Unearthing modes of climatic adaptation in underground storage organs across Liliales

Carrie M. Tribble[1,2,3,*], Michael R. May[1,2], Abigail Jackson-Gain[1,2,4], Rosana Zenil-Ferguson[3], Chelsea D. Specht[5], and Carl J. Rothfels[1,2]

[1]*Department of Integrative Biology University of California, Berkeley, CA 94709, USA*

[2]*University Herbarium, University of California, Berkeley, CA 94709, USA*

[3]*Current address: School of Life Sciences, University of Hawai'i at Mānoa, Honolulu, HI, 96822, USA*

[4]*Current address: Departamento de Geología, Facultad de Ciencias Físicas y Matemáticas, Universidad de Chile, Santiago, Chile*

[5]*School of Integrative Plant Sciences and L.H. Bailey Hortorium, Cornell University, Ithaca, NY 14853 USA*

Abstract The evolution of major innovations in life history strategies (how organisms gather and store energy and reproduce) is a primary theme of biodiversity research. In one remarkable example of a life history innovation, certain plants—geophytes—retreat underground using underground storage organs (USOs), and thus survive extended periods of unfavorable conditions. Geophytes have evolved multiple times independently across all major vascular plant lineages. Even within closely related lineages, however, geophytes show impressive variation in the morphological modifications (i.e. 'types' of USOs) that allow them to survive underground. With all this variation, it is unclear to what extent geophytes with different USO morphologies have converged in climatic niche, or have evolved distinct niches. To test this hypothesis, we extended existing phylogenetic comparative method approaches to test for links between hierarchical discrete traits and adaptation to environmental variation. We inferred a phylogeny of 621 species of Liliales and used the phylogeny and analysis pipeline to test the relationship between underground morphologies and different adaptive optima of climate seasonality. Contrary to expectation, plants with the same USO type do not share climatic niches more than expected by chance, with the exception of root morphology, where modified roots are associated with lower temperature seasonality. These findings suggest that root tubers may be adaptations to different climatic conditions than other types of USOs. Thus, the tissue type and developmental origin of the structure may influence the way it mediates ecological relationships and draws into question the appropriateness of ascribing broad ecological patterns uniformly across geophytes. This work provides a new framework for testing adaptive hypotheses and for linking ecological patterns across morphologically varying taxa. [Macroevolution, geophytes, climatic niche evolution, adaptation]

Introduction

The evolution of major innovations in life history strategies (how organisms gather and store energy and reproduce) is one of the primary themes of biodiversity research (Adler et al., 2014; Enquist et al., 1999). In one remarkable example of a life history innovation, certain plants can retreat underground by producing the buds of new growth on structures below the soil surface (Raunkiaer et al., 1934) while also storing nutrients to fuel this growth in highly modified, specialized underground storage organs (USOs). These plants are collectively referred to as geophytes. Geophytes have evolved independently many times across the plant tree of life, including in diverse and distantly related lineages within the ferns and flowering plants (Tribble et al., 2021). Even within closely related lineages, geophytes show remarkable variation in the particular morphological modifications that allow them to survive underground. By differentially modifying leaves, stems, and roots, geophytes produce complex storage structures through distinct developmental and evolutionary means (reviewed in Tribble et al., 2021).

Previous work has suggested that the geophytic habit is correlated with more seasonal climatic conditions and higher-disturbance regimes (Cuéllar-Martínez and Sosa, 2016; Sosa et al., 2016; Sosa and Loera, 2017; Howard et al., 2019) and may be an adaptation to these conditions (Rees, 1989). Geophytes are particularly diverse in seasonally dry climates such as Mediterranean ecosystems, where they survive hot, dry summers underground and emerge during cool, dry winters to photosynthesize and reproduce, a pattern particularly prominent in the Cape region of South Africa, where almost 15% of native plant species are geophytic (Parsons and Hopper, 2003). Geophytes are also common in deciduous woodland habitats, where their USOs fuel quick spring regrowth to maximize photosynthetic opportunities before trees have regrown their canopies in spring (Whigham, 2004).

In a recent study of monocotyledonous geophytes, Howard et al. (2019) found that geophytism is correlated with areas of lower temperature and precipitation and higher temperature variation. Howard et al. (2019) also tested for different climate preferences among geophytes with different categories of USOs, and were unable to recover significant correlations with climate, with the exception of an association between rhizomatous geophytes and areas of increased temperature variation. The authors suggest that more detailed morphological data, as well as data that takes into account the developmental origin of USOs (leaf, stem, or root tissue), may be necessary to address within-geophytic variation in environmental preferences.

While this body of work provides compelling evidence for correlations between seasonal climates and the geophytic habit, previous phylogenetic-based research has relied on methods that fail to account for the complexities of morphological and adaptive evolutionary processes. While the goal of many phylogenetic comparative methods is to model the evolutionary relationships between (often multiple) traits and species, incorporating diverse data types into a cohesive analytical framework is often stymied by underlying differences in how different types of traits are expected to evolve across a tree. Specifically, including continuous and discrete traits in a single analysis is a longstanding statistical challenge in phylogenetics. Phylogenetic generalized linear models (Garland Jr et al., 1993), such as the phylogenetic ANOVA model used in Howard et al. (2019), are flexible for accommodating different data types (such as discrete morphology data and continuous climatic variables) by modifying the expected variance structure of continuous response variables using the phylogeny and an assumed model of continuous trait evolution. However, the continuous trait models commonly used to establish this expected variance structure are generally considered non-adaptive (e.g., Brownian motion; Felsenstein, 1985), whereas the studies that use such methods are often motivated by adaptive hypotheses. For example, if species have evolved to occupy different climatic niches, then the Brownian motion process, which represents a

random walk—often interpreted as neutral continuous trait evolution—will likely do a poor job of modeling how climatic niche axes have evolved across the phylogeny.

To date, no study has explicitly tested if geophytes with different USOs are adapted to particular climatic niches within an adaptive evolutionary framework that models discrete trait evolution over time. Furthermore, more work is needed to test the extent to which the geophytic life history strategy is non-uniform and if plants with different USOs converge on different climatic realized niches (the set of climatic conditions that describe where the organism successfully lives and reproduces; Holt, 2009).

If geophytes with independently-evolved and developmentally-distinct morphologies converge on the same climatic niche, then the diverse types of USOs may represent different evolutionary paths towards an effectively similar ecological strategy: retreating underground. This result would imply that the diversity of underground forms are due to developmental or genetic differences in the ancestors of geophytes that predisposed plants to modifying particular types of tissue in different ways when presented with the same types of environmental conditions. Conversely, if plants with different USOs occupy different climatic niches, variation in underground morphology may underlie differences in how these plants relate to their environment. In this case, geophytes may not be a uniform life-history strategy at all, but rather a set of different morphologies that allow plants to survive in different conditions.

Addressing these questions requires a nuanced and detailed approach to the evolution of both morphology and climatic niche. Some types of USOs may be more close in morphological and developmental space because they are modifications of the same type of tissue. For example, corms and rhizomes are both modifications of stem tissue, while in bulbs, the primary storage tissue is derived from modified leaves (see Figure 1). A model that represents transitions between corms and rhizomes in the same way that it represents transitions between rhizomes and bulbs effectively erases the complexity of these morphologies and ignores the role that shared developmental mechanisms may play in morphological disparification. Additionally, a non-adaptive model of climatic niche axes is inappropriate for testing adaptive hypotheses. The Ornstein Uhlenbeck (OU) process modifies Brownian motion such that trait values are expected to evolve towards an optimum, θ (Hansen, 1997; Butler and King, 2004), approximating the evolution of a continuous trait towards one or more adaptive peaks. Further modifications of these processes allow for the strength of selection towards those peaks, the number of peaks, and the baseline rates of evolution to vary (Beaulieu et al., 2012; Uyeda and Harmon, 2014). To test if plants with the same type of USO are evolving towards a shared optimal climatic niche, We develop a new analytical pipeline that capitalizes on recently-developed comparative methods that allow explicit testing of adaptive hypotheses (OU models; Uyeda and Harmon, 2014) and the incorporation of complex, nested relationships in morphological characters (Tarasov et al., 2019).

Representation of diverse geophytic morphologies is particularly high in the order Liliales, which contains roughly 1200 species distributed across the globe (Givnish et al., 2016), including geophytic taxa with a striking diversity of USOs. Liliales is an excellent group in which to test if geophytic organs are adaptation to different climatic niches, as there appear to have been many transitions between geophytes with diverse USOs, encompassing most of the main types of underground organs found across plants.

In this study, we build a species-level phylogeny of roughly 50% of the taxa in Liliales by capitalizing on the growing availability of published genetic data (Benson et al., 2018), advances in supermatrix construction (de Queiroz and Gatesy, 2007), and model-based tree-building algorithms (Ronquist et al., 2012), which collectively have widened the scope of phylogenetic recon-

struction and allowed for increasingly large trees and thus larger datasets necessary to obtain statistical power for these more complex phylogenetic comparative methods. We use this phylogeny and my newly-developed analysis pipeline to test the relationship between underground morphologies and modes of evolution in climate seasonality. Specifically, we hypothesize that different USOs are adaptations to specific climatic niches and thus species with those USOs should correspond to different climate values in analyses.

Materials and Methods

Data

We generated three data sets for downstream analysis: a distribution of species-level phylogenies including 50% of the species in Liliales, a modeled climatic niche for each species based on 19 climatic variables (Fick and Hijmans, 2017), and a detailed underground morphology database for all species.

Phylogeny

We used SUMAC 2.0 (Freyman, 2015) to download gene regions from NCBI GenBank (Benson et al., 2018) for all species in the order Liliales. We targeted genes that clustered with specific guide sequences to specifically obtain gene regions for 10 commonly sequenced genes in Liliales (Supplemental Table 1). We filtered the resulting sequences using custom python scripts to remove gene regions with especially high percentages of missing data (fewer than 150 taxa out of 621), remove sites with more than 95% missing data, align and edit sequences, and rename taxa with taxonomic errors. All regions were aligned using MAFFT v7.271 (Katoh and Standley, 2013); some alignments (ITS, psbA, and rpl16, and trnL-trnF spacer) failed to align well under MAFFT and were subsequently aligned using PASTA (Mirarab et al., 2015) to improve alignment accuracy. We concatenated the filtered and edited alignments using Sequence Matrix (Vaidya et al., 2011). We reconstructed the phylogeny using `MrBayes v3.2.6` (Ronquist et al., 2012) on CIPRES (Miller et al., 2011) with two independent runs of four chains, partitioned by gene region, each under the GTR $+ \Gamma$ model with default priors. We constrained tree space to the known family-level relationships within the order according to the Angiosperm Phylogeny Website (Stevens et al., 2016) to reduce run times.

For downstream analyses, we randomly selected five trees from the posterior distribution; performing analyses over a distribution of trees accounts for phylogenetic uncertainty, which may be particularly important in large supermatrix approaches with high percentages of missing data and low support for some branches of the tree. We dated each of the selected trees in `R` (R Core Team, 2013) using the `chronos()` function from the `R` package `ape` (Paradis and Schliep, 2019)—an implementation of the penalized likelihood approach (Sanderson, 2002)—using data from two fossils and a secondary calibration (see Supplemental Table 2; Iles et al., 2015; Givnish et al., 2016).

Climate

We modeled the climatic niche of each species in Liliales using a newly developed `R` pipeline, Climate and Niche Distribution Inference (`CaNDI`, manuscript in preparation, all code available on GitHub at https://github.com/abbyj-g/candi) that gathers

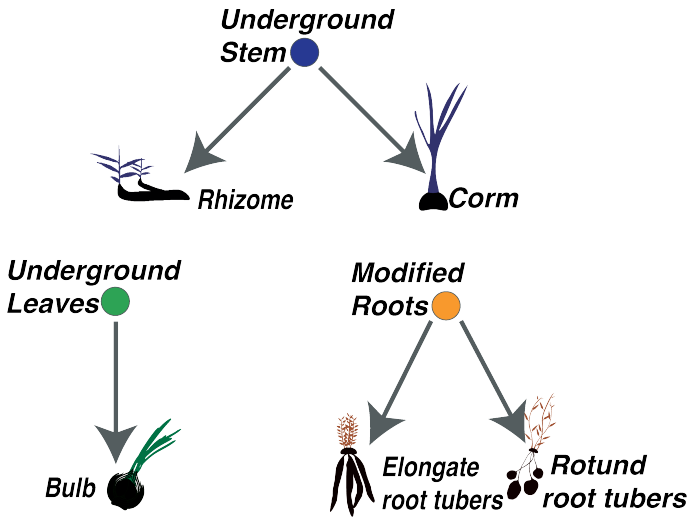

Figure 1: Hierarchy of morphological states used in PARAMO analysis.

and cleans species occurrences, downloads climate data (among other variables), and estimates niches for hundreds of taxa at a time. `CaNDI` takes as input a list of species, queries the Global Biodiversity Information Facility (GBIF; Flemons et al., 2007) and the Botanical Information and Ecology Network (BIEN Maitner et al., 2018) for occurrence records, and cleans those records using a series of filters designed to remove latitude and longitude records that fall outside of the species' native range, exactly at 0°, 90°, or 180°, or in the ocean. `CaNDI` then passes these climate data and occurrences to MaxEnt (Phillips and Dudík, 2008) to estimate the climatic niche. For each species, `CaNDI` returns the probability of occurrence across the landscape.

We used climate data from the WorldClim database (Fick and Hijmans, 2017): 19 bioclimatic variables which describe various aspects of temperature and precipitation. Collinearity of predictor variables does not affect model performance (except in cases of model transfer; Feng et al., 2019), so we included all 19 variables in niche estimation. To obtain a single estimate of the optimal value for each climate variable, we selected the value that corresponded to the part of the species' range with the highest probability of occurrence. Downstream analyses focused on the two axes of the multidimensional niche that describe seasonality: seasonality of precipitation and seasonality of temperature.

Morphology

We used morphological data from Kew's World Checklist of Selected Plant Families (WCSP, 2020) to describe the USOs associated with species in Liliales. For taxa listed as tuberous, we referred to morphological literature (Kubitzki and Huber, 1998; Sanso and Xifreda, 2001; Pate and Dixon, 1982) for more detailed descriptions of their USOs, as the WCSP uses tuber as a catch-all category, including for corms, root tubers, and other organs. The final coding scheme consisted of the presence and absence of eight characters, grouped into three hierarchical clusters based on tissue type (leaf, stem, and root; see Figure 1).

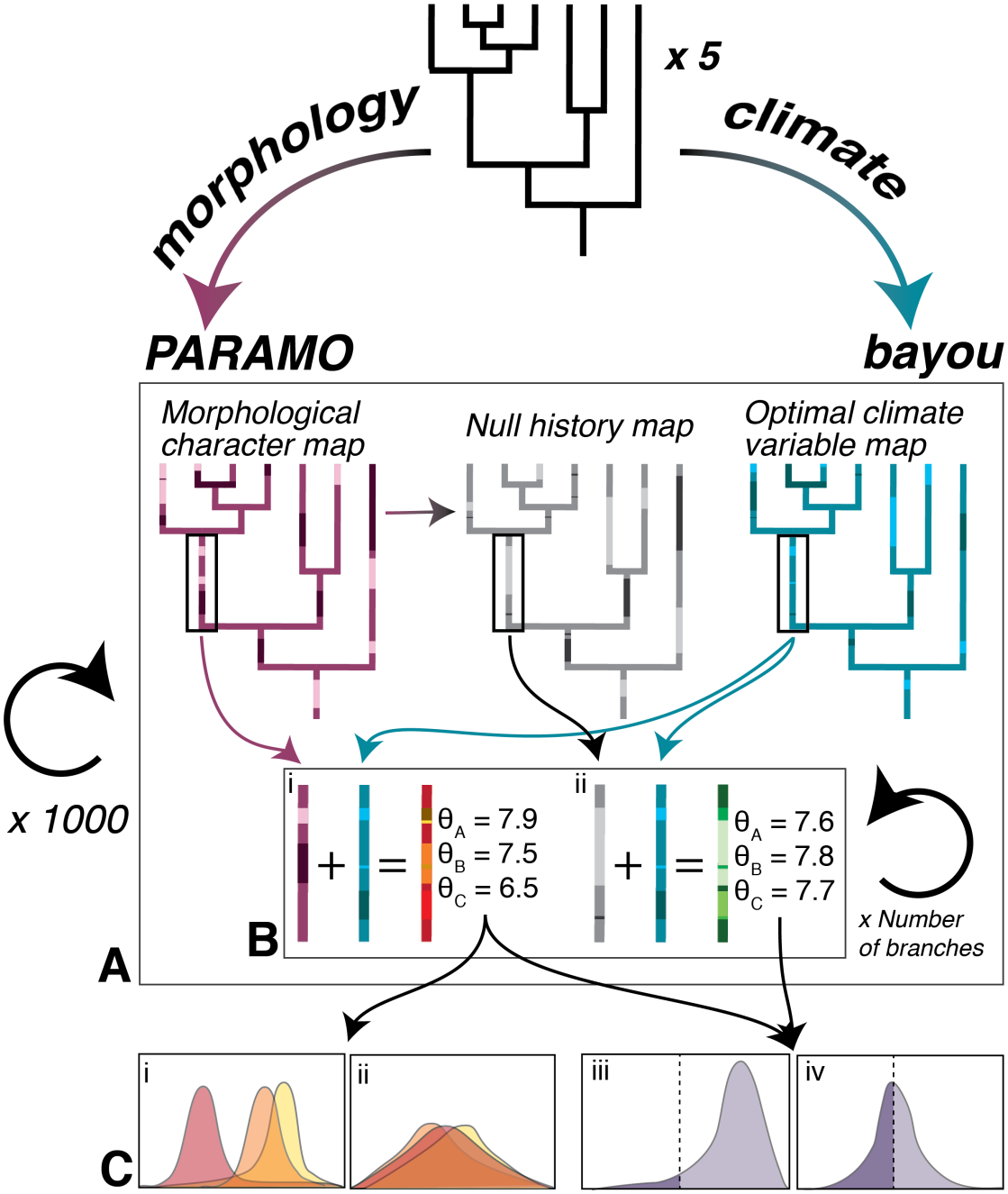

Figure 2: Schematic representation of methods used in this study. For each of five phylogenies sampled from the posterior distribution, I: A) used PARAMO and `bayou` to generate 1000 stochastic maps of the morphology and estimated climatic niche optima (θ), respectively, and simulate 1000 null histories (see Methods: Hypothesis testing) using the estimated evolutionary models from PARAMO. B) We combined each of the 1000 optimal climate variable maps with a morphological character map, and separately with a null history map, to create two sets of composite maps that show the state-specific θs per character state. C) We plot the densities of all estimated state-specific θs (i, ii) and the distribution of a test statistic (iii, iv; see Methods: Hypothesis testing). Plots i and iii represent a case where we would reject the null model, while in plots ii and iv we would fail to reject the null.

Analysis

We combined the climate data, morphology data, and phylogeny in a novel analysis pipeline that integrates newly-developed methods for modeling continuous characters (such as climate) and discrete characters (such as morphological categories). This novel pipeline models the continuous variables (climate) and the discrete variable (morphology) independently, and then asks if variation in adaptive optima for continuous characters are explained by the state of the discrete trait, allowing for complex models of the discrete trait and for an imperfect correspondence between adaptive optima and the discrete trait. We applied this pipeline to test the hypothesis that underground morphology (discrete) explains the patterns of adaptive optima in two axes of climatic niche (continuous).

Climatic Data Analysis with `bayou`

The multitype Ornstein-Uhlenbeck (OU) process implemented in the `R` package `bayou` (Uyeda and Harmon, 2014) models the evolution of a continuous character under an OU process, but allows the optimum of the OU process to vary across the branches of the phylogeny (Butler and King, 2004). This method, an extension of the Brownian motion process (Felsenstein, 1985), models the number and placement of adaptive regimes across the branches of the phylogeny, where each adaptive regime is characterized by a unique optimal continuous trait value, θ; the rate of evolution, σ^2, and the strength of selection, α. These regimes and their associated parameter values are sampled in proportion to their posterior probabilities using reversible-jump MCMC (Green, 1995). We use `bayou` to describe the modes evolution of climatic niche in Liliales.

For both temperature and precipitation seasonality we log-transformed the variable and ran `bayou` for 3.5 million generations using the priors specified in Supplemental Table 3. We assessed convergence using the `R` package `coda` (Plummer et al., 2006) and discarded the first 1% of samples as burnin. We drew 1000 samples from the posterior distribution of adaptive regimes for each climatic niche variable to use in subsequent calculations. Each of these samples contains a history of adaptive-optima evolution, which maps the adaptive optima along branches of the phylogeny.

Morphological Ancestral State Reconstructions with PARAMO

We used the PARAMO pipeline (Tarasov et al., 2019) to reconstruct the evolution of underground morphologies using hidden, structured Markov models and stochastic mapping. The hidden states represent the 'predisposition' to evolve USOs of different tissue types, incorporating the hierarchy of states illustrated in Figure 1. Each cluster (leaf, stem, and root) corresponds to a distinct evolutionary model such that the clusters evolve independently. First, for each of the three clusters, we estimated evolutionary rates using the `R` package `corHMM` (Beaulieu et al., 2013). We then reconstructed their evolutionary histories by simulating the evolution of each cluster 1000 times under the inferred model of evolution and conditioning on the observed data at the tips, a process termed stochastic mapping (Nielsen, 2002; Huelsenbeck et al., 2003). Each simulation produces a single stochastic map that illustrates a possible scenario of character evolution under the inferred model. PARMO performs simulations using the `make.simmap()` function in `phytools` (Revell, 2012). We produced a set of 1000 combined phenotype maps by overlaying the maps of each cluster. In isolation, each cluster only contains information about some of the USOs used the study. The combined phenotype map illustrates the history of underground morphology as the entire underground phenotype with many character

states.

Calculating State-Specific Climatic Optima

For a given `bayou` sample and a given morphological stochastic map, we calculated the optimal climate value for each morphological trait weighted by the amount of time spent in the particular combination of optimum and morphological trait (Figure 2B). As the observed morphology rather than the hidden states are expected to affect an organism's relationship to its environment, we collapsed the full set of character states (observed and hidden) into the observed states by combining the state-dependent optima of states with the same observed state but different hidden states. This procedure produced a set of state-specific optimal values per character state for each of 1000 stochastic map pairs (1000 stochastic map simulations from PARAMO and 1000 stochastic map samples from the `bayou` posterior) and for each of the 5 trees, for a total of 5000 state-specific optima per comparison. Some states were visited infrequently during stochastic mapping and thus have high percentages of missing data, which makes estimates of their state-specific optima more uncertain. To avoid these uncertain estimates, we dropped from analysis those states represented in less than 50% of the samples. We performed these comparisons for both precipitation and temperature seasonality and for the three morphological clusters (leaf, stem, and root) and the combined morphological phenotype, for a total of eight comparisons.

Hypothesis Testing

Our null hypothesis is that plants with different USOs are not evolving towards different climatic niche optima; in other words, state-specific climatic niche optima (θ) are equal. Correspondingly, under the alternative hypothesis, state-specific θs are different. However, for any finite dataset, state-specific θs will be different, even under the null hypothesis, and this issue is exacerbated by the phylogenetic structure to the inferred θ values. To account for this structure, and the finite sample, we calculated a test statistic that summarizes the overall difference between any particular set of state-specific θs. We simulated the distribution of that test statistic under the null model and checked whether the differences generated under the null model are less than the differences of the empirical estimates.

We simulated 1000 null histories of the three characters using the `sim.history()` function in the `R`package `phytools` (Revell, 2012) and the estimated Q-matrices from the empirical analyses. This procedure differs from stochastic mapping in the empirical analysis in that simulations are not conditioned on the observed character data. As in the empirical analysis, we also combined the three characters to produce 1000 null histories of the combined phenotype.

To calculate the test statistic, for a given vector of state-specific θ values, we calculated the pairwise distance between all state-specific θ, which results in a distance matrix, D:

$$D = \begin{pmatrix} d(\theta_{1,1}) & d(\theta_{1,2}) \\ d(\theta_{2,1}) & d(\theta_{2,2}) \end{pmatrix},$$

where $d(\theta_{i,j})$ is the distance between θ_i and θ_j:

$$d(\theta_{i,j}) = \sqrt{(\theta_i - \theta_j)^2}.$$

We then measure overall amount of difference using the Frobenius norm, $F(D)$, which summarizes the magnitude of the distance matrix. For a given `bayou` sample i, we calculate, S_i, the difference between the $F(D_i)$ given the stochastic map and $F(D)_i$ given the null history. we then compute S_i for each `bayou` sample; if 0 is in the 95% credible interval of S, then the null cannot be rejected.

Sensitivity of Results to Tree Selection

We performed all the above analyses on five trees randomly sampled from the posterior distribution of trees. To determine if our results are sensitive to particularities of the sampled trees, we repeated the entire procedure for five different trees. The results did not change meaningfully between these two sets of analyses (Supplemental Figure 1), so we present results based on the original five trees.

Data and Code Availability

All code is freely available on GitHub: https://github.com/cmt2/underground_evo and all data will be made available on Dryad prior to publication.

Results

Across all five trees, the ancestor of Liliales is estimated to have a rhizome with no leaf or root modifications (Figure 3 A–D shows the reconstructions for one tree), in agreement with previous work (Patterson and Givnish, 2002; Howard et al., 2019). For both temperature and precipitation seasonality, the estimated optima at basal branches in the phylogeny are highly seasonal, with subsequent shifts into less seasonal optima along more recent branches (Figure 3 E and F).

Figure 4 illustrates the distribution of estimated state-specific optima. Overall, the distributions of state-specific optima are more dispersed across the temperature seasonality axis than the precipitation seasonality axis. For the leaf cluster, the estimated bulb-averaged optima are more seasonal than those for no bulb. For the stem cluster, both rhizomes and corms are estimated to be less seasonal than non-stem modification, though for precipitation just rhizomes are estimated to be less seasonal than the other states. In the root cluster, root tubers (especially rotund root tubers) have lower temperature seasonality than no root tubers, but for precipitation, rotund and no root tubers overall overlap in their distributions while elongate root tubers are estimated to be more seasonal. In the combined phenotype for temperature, the most seasonal state for precipitation is bulb with rhizome and the least seasonal state is rhizome and rotund root tubers. For precipitation seasonality in the combined phenotype, most state-specific optima have highly overlapping distributions, though it appears that rhizomes are slightly less seasonal and rhizomes with elongate root tubers are slightly more seasonal than other distributions. For both temperature and precipitation, the distribution corresponding to no modified underground organs (non-geophytes) falls out intermediate along both climatic niche axes and overlaps with many other states, signifying that non-geophytes are not more or less seasonal than geophytes.

While the state-specific optima curves in Figure 4 appear distinct for many of the morphology-climate comparisons, across both climate variables, no distributions are more different than expected by chance (p-values 0.237 - 0.581), with the exception of the root cluster for temperature seasonality, which is marginally significant (p-value 0.053; Figure 5).

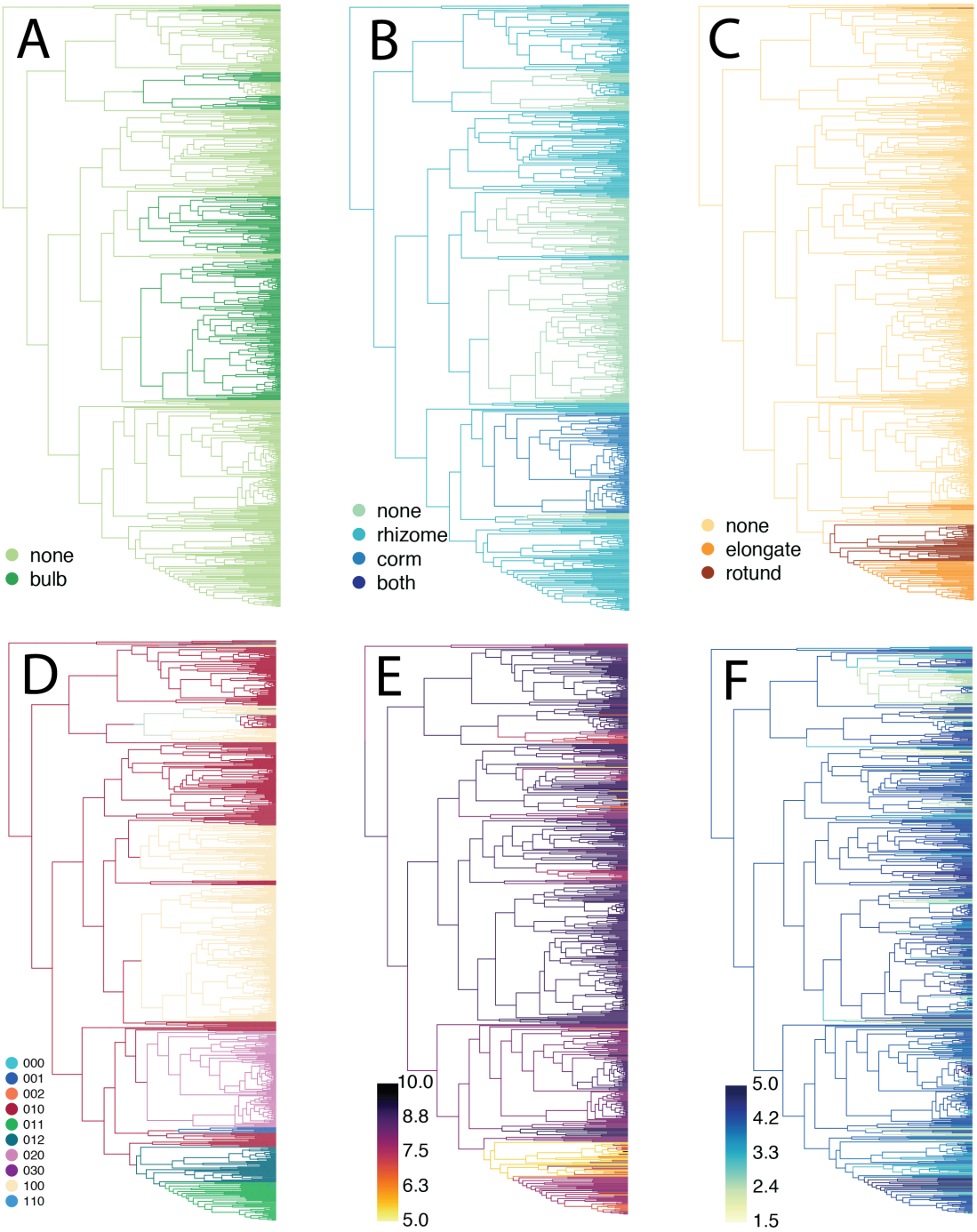

Figure 3: Stochastic maps of intermediary results for one of the trees used in analysis: A) leaf character from PARAMO, B) stem character from PARAMO, C) root character from PARAMO, D) combined phenotype character from PARAMO, E) posterior mean branch-specific θ for temperature seasonality F) posterior mean branch-specific θ for precipitation seasonality. In A, B, and C, the "none" category applies to absence of modifications of the relevant tissue type; those taxa may have modifications of the other tissues.

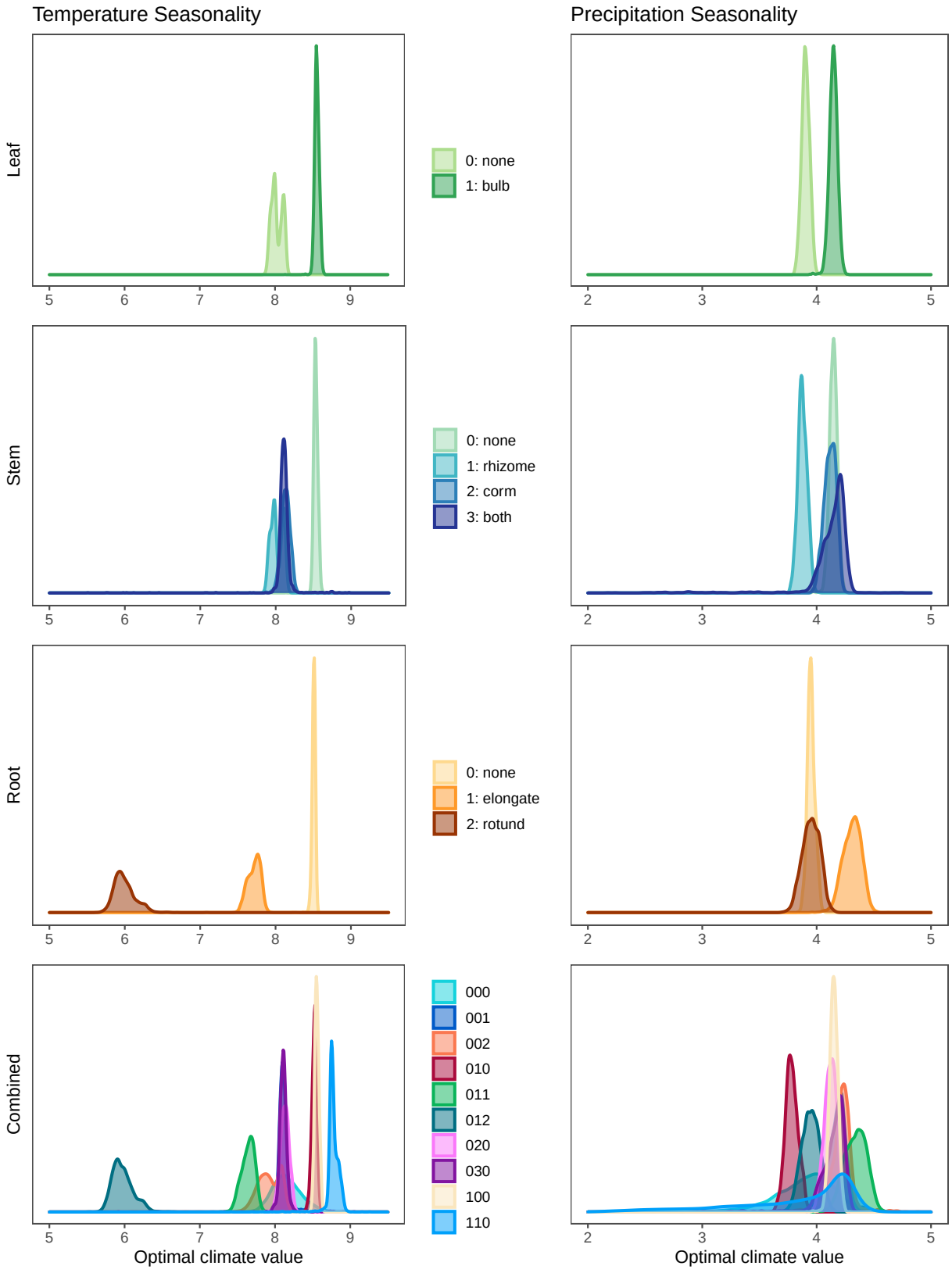

Figure 4: Posterior distributions of state-specific θs. For the combined morphology densities, digits in the state labels refer to states for each cluster. The first digit corresponds to the leaf state, the second digit corresponds to the stem state, and the third digit corresponds to the root state. For example, the 000 state refers to the absence of any USO (non-geophytes), and 012 refers to rhizomes and rotund root tubers.

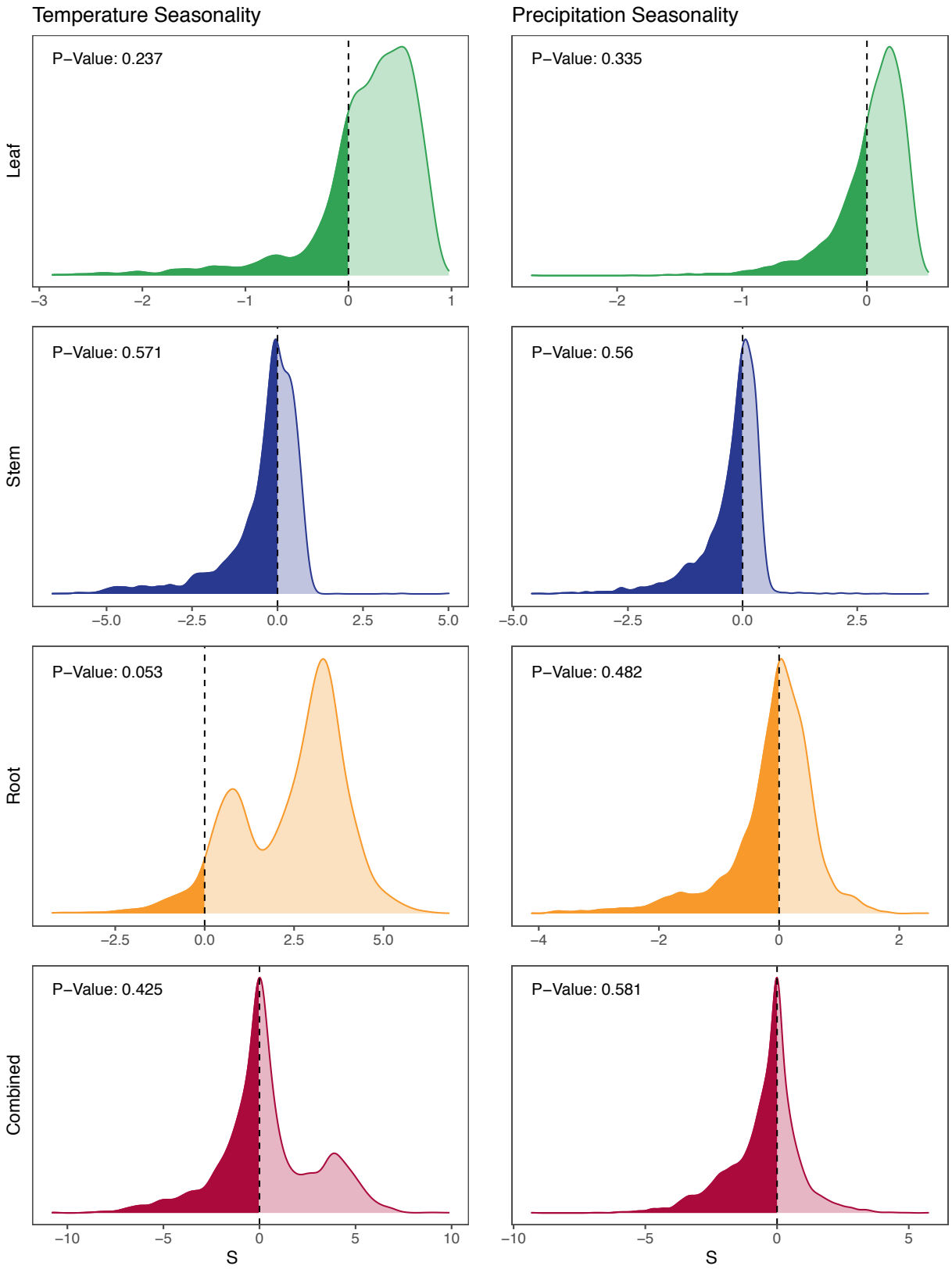

Figure 5: Test statistic (S) distributions. Distributions with 95% > 0 are considered evidence for a statistically significant difference in state-specific θ. P-Values correspond to the percent of values equal to or less than zero.

Discussion

Getting at the root of the problem: are USOs adaptations to particular climatic niches?

Together, these analyses demonstrate that plants in Liliales with the same underground storage organ do not share similar climatic niches more than expected by chance, with the exception of root morphology, where the presence of modified roots, especially rotund root tubers, is associated with lower temperature seasonality. Furthermore, non-geophytes in Liliales are not associated with more or less seasonal climates than their geophytic counterparts. While many of the state-specific optima curves (Figure 4) appear distinct, the null model suggests that these differences could be observed even with no correspondence between the trait and climate (Figure 5), due to the phylogenetic structure of environmental niche preference in the underlying data. This null model represents that case where a simulated discrete trait evolves under the same evolution model as the estimated models for USOs, but without the observed pattern at the tips. Any correspondence between the simulated traits and climate is due to chance, the distribution of the climate data on the tree, and/or the rates of evolution and stationary frequencies of the model, rather than the distribution of morphology across the tree.

These findings suggest that root tubers may be an adaptation to distinct ecological conditions or that they experience physiological constraints that restrict root tubers to particular climatic niches, distinguishing them from the other USOs included in this study. Unlike corms, rhizomes, and bulbs, root tubers are rarely the source of perennating underground buds. Most geophytes in Liliales (liliid geophytes) with root tubers also have rhizomes (e.g., *Bomarea*, *Alstroemeria*), which may serve as the source of underground buds while the root tubers store nutrients and water (Tribble et al., 2021). Many geophytes regenerate their USOs annually (especially bulbs and corms; Pate and Dixon, 1982; Kamenetsky and Okubo, 2012), and thus the processes of nutrient flow between the USO and the above ground plant are necessarily linked to the same seasonal cycles. Partitioning growth and storage between organs (as in the case of the species with tuber and rhizomes) may be particularly advantageous in climates with less temperature seasonality, as it allows for the continuous production of aerial shoots and the periodic replacement of stored nutrients as needed. Alternatively, places with less seasonal temperatures may be less likely to reach the freezing point, and root tubers may be particularly maladapted to frost compared to USOs derived from stem or leaf tissue.

These results differ from previous work in three primary ways. First, while we found no significant difference in environmental niche geophytes and non-geophytes, prior work suggested that being geophytic is associated with lower temperatures and precipitation and higher temperature variation compared to not being geophytic (Howard et al., 2019). The sparse representation of non-geophytes in Liliales indicates that these results may not reflect generalizable patterns of climatic niche occupancy between geophytes and non-geophytes, as these data likely lack the power to make this distinction. Liliales contains many taxa with diverse USOs, but non-geophytes are limited to a few exceptional plants, including *Alstroemeria graminea*, an annual native to the Atacama desert, and *Campynema lineare*, a curious herb distantly related to the rest of the order. Secondly, previous work found that rhizomes are correlated with increased temperature variation and found no evidence for difference niches between tuberous and non-tuberous taxa (Howard et al., 2019), while my analysis found no significant association between rhizomatous or non-rhizomatous taxa but instead suggests that taxa with root tubers are evolving towards lower optimal values of temperature seasonality. Thirdly, Patterson and Givnish (2002) found evidence that in the core Liliales, convergence on bulbs correlated with

independent transitions into seasonal and high light intensity habitats, but while we also find evidence for several independent transitions to bulbs, the association between bulbs and increased seasonality is not statistically significant in my results (P-Values 0.237 and 0.335). The present study differs from prior work in terms of level of detail for morphological characters, number of included climate variables, methods of analysis, and taxonomic scope, so it is possible that differences are due to any combination of these factors. In particular, the Howard et al. (2019) study does not distinguish between root tubers and stem tubers, and thus may not have recovered the association we find here.

In Liliales, rotund and elongate root tubers are mostly found in a few clades (namely *Bomarea*, *Alstroemeria*, *Burchardia*, and a few additional taxa), so it is possible that the strong association between decreased temperature seasonality and the presence of root tubers is driven by an unmeasured trait that happens to co-occur in those clades. For this reason, extrapolating my results to non-liliid geophytes may not be appropriate, so follow-up studies should address variation in root morphology and climate in other clades, particularly in groups with many independent transitions between the absence and presence of root tubers and between different root tuber morphologies. *Asparagus* would be a particular appropriate system in which to further test these associations, as root morphology is highly variable in the genus (Leebens-Mack, comm). However, there are few other clades known for well-characterized variation in the presence and absence of root tubers or for variation in root tuber morphology. Root morphology is vastly undercharacterized in many plant clades (Tribble et al., 2021; Janzen et al., 1975), so this work also motivates increased morphological characterization of underground morphology and root morphology in particular and demonstrates the importance continued emphasis on classic botanical techniques for understanding biodiversity. Studies that characterize the functional ecology and physiology of root tubers will likely yield important insights into how and why they differ from other USOs.

Applicability of analysis pipeline for future comparative studies

This project demonstrates the utility of adapting existing, complex evolutionary models to address a long-standing challenge in statistical comparative biology: modeling discrete and continuous traits. The methodological pipeline presented in this study uses PARAMO to estimate the evolutionary history of a discrete trait while taking into account nested relationships between character states and `bayou` to estimate regimes of continuous trait evolution (including number and location of optimal continuous trait values). It then combines these estimates by calculating the average optimal values for each discrete trait and asks if these averaged optima are more different than expected under a null model.

This pipeline has several advantages over existing methods. First, the model parameters have clear biological interpretations. Densities from Figure 4 show the distributions of probable optimal phenotypes (θ) of the continuous trait by discrete category. These θs correspond to peaks in the adaptive landscape of the continuous trait, so the estimated parameter values have a clear and direct link to adaptive evolutionary theory, unlike the estimated effect sizes from linear models. Second, the methods directly model the evolutionary processes of both the continuous and discrete traits and thus are more appropriate for addressing hypotheses of adaptive evolution. Third, the pipeline can accommodate any number of models for discrete traits. While we use PARAMO (Tarasov et al., 2019) to model the nested relationships between USOs derived from the same tissue, any model of evolution which can be simulated under could generate stochastic map histories and thus be incorporated into the pipeline. In some empirical cases, not including the present study, traits are explicitly defined in ontologies (e.g. `phenoscape.org`), and PARAMO uses these

definitions directly to establish the hierarchy between character states. As the use of ontologies in comparative biology becomes more common, incorporating hierarchies codified in ontologies will greatly expand the ability of researchers to test increasingly complex hypotheses, including how developmental processes impact trait evolution and the relationships between traits. This modularity means that the pipeline is flexible and can incorporate a wide range of plausible empirical datasets and hypotheses and thus could be adopted widely across macroevolutionary research.

Finally, this method allows for imperfect correspondence between the estimated quantitative regimes and the discrete states. Quantitative trait evolution, and climatic niche evolution in particular, is complex and almost certainly explained by many other variables. It would be inappropriate to force our model to test between either no correspondence or full correspondence between the quantitative trait evolution and the state of a discrete trait. State-dependent speciation and extinction methods may provide a useful metaphor; the addition of hidden states allowed these models to portion the variance of speciation and extinction rates between the observed trait of interest and an unobserved hidden state (Rabosky and Goldberg, 2015). Similarly, our method examines the distributions of state-dependent optima without requiring that the continuous trait regimes and the discrete trait vary perfectly together over the tree. We test for significance by comparing these observed distributions to null distributions that are built from our null expectation of random evolution of the discrete trait. We thus avoid the "straw-man effect" described in May and Moore (2020), in which any variation in the process of quantitative trait evolution is spuriously attributed to the discrete trait, because the null hypothesis is that there is no variation at all.

All code and scripts used in this pipeline are publicly available on GitHub at https://github.com/cmt2/underground_evo to facilitate adoption of the methods.

Conclusions

The results of this study support differences in the optimal climatic niches of plants with some underground storage organs, namely between plants with and without root tubers. Having root tubers, particularly rotund root tubers, is associated with lower temperature seasonality in Liliales, suggesting that root-derived underground morphologies may experience different ecological constraints or differentially mediate ecological interactions than do shoot-derived USOs. This implies that the developmental origin of the structure influences the way it mediates ecological relationships and draws into question the appropriateness of ascribing broad ecological patterns uniformly across geophytes. This work highlights an important clade of geophytes within the monocotyledons, and zooming in on this specific group allows us to be detailed in describing morphology and may illuminate clade-specific trends not detected in a larger analysis. Furthermore, this study serves a proof-of-concept for a new analysis pipeline that models the adaptive evolution of a continuous trait based on the hierarchical, nested evolution of a discrete trait. This pipeline is applicable across many areas of evolutionary biology and may serve as a model for future hypothesis-driven comparative research.

Author Contributions

CMT designed and executed the study. CMT collected data. CMT and AJ-G performed analyses, and MRM advised on statistics. CMT, CJR, CDS, and RZ-F guided interpretation of results.

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