Compositional shifts associated with major evolutionary transitions in plants

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Summary

- Conflict across genes trees and between morphological characters has been associated with several evolutionary innovations across the tree of life. Here, we examine heterogeneity in composition across a large transcriptomic dataset of plants in order to better understand whether locations of shifts in composition are shared across gene regions and whether directions of shifts within clades are shared across gene regions.
- We estimate mixed models of composition for both DNA and amino acids across a recent large scale transcriptomic dataset for plants.
- We find shifts in composition across both DNA and amino acid datasets with more shifts detected in DNA. We find that Chlorophytes and lineages within experience the most shifts. However, many shifts occur at the origins of land, vascular, and seed plants. While shifts in these clades across genes do not typically share the same composition, they tend to shift in the same direction, reflecting potential mutation bias exacerbated by life history changes and population size.
- Compositional heterogeneity has been highlighted as a potential problem for phylogenetic analysis, but the variation presented here highlights the need to further investigate these patterns for the signal of biological processes.

Plain language summary

We demonstrate that many nucleotide and amino acid compositional shifts in plants occur at the origins of major clades and while individual genes do not share the same composition, even at

shifts, they often shift in the same direction. We suggest that these patterns warrant further exploration as to the biological processes that may be the cause.

Keywords: composition, heterogeneity, land plants, evolutionary transitions, transcriptomes

Introduction

Heterogeneity in the patterns and processes of molecular evolution is common through time and between lineages. For example, topological conflict between different gene regions has been demonstrated to be common across the tree of life, reflecting population processes including introgression and incomplete lineage sorting (Maddison, 1997; Rokas et al., 2003; Smith et al., 2015). An additional widely recognized form of heterogeneity is in composition: changes in the proportion of different states, such as nucleotide bases or Amino Acids (AAs), between lineages and through time, which emerges from the interplay between mutation, gene conversion, drift and selection (Eyre-Walker & Hurst, 2001; Lynch, 2007). Compositional differences are also expressed at the site-level, with different protein sites preferring different AAs (Lartillot & Philippe, 2004; Wang et al., 2008; Le et al., 2008), and genome-wide, with different composition between different regions within the same genome (Lynch, 2007). Different lineages are also known to favor different synonymous codons, leading to compositional bias at the codon level (Chen et al., 2004; Plotkin & Kudla, 2011). These differences are tree-heterogeneous and interactive, so that different sites and loci might experience different compositions in different lineages at different times.

Previous research has focused on the impact of heterogeneous composition on phylogenetic inference, identifying how clade-specific biases in nucleotide base composition can produce false groupings of evolutionarily distant but compositionally similar taxa (Foster, 2004; Cox, 2018; Sousa et al., 2020). Another, less well-explored, avenue is the ability for heterogeneity in composition to provide a window into the molecular and population processes impacting the genome. A separate body of research has addressed the role and influence of

these processes on genomes in multiple clades (Duret & Galtier, 2009; Weber et al., 2014; Clément et al., 2015; Clément et al., 2017). Mutation pressure is thought to explain some genomic patterns (Lynch, 2007), such that changes in composition might reflect important shifts between the balance of mutation and drift, and hence effective population size. GC-Biased Gene Conversion (gBGC, where during recombination-associated gene conversion events, GC alleles act as the donor more often than expected at random) also influences genome-wide GC content, and changes in recombination rate might therefore change compositions across the tree (Marais et al., 2004; Duret & Galtier, 2009; Muyle et al., 2011; Weber et al., 2014). Because BGC occurs during meiosis, changes in effective population size might drive changes in composition via an increase in the efficacy of BGC (Weber et al., 2014), and increases or decreases in generation time could change composition both by changing mutation rate and changing the number of meiotic, and hence the number of BGC, events (Romiguier et al., 2010; Weber et al., 2014). On the other hand, non-demographic processes can also contribute to compositional change (Clément et al., 2017). Selection on codon usage for translational accuracy and efficiency could explain compositional changes (Qiu et al., 2011). Compositional bias could also affect codon usage and eventually, AA preference (Knight et al., 2001; Qiu et al., 2011). Selection for particular AAs might also influence composition (Błażej et al., 2017). Compositionally mediated changes in codon usage might also influence gene expression (Zhou et al., 2016). Macrogenomic changes, such as Whole-Genome Duplication (WGD) and biased retention or loss, could also create dramatic changes in composition (McGrath et al., 2014; Veleba et al., 2014). In plants, empirical patterns in various clades, such as the GC-richness of Commelinid monocots, have been described and explained by mutation, selection and BGC (Qiu et al., 2011; Clément et al., 2015; Clément et al., 2017). Because shifts in base composition bias tend to be linked with such crucial evolutionary parameters as generation time and population size, they may also shed light on major evolutionary transitions in the plant tree of life.

Models of molecular evolution typically consist of two components: relative transition rates between states, and the composition of those states. State compositions of nucleotides or AAs are typically modeled at equilibrium, assuming a process that does not vary between sites or across time (Yang, 2014). These assumptions can be relaxed, including partitioned models (Lanfear et al., 2012), and models which allow the equilibrium composition to vary across sites (Lartillot & Philippe, 2004; Le et al., 2008), or across the tree (Galtier & Gouy, 1998; Foster, 2004), or allow substitution models and compositions to vary across branches (Jayaswal et al., 2011; Zou et al., 2012; Jayaswal et al., 2014). Methods relaxing these assumptions have been a major focus for phylogenetic inference of ancient nodes across the tree of life (Sousa et al., 2020; Redmond & McLysaght, 2021; Li et al., 2021). Phylogenetic inference can be sensitive to composition biases across clades, with conflicting resolutions drawn from homogeneous vs heterogeneous models. If molecular and population processes are driving the patterns accounted for by heterogeneous phylogenetic models, these models could be used to detect the signal of changing evolutionary processes across the tree.

Instead of focusing on the resolution of relationships within plants, we concentrate on examining the extent to which there are compositional shifts across nodes and gene regions. One shortcoming to the application of phylogenetic methods to the detection of compositional shifts is that tree-heterogeneous methods typically require the branches of interest to be specified a priori. Consequently, a number of efforts have been made to relax this restriction, such as testing all branches in the tree, or by investigating summary statistics of the substitution process, or other methods (Blanquart & Lartillot, 2006, 2008; Dutheil et al., 2012). Alternatively, Bayesian MCMC jump methods have been developed that allow for uncertainty in the number and placement of shifts in composition (Foster, 2004; Gowri-Shankar & Rattray, 2007). However, computational methods that allow for integrating over the uncertainty of their placement are too burdensome for large genomic datasets with hundreds of taxa and hundreds of gene regions. In parallel, research has focused on detecting shifts in the rate of diversification

or phenotypic evolution across the tree (Alfaro et al., 2009; Uyeda & Harmon, 2014; Mitov et al., 2019). One such class of method uses stepwise model selection with information criteria to automatically partition the tree into different regimes (Alfaro et al., 2009; Mitov et al., 2019), but barring Dutheil et al. (2012), such approaches are not commonly applied to molecular data.

Here, we extend methods that allow composition to vary across the tree by implementing an algorithm that detects compositional shifts by comparing models of different dimensions using information criteria. We apply our method to a large collection of orthologs of coding regions from across the Viridiplantae clade (Leebens-Mack *et al.*, 2019) and, instead of targeting the impacts of composition on topological resolution, we focus on identifying compositional shifts on individual gene regions.

Methods

Dataset

We analyzed the nucleotide and AA data from the 1000 transcriptome project data release available at https://github.com/smirarab/1kp (Leebens-Mack *et al.*, 2019) to identify patterns in compositional heterogeneity across plants. For nucleotide data, we used the “unmasked and FNA2AA” data and filtered for columns containing at least 10% of data using pxclsq from phyx (-p 0.1) (Brown *et al.*, 2017). We chose these alignments instead of those for which trees were already inferred in order to include third codon positions for composition analyses. We ran an analysis to detect compositional shifts in both the nucleotide (the cleaned alignments of all three codon positions and our inferred trees) and AA data (using the available alignments and trees). For these alignments, we conducted phylogenetic analyses using IQ-TREE v1.6.6 (Nguyen *et al.*, 2015) under the GTR+G model of evolution. For AAs, we used the “masked FAA" data and the corresponding trees inferred as part of the original study. We analyzed the AA using the JTT model of evolution.

Because of the non-homogeneity of the compositional model, our analysis required rooted trees. Perfect rooting was not required and would have been prohibitive considering the

variation and non-monophyly of many taxonomic groups in each gene tree. In order to accommodate this, we rooted using pxrr from phyx, applying the ranked option with the following taxa in order: UNBZ, TZJQ, JGGD, HFIK, YRMA, FOMH, RWXW, FIKG, VYER, LDRY, VRGZ, ULXR, ASZK, JCXF, QLMZ, FSQE, DBYD, VKVG, BOGT, JQFK, EBWI, FIDQ, QDTV, OGZM, SRSQ, RAPY, LLEN, RFAD, NMAK, VJED, LXRN, APTP, BAJW, IAYV, IRZA, MJMQ, ROZZ, BAKF. The ranked option searches through the list of taxa and roots on the first one present.

Detection of compositional heterogeneity

We developed an algorithm to detect locations of shifts in stationary frequencies in state composition that we describe below. The method is generalized to any state model, and so proceeds in the same way for nucleotides or AAs. It requires a rooted tree and matching alignment as input. First, the method estimates a maximum likelihood root composition for the entire dataset. Next, the tree is traversed postorder, and a maximum likelihood composition is estimated for the subtree subtending each node, if that subtree contains more than a user-specified minimum number of tips. In this work, we considered any subtree containing at least 10 tips. Using this composition for the focal node and subtree, and the root composition for the remainder of the tree, we calculate a likelihood and the Bayesian Information Criterion (BIC: Schwarz, 1978). Once a model for every eligible subtree has been estimated, we order subtrees by their BIC (i.e., by their relative improvement in fit over the base model), add them to the model configuration, calculate a new likelihood and BIC for the whole tree and add the sub-model if the new BIC is lower (i.e., the model provides a better fit). To improve computational efficiency, we discard models if their BIC score is greater than the current model by an arbitrary cutoff (we assigned a cutoff of 35). Our method has been implemented in both Golang (for flexibility) and C (for speed), and the source code is available at https://git.sr.ht/~hms/janus and https://git.sr.ht/~hms/hringhorni, respectively. An example is presented in Supp. Fig. 1.

Accommodating model uncertainty

One common challenge in information criterion (IC) based approaches to model comparison is their tendency to overfit, sometimes favoring models of higher complexity than the generating model. Our solution to this tendency was to assess statistical uncertainty in each model shift by estimating the relative support for the model that includes the shift vs the model without the shift. We performed these tests using BIC weights (*wBIC*), comparing, for each putative shift, the BIC of the full model containing all inferred shifts to one dropping each individual model shift. The strength of support for each inferred shift was thus calculated by calculating the relative BIC of each candidate model *i* (in this case, shift vs no shift):

$$\mathrm{relBI}C_{shift} = e^{\left(BIC_{shift} - BIC_{noshift}\right) \times 0.5}$$

And assessing support for the shift as the ratio of the ratio of that model over the sum of all *i* candidate models:

$$wBIC = \frac{relBIC_{noshift}}{\left(relBIC_{noshift} + relBIC_{shift}\right)}$$

This calculation yields an index between 0 and 1, where values closer to 0 indicate weaker support for the shift, and values closer to 1 indicate stronger support. Using the reasoning that spurious shifts will likely typically be poorly supported, we removed shifts with wBIC support values below 0.95.

Simulations

We conducted several simulations to validate the performance of our algorithm in detecting model heterogeneity. Phylogenies were simulated under a birth-death model with phyx using the pxbdsim command with defaults, except varying the size of the tree between 100 and 250 tips, and root height set to 0.75 with pxtscale from phyx. Nucleotide and AA alignments were simulated using a nucleotide simulator which allows for shifts in composition across the tree, under JC+G (with α = 1 for rate heterogeneity) for nucleotides and JTT with no rate variation for AAs, with a single randomly positioned compositional shift per tree. Phylogenies were then reconstructed with IQ-TREE under the GTR+G model of evolution for nucleotide alignments and

the JTT+G model for AA alignments. For each simulation set, we simulated 100 replicates. Root height was set to 0.75. Alignment lengths were 1000 for nucleotides and 300 and 1000 for AAs.

Summarizing compositional heterogeneity

We summarized the results from these analyses in several ways. Directly comparing model shifts across genes was complicated by extensive gene tree conflict. We compared the distribution of model shifts by pairwise comparison of tips on the species tree inferred in the original paper (Leebens-Mack *et al.*, 2019), recording the number of times that two tips were descended from a node with a shared model, and plotted this in a heatmap on the species tree (Supp. Fig 2). Secondly, we defined major clades in the species tree, and recorded to which groups each tip descending a model shift in each gene tree belonged. We counted the number of tips from each taxonomic group, and further counted the number of tips within those taxonomic groups which were not included in the model shift (i.e., either the model shift occurred nested within that group, or those tips were placed polyphyletically in the tree due to conflict). We manually assessed these mismatches and the position of the model shift on the gene tree and assigned the shift on the species tree to occur either i) at the node defining a major clade (assuming mismatching tips are errors), which we summarize as occurring at the origin of the clade or ii) descending a node defining a major clade, which we summarize as occurring within the clade. For individual genes, we plotted model shifts on the tree and changes in parameter estimates between models. To characterize the direction and size of parameter shifts, we used a Principal Components Analysis where each row was a single sequence and each column was the frequency of one state for that sequence (i.e., 4 columns for nucleotides and 20 for AAs). We projected every gene tree onto the same set of axes for the first two PCs and colored each point (representing a single tip), by the model from which it was descended.

Results

Simulations

Our simulations demonstrate that, given sufficient data (i.e., alignments of sufficient length), our method has acceptable false positive and negative rates (Table 1). False positive rates were negligible after removing shifts that were poorly supported by BIC. In general, we consider the false positive rates to be of more concern than false negatives rates, but the latter were also negligible in our simulations. The highest rates of false positives were observed in short (300 site) AA alignments, which were diminished but not entirely alleviated by taking uncertainty into account. False positive rates were generally elevated when species tree reconstruction error existed in the simulated data.

Table 1. Results of simulations for both nucleotide and amino acid data

# of shifts	# tips	Nuc/AA	Len	False +	False + unc	False +(rec)	False +(rec) unc	False -	False - unc	False - (rec)	False - (rec) unc	Avg. RF
0	100	Nuc	1000	0	0	0	0	0	0	0	0	9.96
1	100	Nuc	1000	0	0	0	0	0	0	0	0	8.76
2	150	Nuc	1000	0.14	0.03	0.09	0.0	0.0	0.02	0	0.02	15.0
2	250	Nuc	1000	0.1	0.01	0.1	0.02	0.01	0.03	0.04	0.07	24.8
0	100	AA	300	0	0	0	0	0	0	0	0	14.32
1	100	AA	300	0.02	0.01	0.11	0.07	0	0	0.02	0.02	15.9
2	150	AA	300	0.03	0	0.18	0.07	0.01	0.01	0.01	0.01	21.34
2	250	AA	300	0.02	0	0.19	0.10	0.02	0.03	0.03	0.01	35.6
0	100	AA	1000	0	0	0	0	0	0	0	0	4.84
1	100	AA	1000	0.01	0	0.03	0.01	0	0	0	0	4.76
2	150	AA	1000	0.18	0	0.19	0	0	0	0.01	0.01	6.82
2	250	AA	1000	0.22	0	0.22	0.01	0	0	0	0	12.0

Phylogenetic patterns of compositional shifts

We applied our method to a large dataset of orthologs derived from genomes and transcriptomes across Archaeplastida. As noted in the original study (Leebens-Mack et al.,

2019), the inferred gene trees contained high levels of conflict. For example, 38% of nucleotide and 32% of AA gene trees contained non-monophyletic seed plants. We searched for compositional shifts in inferred gene trees from nucleotide and AA data. We detected multiple shifts in both datasets, with many more shifts detected for nucleotide data (**Figure 1**). The phylogenetic location of these shifts differed between different trees, and we observed a great deal of gene tree conflict between the individual orthologs and the species tree, complicating the localisation of shifts. Nevertheless, general patterns did emerge when comparing shift locations to the species tree (**Figure 2, Figure 3**). Many nucleotide shifts were detected at the Embryophyta node, corresponding to the transition of land plants; at the Tracheophyta node corresponding to the evolution of vascularity; at the Spermatophyta node corresponding to the evolution of seeds and at the Angiosperm node corresponding to the evolution of flowers. Many nucleotide shifts were also detected at the base of and within Chlorophytes. By contrast, AA shifts were enriched at the Spermatophyta and Angiosperm nodes and were similarly common at and within Chlorophytes. Several shifts were identified within the named clades we select in **Figure 2**, which might themselves occur at named clades. However, our sampling or the conflict in the gene tree precluded identification.

Direction of compositional shifts

The direction of compositional shifts (i.e., which state frequencies increased or decreased between a parent and child model) differed both within and between genes. While specific compositional values may not be shared by many genes, we noticed a tendency for shifts at comparable nodes to occur in similar directions (**Figure 3**). The root nodes of angiosperms, chlorophytes, and embryophytes each displayed many nucleotide composition shifts that were heavily directionally biased (**Figure 1**). The root node of angiosperms displayed a similarly biased pattern. Several nodes displayed similarly biased amino acid compositional shifts. These biased shifts were highly evident at the origin of Tracheophyta, angiosperms,

Zygnematophyceae, Spermatophyta, Embryophyta, and chlorophytes. The only clade that we examined with relatively unbiased amino acid composition shifts was Rhodophyta.

To determine whether patterns in the direction of nucleotide compositional shifts were related to codon usage bias, we examined codon usage for each model within each gene. We noted several patterns. Firstly, codon usage was strongly biased within each residue, and there is a tendency for land plants to feature more AT-rich codons. Additionally, clades nested within land plants (e.g., Embryophyta, Tracheophyta) tend to be more AT-rich than other clades (e.g., Bryophytes). Gymnosperms showed the highest degree of codon usage bias, favoring AT-rich codons.

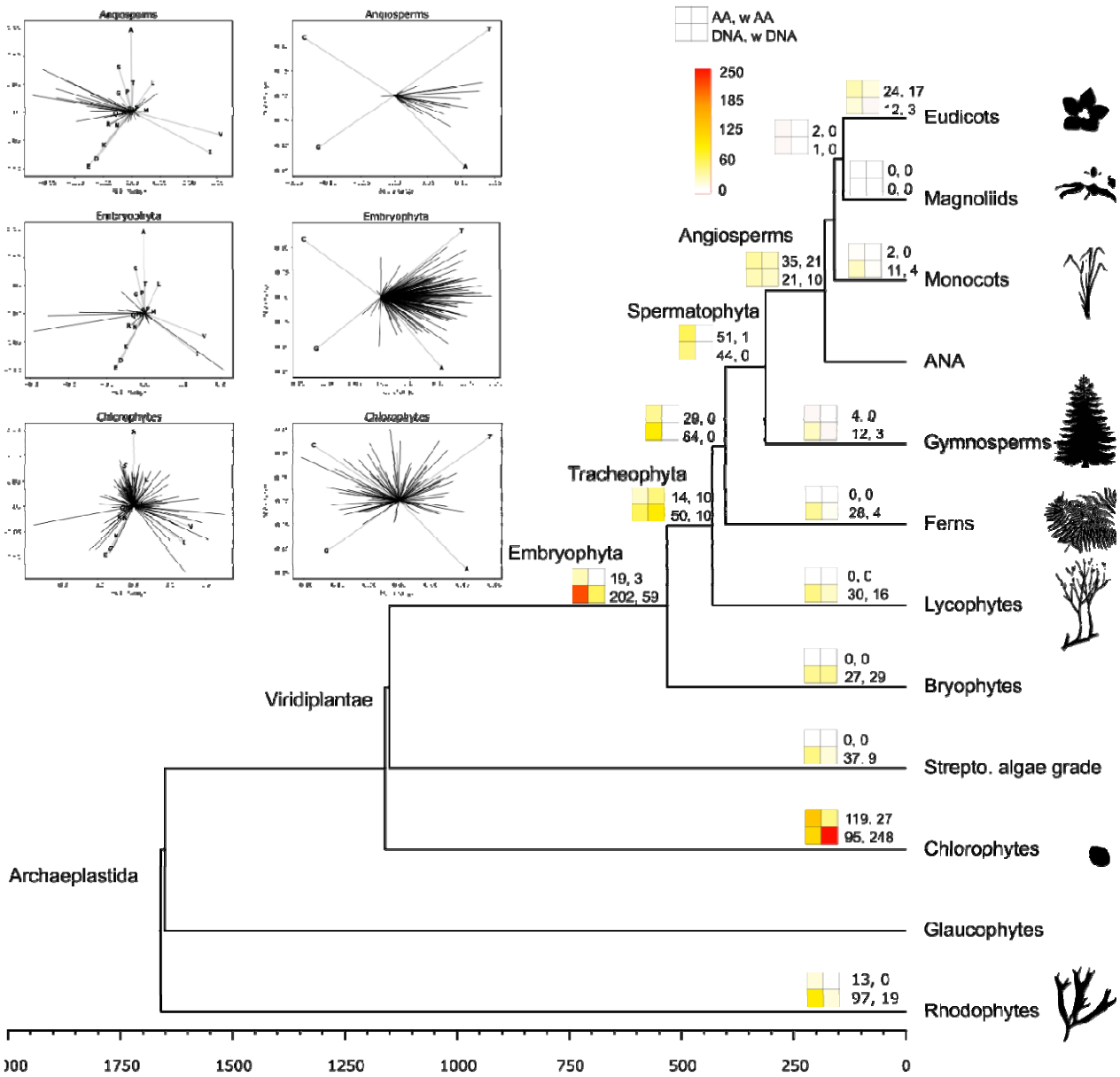

Figure 1. Summarized results for AA and DNA. Shifts that are found within (i.e., not at the base of) the marked clade are also denoted. Plots on the left denote vectors of composition shifts for both AA (left) and DNA (right) for Angiosperms, Embryophyta, and Chlorophytes. For the complete set, see Supp Fig. 3.

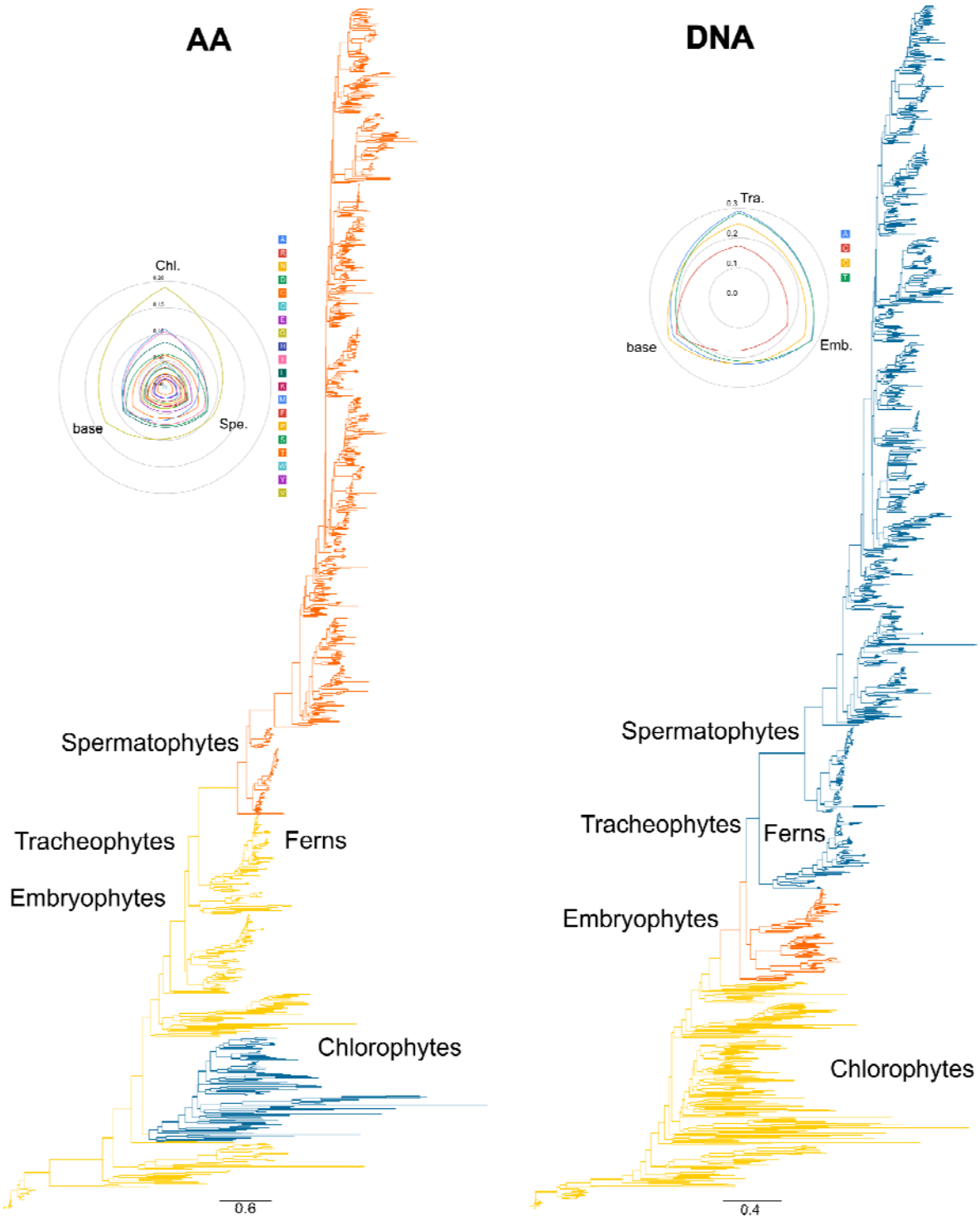

Figure 2. Ortholog 5936 results from both AA and DNA datasets. Colors are meant to identify shifts and do not denote shared models between AA and DNA results. Model results are presented in radar graphs.

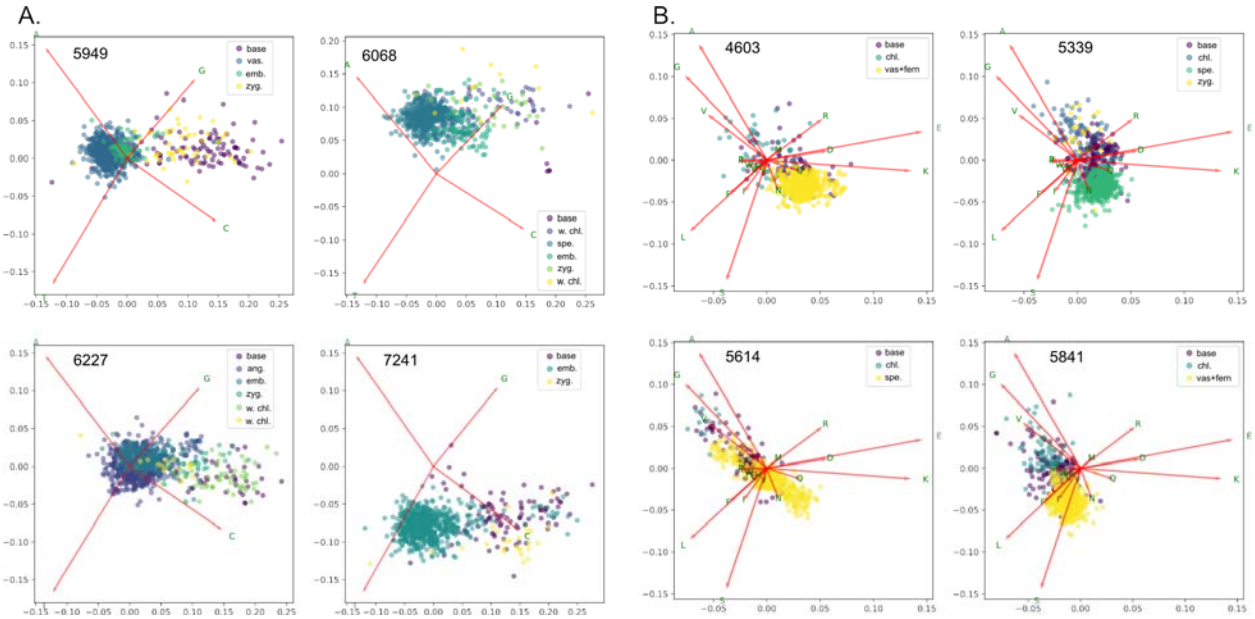

Figure 3. Principal component analyses of four DNA datasets (A) and four AA datasets (B) with each point representing one taxon and colors denote shared shifts within the dataset. The PC loadings are based on the entire DNA and AA datasets respectively to allow for easier interpretation.

Discussion

Life history. The results of the analyses of the direction of the compositional shifts, the phylogenetic position of the shifts, and the composition bias suggest a connection to more extensive bias within nested clades of land plants. For example, when there are shifts associated within vascular plants, they tend to be more biased and more AT rich than compositional patterns within the rest of embryophytes and others. This suggests a potential pattern related to the major life history changes that have occurred throughout the green plant phylogeny including movement onto land, evolution of vascularity, development of seeds, and evolution of flowers. Biased gene conversion can favor the proliferation of GC alleles during meiotic recombination, such that decreasing generation time could lead to increased GC-

richness, while increasing effective population size is expected to increase the efficiency of gBCG (Duret & Galtier, 2009; Weber et al., 2014). On the other hand, mutation tends to be biased toward AT (Lynch, 2007). Faster generation times produce a higher mutation rate and smaller effective population sizes increase the probability that mutations will be fixed by drift, such that changing population size and generation time could also lead to increased AT-richness (Romiguier et al., 2010). In the case of land plants, it may be the case that slower generation time of many of these plants in relation to their non-land relatives is related to population size. Of note, are the gymnosperms which tend to have higher composition bias but fewer phylogenetic shifts. One possible explanation for this pattern is gymnosperms' lower data availability. However, a biologically rooted potential cause could be their slower generation time, which may have prevented them from reaching compositional consistency between lineages (Lanfear et al., 2013). This would yield weaker signals for our methods to detect shifts.

Our expectations under a model of mutation bias and gBGC is that populations with slower generation time and smaller effective population sizes will have lower GC-richness and higher AT-richness at equilibrium because of AT-biased mutations and a lower rate and a lower efficiency of gBGC. Based on this, our results are consistent with many major changes in traits and life history across the Viridiplantae being associated with longer generation times and/or reductions in effective population size. This pattern seems likely to be true of gymnosperms, which are large trees with slow generation times (De La Torre et al. 2017) and our results suggest that it is true of angiosperms and other lineages.

Although BGC and mutational bias seem likely to be significant factors shaping compositional variation, they interact with other processes, such as selection, that might contribute to compositional biases in both AAs and nucleotides (Jobson & Qiu, 2011; Muyle et al., 2011; Błażej et al., 2017; Clément et al., 2017). One driver for the focus on gBGC is that it mimics the

pattern of selection on segregating variation and may thus mislead methods designed to detect selection (Mugal et al., 2015). However, it is increasingly clear that selection can change composition in various ways, including via selection on codon usage (Qiu et al., 2011). Our analysis of codon usage indicated strong biases in preferred codons. Although it can be difficult to determine if codon usage determines composition or vice versa, this signal might also influence our results. Additionally, many of the nodes we highlight with frequent shifts are thought to coincide with WGDs (Jiao et al., 2011; Leebens-Mack et al., 2019). However, because our analysis uses single orthologues, it seems unlikely to strongly influence our results unless many orthologues contain hidden duplications and loss. Due to the necessarily coarse nature of our investigation, it is difficult to comment on how different processes might contribute to the patterns we observe. Such a distinction is a goal of further modeling efforts (Kostka et al., 2012), and will undoubtedly be important in more focused studies of single organisms or loci.

Phylogenetic resolution. The phylogenetic resolution of several deep nodes differs between genes in the DNA and amino acid datasets, and some shifts associated with deep nodes are associated with those alternative resolutions of major clades. For example, in many genes, the Bryophytes are non-monophyletic and shifts are associated with the nodes surrounding this conflicting relationship. For example, in gene region 6401, the Bryophytes form a grade with a shift shared by a clade of liverworts and the rest of vascular plants. The amino acid phylogeny of the same gene has no significant shift in the molecular composition. Other examples include lycopods sister to ferns versus ferns sister to seed plants–the latter is associated with shifts in molecular evolution 29 times in amino acids and 68 times in nucleotides. While this analysis is not focused on the phylogenetic resolution of these major clades, other studies have demonstrated that heterogeneity can alter phylogenetic reconstruction. The analyses here underscore the importance of that consideration in future studies.

Data quality. The datasets used here have several challenges that may be the result of the large and complex nature of the underlying data quality. We note this problem primarily because as many new genomic and transcriptomic datasets become available, as in this study, researchers will be tempted to address large scale questions taking advantage of these enormous datasets. However, caution should continue to be exercised as errors in homology or contamination are still prevalent. In this case, we are limited in interpretation of the phylogenetic location of many of the shifts because of the non-monophyly of many clades that are well established. For example, 38% of the nucleotide gene trees and 32% of amino acid gene trees have non-monophyletic seed plants. This presents several challenges, but primarily, in summarizing the phylogenetic placement results, we had to accept that there may be outlying taxa that make strict monophyly difficult to enforce.

We highlight this problem not to single out these data or the original analyses as we recognize that many large-scale datasets inevitably face challenges when cleaning data. Instead, we want to underscore the importance of homology and orthology analyses in the construction of single gene alignments and gene trees. While errors like this may not greatly impact species-tree analyses, especially if they are mostly random between gene trees, they can dramatically limit the utility of these data for other analyses.

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Author Contribution: SAS, CPF, and NWH contributed to the conception, programming, and writing of the manuscript.

Data Availability

The alignments for both DNA and amino acid datasets are available through the resources of the original data release paper. The gene trees for DNA were generated as part of this study and are available from DataDryad. The code is available through github and sourcehut linked above.

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