeny under both the Dayhoff and JTT models with branch-length scaling factors (-s) set to 1, 5, 10, 15, and 20.

```
raxmlHPC -p 420 -m PROTGAMMALG -n Chang.1.LG -s Chang.PAM.1.phy
```

2.1.2 Use the program TOPD/FMTS (Puigbó et al. 2007) to compute Robinson-Foulds distances (RFDs) between LG-generated trees and the tree from Chang et al. (2015) that was used to simulate the data. We will compare these RFDs to those from comparisons between the trees estimated using recoded amino acids.

```
cat Chang_orig_phylobayes.tre RAxML_best* > Chang_all_trees.tre
```

```
perl topd_v4.6.pl -f Chang_all_trees.tre -m split -r no
```

2.2 Effect of model fit on recoding vs. non-recoding using data simulated w/ estimated model

2.2.1 Simulate the evolution of amino acids along the phylogeny produced in Chang et al. (2015) using Seq-Gen and apply model parameters inferred from the data (i.e., amino acid frequency, alpha parameter, and transition rates taken from the main ML tree of Chang et al. 2015). We asked the authors of Chang et al. (2015) for their RAxML output files, but they did not have these available, so we will reestimate these values using the exact RAxML run that was used in the original study. The branch-length parameter (-s) for simulations will be set to 1, 5, 10, 15, and 20.

```
seq-gen -z420 -mGENERAL -r[rates] -f[frequency] -a[alpha] -n1000 -s1 -or
Chang_orig_phylobayes.tre  > Chang.mismatch.1.phy
```

```
seq-gen -z420 -mGENERAL -r[rates] -f[frequency] -a[alpha] -n1000 -s5 -or
Chang_orig_phylobayes.tre  > Chang.mismatch.5.phy
```

```
seq-gen -z420 -mGENERAL -r[rates] -f[frequency] -a[alpha] -n1000 -s10 -or
Chang_orig_phylobayes.tre  > Chang.mismatch.10.phy
```

```
seq-gen -z420 -mGENERAL -r[rates] -f[frequency] -a[alpha] -n1000 -s15 -or
Chang_orig_phylobayes.tre  > Chang.mismatch.15.phy
```

```
seq-gen -z420 -mGENERAL -r[rates] -f[frequency] -a[alpha] -n1000 -s20 -or
Chang_orig_phylobayes.tre  > Chang.mismatch.20.phy
```

divide.pl (For all 5 commands above, divide the 1000 datasets outputted by Seq-Gen into separate phylip files)

```
perl divide.pl Chang.mismatch.1.phy Chang.mismatch.1
```

2.2.2 Convert simulated sequences to Dayhoff-6 recoded datasets using the script chunkify2.pl. Chunkify2.pl also generates scripts to perform maximum-likelihood analyses in RAxML for each non-recoded dataset under the Dayhoff model and for each recoded dataset under the MULTIGAMMA multi-state model with GTR.

*Note: Chunkify2.pl requires the user to input data on servers being used and processors available for each server (this is hard-coded in the script and should be altered when reproducing results on another machine).

```
perl chunkify2.pl
```

To execute the scripts generated from chunkify2.pl for maximum-likelihood analyses of both recoded and non-recoded datasets:

```
ls -1 servername* | perl -ne 'chomp; print "sh $_ &\n";' | sh
```

2.2.3 Concatenate trees from section 2.2.2 into one tree file along with the true tree used for simulation, then calculate RFDs in TOPD/FMTS to compare differences between trees. Box plots will be used to visually compare RFDs and t-tests will be used to determine if there are significant differences in RFDs between non-recoded and recoded trees in R (R Core Team 2017). The script compare_trees_to_sim.pl.v04 performs these analyses.

*Note: The directories in which to perform these analyses are hardcoded and should be altered when reproducing results on another machine.

```
perl compare_trees_to_sim.pl.v04
```

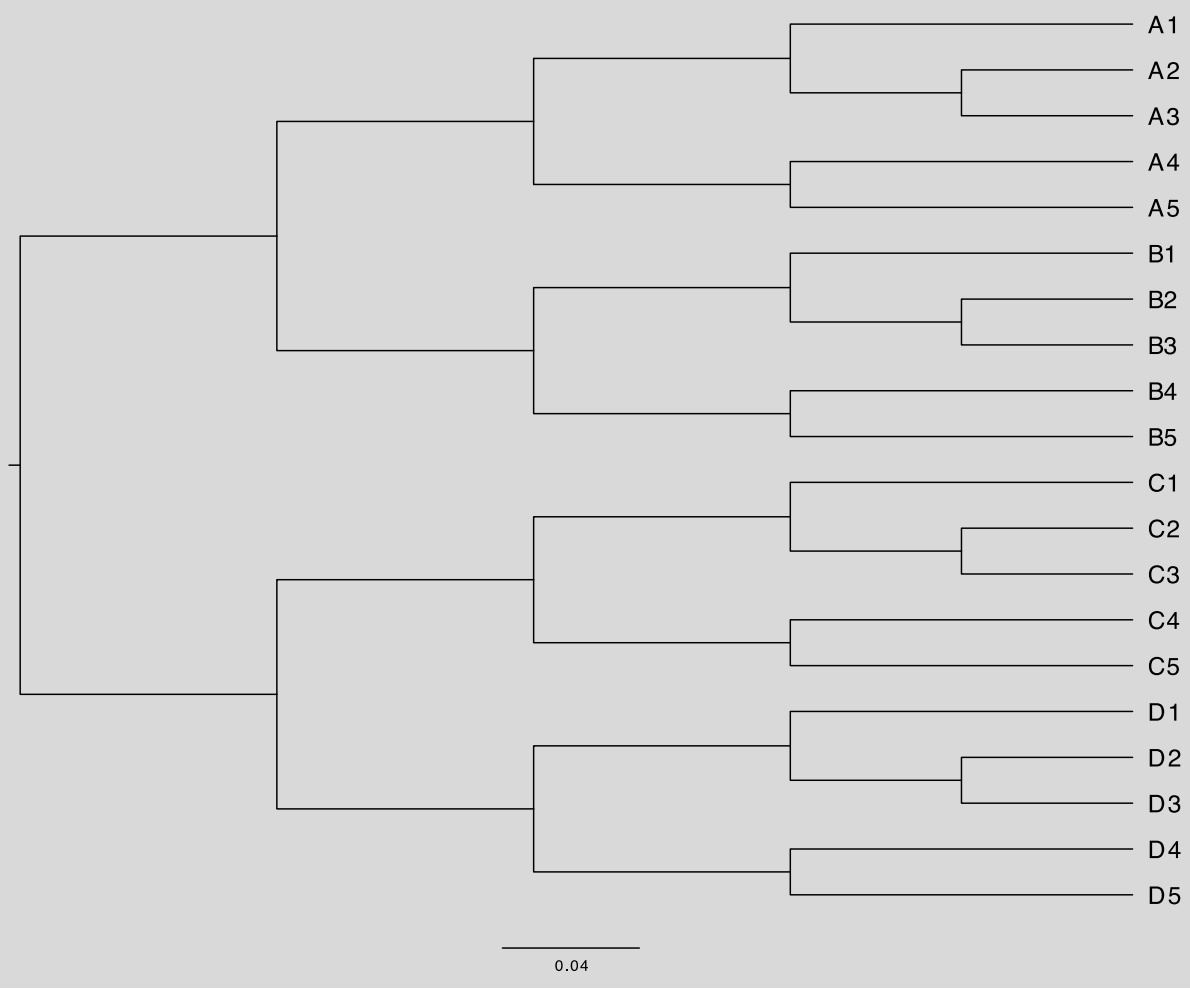
2.3 Effect of compositional heterogeneity

2.3.1 Simulate the evolution of amino acids 1,000,000 times along a twenty-taxa tree (comp_het.tre) (shown below) using P4 (Foster 2004) to produce compositional heterogeneity. We will make the taxa in clade A and C compositionally homogeneous, and the taxa in B and D compositionally homogeneous.

```
python sim_hetero_data.py
```

2.3.2 Simulate the evolution of amino acids 1,000,000 times along the same twenty-taxa tree (comp_het.tre) to produce a compositionally homogeneous dataset using P4. We will fix amino acid frequencies as uniform for all taxa.

```
python sim_homo_data.py
```



2.3.3 To ensure that taxa from clades A and C are compositionally different from the taxa in clades B and D in our compositionally heterogenous datasets, we will compute amino acid frequencies using the script comphet.pl and determine the mean frequency of each amino acid for all the taxa in each of these clades. We will then sum the differences of mean frequencies for each amino acid between clades A and C and between B and D, and calculate the absolute difference of AC from BD. We call these differences a compositional heterogeneity index, or comp-het index for short. We will select the 1,000 trees that have the greatest comp-het index out of the 1,000,000. We will then sum these 1,000 comp-het indices and use this sum as a test statistic in a Monte Carlo simulation. Using this same script, we will compute 1,000 sums of comp-het indices that are randomly selected from the 1,000,000 homogeneous datasets and calculate the number of these sums that are greater than our test statistic. If our test statistic is not significant (i.e. we identify more than 50 sums greater than our test statistic), we will bias our datatset to be compositionally heterogeneous using Seq-Gen as described in our phylotocol version 1.0.

$$\textbf{comp-het index} = \left| \sum_{i=A}^{Y} i = |F_{A} - F_{C}| - \sum_{i=A}^{Y} i = |F_{B} - F_{D}| \right|$$

```
perl comphet.pl
```

2.3.4 We will recode the 1,000 heterogenous datasets selected from section 2.3.3 using Dayhoff-6 recoding and perform maximum-likelihood analyses in RAxML for both the recoded and non-recoded datasets under the Dayhoff model using the script chunkify_comp.pl (performs the same as chunkify2.pl but with parameters specifically set for this dataset).

```
perl chunkify_comp.pl
```

To execute the scripts generated from chunkify_comp.pl for maximum-likelihood analyses of both recoded and non-recoded datasets:

```
ls -1 servername* | perl -ne 'chomp; print "sh $_ &\n";' | sh
```

2.3.5 We will use the script compare_comp_trees.pl to calculate RFDs in TOPD/FMTS to compare differences between recoded and non-recoded trees against the true tree used for simulation. Box plots will be generated to visualize RFD results and t-tests will be used to determine if there are significant differences in RFDs between non-recoded and recoded trees in R.

*Note: The directories in which to perform these analyses are set in the script and should be edited when reproducing results on another machine.

```
perl compare_comp_trees.pl
```

3 WORK COMPLETED SO FAR W DATES

We have not performed any additional tests after completing the work from the Hernandez_Ryan_2018_RecodingSim repo.

4 LITERATURE REFERENCES

Chang, E. S., Neuhof, M., Rubinstein, N. D., Diamant, A., Philippe, H., Huchon, D., & Cartwright, P. (2015). Genomic insights into the evolutionary origin of Myxozoa within Cnidaria. *Proceedings of the National Academy of Sciences of the United States of America*, *112*(48), 14912–7. https://doi.org/10.1073/pnas.1511468112

Hrdy, I., Hirt, R. P., Dolezal, P., Bardonová, L., Foster, P. G., Tachezy, J., & Martin Embley, T. (2004). Trichomonas hydrogenosomes contain the NADH dehydrogenase module of mitochondrial complex I. *Nature*, *432*(7017), 618–622. https://doi.org/10.1038/nature03149

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Stamatakis, A. (2014). RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics*, *30*(9), 1312–1313. https://doi.org/10.1093/bioinformatics/btu033

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5 PHYLOTOCOL AMENDMENT HISTORY

Version	Date	Significant Revisions
1.1	26 June 2018	Changed methods in section 2.3