## Title

# Temperate-tropical transitions are linked with shifts in the structure of evolutionary integration in Vitaceae leaves

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## Abstract

Understanding how the intrinsic ability of populations and species to meet shifting selective demands shapes evolutionary patterns over both short and long timescales is a major question in biology. One major axis of evolutionary flexibility can be measured by phenotypic integration and modularity. The strength, scale, and structure of integration may constrain or catalyze evolution in the face of new selective pressures. We analyze a dataset of seven leaf measurements across Vitaceae to examine whether the structure of macroevolutionary integration is linked to transitions between temperate and tropical habitats by examining how the structure of integration shifts at discrete points along a phylogeny. We also examine these patterns in light of lineage diversification rates to understand how and whether patterns in the evolvability of complex multivariate phenotypes are linked to higher-level macroevolutionary dynamics. We found that shifts in the structure of macroevolutionary integration in leaves coincide with early colonization events into new climates and that lineages that are more climatically labile are more weakly integrated overall. These more evolutionarily flexible lineages also had higher lineage turnover, suggesting a link between shifting vectors of selection, internal constraint, and lineage persistence in the face of changing environments.

## Introduction

Phenotypic traits often covary. The causes, consequences, and biological significance of trait covariation are complex and manifest distinct patterns across levels of temporal and biological scale. Trait covariation provides a numeric basis for partitioning the phenotype into semi-autonomous regions, where suites of traits internally covary, but are independent from one another. This is referred to as modularity (Wagner et al., 2007). The evolution of modularity and its relationship to major unanswered questions in evolutionary theory has long been intuited, but few empirical links have been drawn between the modular patterns that emerge at different levels of the biological hierarchy. Examples at a handful of these levels follow.

Trait covariation has long been used to characterize internal constraints on adaptation within populations of organisms (e.g., Cheverud 1984, 1988; Wagner and Altenberg 1996). At this level, trait covariation is typically thought to reflect the genetic variance/covariance (VCV) matrix, i.e., the additive genetic variance shared between each trait pair (Cheverud 1988). The biological significance of this is straightforward. Trait pairs that share a lot of underlying genetic architecture will be constrained in their evolution by the functional demands of each other. The consequences of covariation on adaptation have been fruitfully explored in *Drosophila*. For example, Chenoweth and colleagues (2010) found that divergence between nine *Drosophila* populations aligned more closely with the orientation of the VCV than with the direction of experimentally induced sexual selection. In another case study, Hansen and colleagues (2003) found that the direction and strength of floral evolution over the short term was strongly predicted by constraints induced by covariation. Numerous similar examples exist (Bolstad et al. 2014, Sztepanacz and Houle 2019). However, results are mixed, with many studies suggesting that directional selection can overcome variational constraints (Beldade et al. 2002, Agrawal and Stinchcombe 2009). Computer simulations have even shown that directional selection itself may lead to the breakup and rearrangement of patterns in covariation (Melo and Marroig 2015). And so, selection-induced shifts in the structure of modularity might help facilitate the emergence of new, perhaps complex, adaptations. It appears sensible, then, to conceive of 'evolvability' – the ability for a population to respond to selection – as an axis that varies as a function of how well constraints are aligned with selection vectors and the capability for covariation patterns themselves to shift (Houle 1992, Hansen and Houle 2008).

Expanding temporal scale outward, the evolution of covariation patterns has repeatedly come up in paleontological and macroevolutionary studies. In these studies, covariation is measured using a diversity of approaches and data sources and so may perhaps be best considered more broadly as reflecting the general structure of modularity that emerges over long evolutionary timescales. It is possible that the origin of new morphologies is facilitated by shifts in the structure of modularity. Qualitative morphological analysis (Vermeij 1973), shifts in patterns displayed by discrete traits (Wagner 2018), and coordinated patterns in evolutionary disparity and rate among suites of quantitative traits (Parins-Fukuchi 2020) have all been used to reach this conclusion. Paleontological work also suggests that shifts in the strength of covariation may mediate long-term trends in phenotypic evolution (Goswami et al. 2015). A parallel but distinct avenue of research has also shown that changes in the strength of correlation between pairs of traits may underlie ecological transitions (Revell and Collar 2009, Revell et al. 2022). All of these diverse contexts are consistent with the population genetic notion that phenotypic innovations may correspond to changes in 'parcellation' and 'integration' (i.e., separating and joining together, respectively) of traits (Wagner and Altenberg 1996), but no explicit links have been drawn. The impact of constraint induced by integration patterns on macroevolutionary patterns, such as lineage survival, are also very poorly known.

Trait covariation has also been explored in the context of ecological community assembly. When measured within plant communities, each aligned along an environmental gradient, trait covariation varies as a function of environmental stress (Dwyer and Laughlin 2017, Brown et al. 2022). This pattern probably results from the functional inviability of some trait combinations within certain climates. In this scenario, lineages with unfavorable trait combinations or covariation patterns are filtered out of some regions. While useful from the standpoint of functional ecology, these studies do not tell us how variation in covariation patterns itself arises, nor how or whether shifts in the structure of covariation may underlie the movement of individual lineages into new ecological contexts. Nevertheless, they make it clear that environmental variation plays a major role in patterns of phenotypic integration. This body of work has clearly explained trait covariation in terms of plant ecology; we seek to address it in terms of plant evolution.

Here, we perform a novel analysis of macroevolutionary integration across Vitaceae (grapes and their relatives) to see whether evolvability in multivariate leaf phenotypes coincides with transitions across habitats. Our interests here follow two major themes: 1) identifying whether changes in covariation have the potential to explain major ecological shifts, and 2) reaching across the biological hierarchy to draw more explicit links between the apparently distinct levels of covariation and evolutionary process (microevolutionary, macroevolutionary, ecological) outlined above. We explored this by applying a novel phylogenetic approach to test for shifts in the structure of covariation in evolutionary divergences across a set quantitative leaf traits measured across Vitaceae, a clade that has undergone multiple transitions between temperate and tropical biomes. Previous work has found that major changes in leaf phenotype coincide with temperate-tropical transitions in *Viburnum* (Schmerler et al. 2012). We sought to ask whether these changes may themselves be facilitated by rearrangements of the structure of evolutionary covariation among leaf traits. As a final goal, we aimed to identify whether the population and quantitative genetic processes that give rise to patterns in the structure of covariation provide any explanatory power over macroevolutionary dynamics in phenotypic disparity and lineage diversification across clades.

**Methods and Materials**

*Data and code.* Leaf measurements across Vitaceae were obtained from Chen (2009). The following seven traits were included: leaf width, leaf length, petiole length, petiole width, distance between secondary veins, tooth location (distance from leaf base), and petiolule length (of lateral leaflets) These morphological data were cleaned and log-transformed prior to analysis. All data files and code used in the study are available at (WILL PROVIDE).

*Phylogeny.* We used the molecular phylogeny of Vitaceae published by Parins-Fukuchi (2018). We applied dates to this phylogeny by including the fossil lineages examined in the aforementioned study as node calibrations using treePL (Smith and O'Meara 2012). We did not include the fossils as tips in the dating and covariation analyses because they represent seeds and therefore would have been uninformative with regard to leaf trait covariation.

*Phylogenetic variation in evolutionary covariation*. We developed a novel approach to examine shifts in the structure of evolutionary covariation across a phylogeny. This approach builds conceptually upon the work of Parins-Fukuchi (2020b), by extending the basic framework to explore 1) how covarying evolutionary patterns between traits themselves shift along a phylogeny, and 2) by modeling the covariance structure more explicitly rather than simply relying on shared patterns in phenotypic disparity across traits.

We will start our explanation of the method using the simplest version of the model: one where the structure of covariation is shared across the entire phylogeny. We first perform an ancestral state reconstruction (ASR) under Brownian motion on each of the traits (Maddison 1991). From here, we estimate directional vectors of change along each branch by subtracting the value at each node from that of its parent. At this point, each trait has been transformed into a set of $2n-2$ ($n$ is the number of lineages included in the phylogeny) vectors of edgewise evolutionary divergence. We then construct a correlation matrix using the vectors for each trait. This measures the magnitude with which each trait pair undergoes coordinated evolutionary changes and the direction of the association (positive or negative). In other words, it gives the covariance between changes in population means. The precise evolutionary interpretation of this matrix, typically referred to as $V$, has been outlined by Felsenstein (1988) using the equation:

$V$=$GCG$ (eqn. 1)

*G* is the genetic covariance matrix, while *C* represents the set of covariances in the selection vectors for each pair of traits. Taken together, *V* is then defined sensibly through a combination of the set of genetic constraints and the effects of coordinated selection regimes. As a side note, Felsenstein (1988) gave an explicit philosophical critique of the method through which we construct *V*. Specifically, he pointed out that ASRs are not true data, but instead inferences drawn from data. While we, of course, agree on a philosophical level, we believe the method is sufficient for our aim of reconstructing broad shifts in evolutionary covariation. Practically speaking, while ASRs are often rife with error, we believe that our own questions can be adequately tackled with estimates containing relatively high error. The most important aspect is identifying positive and negative correlations that particularly stand out and how that structure changes across a tree. It is also worth noting that many phylogenetic comparative methods have arisen since the time of Felsenstein's writing that use essentially the same information we use here – traits mapped to a phylogeny – to derive robust historical inferences. We are therefore confident in our approach given our purposes. Detailed examination of each pairwise trait relationship, or a full breakdown and interpretation of the *G* and *C* components, may benefit from more careful methodological consideration, or at least further validation that the resulting covariances are numerically robust to this approach. However, such fine-scaled analysis is not included among our goals at present.

Estimating *V* is fundamental to our approach. It provides a natural link between the population genetic conceptualization of covariation and modularity, defined ultimately by *G*, and the patterns observed over deeper timescales, including those explored by paleontologists and macroevolutionists. If we observe shifts in *V*, we are forced to acknowledge the likely reality that those shifts are at least partially facilitated by shifts in *G*. This is because we know, over shorter timescales, that selection tends to be inhibited if it is misaligned with *G*. Of course, the reality is that *C* also likely shifts during major ecological transitions. Any heterogeneity must therefore be considered as the sum of these population processes.

To consider the possibility that the structure of modularity has shifted across the phylogeny, we defined a heterogeneous model structured as a phylogenetic mixture of multivariate normal distributions. In the single *V* model, we model *V* as a single multivariate normal distribution with a mean vector of expected changes equal to zero (this is the expectation under Brownian motion) and covariance matrix equal to *V*. In order to more fairly compare *V* across the tree, we placed all traits on the same scale and rescaled all estimated covariance matrices to correlation matrices. Examining covariance matrices instead may also be a useful application of the method, by searching simultaneously for shifts in both evolutionary rate and covariation patterns, but was not our goal here. The probability density function of this distribution gives us a likelihood function with which to evaluate the evidence in favor of our model. To find the best-supported set of covariance regimes, we employed an automated search algorithm based on that implemented by Smith et al. (2022). A summary of the algorithm follows.

*The search algorithm.* The algorithm requires a specified minimum size threshold, defined by the number of tips, for clades to be considered. For example, if we specify 10, clades with 10 or more tips will be considered as possible shift points. This greatly improves computational efficiency and also helps protect against estimating poorly defined covariance matrices on very small clades. For every clade that meets this size criterion, a covariance matrix is then estimated using *only* the edges subtending the clade root. The algorithm then proceeds as follows: evaluate the likelihood of a combined model, whereby the data are characterized using two multivariate normal distributions, one encompassing the proposed shift and the other encompassing the rest of the taxa in the tree. Calculate the Akaike information criterion (AIC) value using this combined likelihood. If the AIC indicates an improvement in fit, save the estimated parameters and AIC scores; if not, discard them. Rank all of the shifts according to their improvement in AIC over the base (single regime) model. Proceed through this ranked list. Retain each model that, when combined with the previously retained models in the ranked list, yields an AIC improvement over the base model. This procedure has the benefit of naturally identifying the optimal shift point in the case where several adjacent nodes all represent possible shift locations. The ranking ensures that the best-supported location will be added first; others will have to add significantly to the explanatory power of the model if they are to be included as a nested shift.

*Environmental habit*. We constructed a dataset characterizing the environmental occupancy of each lineage, as defined by freezing tolerance. First, we generated a dataset of spatial occurrences across Vitaceae by gathering data from the Global Biodiversity Information Facility (GBIF– https://www.gbif.org/). We then extracted climate data across these locales using the Bioclim dataset (https://www.worldclim.org/data/bioclim.html). We defined any lineage as freezing tolerant for which 2.5% or more of occurrences across their sampled geographic range experience minimum temperatures at or below zero degrees celsius during the coldest month of the year. We then performed a parsimony analysis to map freezing tolerance to the phylogeny in order to compare the location of shifts in covariation structure to those in the environment.

*Diversification rate analysis*. We estimated lineage-specific diversification rates using MiSSE (see Vasconcelos et al. 2022a), a likelihood-based, hidden state only, state-speciation and extinction model implemented in *hisse* (Beaulieu and O'Meara 2016). Within MiSSE there are 52 possible models to evaluate, so we used the function *MiSSEGreedy*() to automate the process of fitting a large set of MiSSE models. The function first runs a chunk of models, determines the “best” based on AIC, then it continues on from that complexity until all models in a chunk of complex models are greater than 10 $\Delta$AIC units than the current best model. In this way, we only evaluated a set of models that are reasonably parameter-rich with respect to the data set. We culled the resulting model set to remove any redundant model fits. For example, if the maximum likelihood estimates are the same for turnover rate in regime *A* and the turnover in regime *B* in a turnover rate varying-only model, it is essentially the same as including a single turnover rate model twice. This would lower the weight of other models as a consequence. It is recommended in these situations to remove the more complex of the two from the set (Burnham and Anderson 2003). For each model, we obtained the marginal reconstructions of the specified hidden states for each node and tip in the tree. We then summarized results based on diversification rates model-averaged across only the tips that survived to the present. For a given model, the marginal probability of each rate regime is obtained for every tip, and the rates for each regime are averaged together using the marginal probability as a weight: a weighted average of these rates is then obtained across all models using Akaike weights.

We used a paired *t*-test to assess whether model-averaged diversification rates are different across the different evolutionary covariation regimes. However, before conducting this analysis we first identified all “cherries” in the tree, which are two tips that are sisters to each other and share the same branch length to the direct ancestral node. Within MiSSE, all sister tips should theoretically inherit the exact rate class probabilities, meaning they have identical tip rates. This could artificially inflate or reduce any means within a given regime. Therefore, as a precaution, we removed, at random, one of the two taxa represented in a cherry (see Vasconcelos et al. 2022a).

## Results and Discussion

*The structure of leaf evolutionary integration and climate shifts*.

A close relationship between leaf form and climate has long been recognized (e.g., Givnish 1987; Spicer et al. 2021). This is reflected by the repeated, independent evolution of particular leaf syndromes and functional traits in similar climates—e.g., more rounded, toothed and lobed leaves in temperate environments (e.g., Schmerler et al. 2012). The widespread use of leaf physiognomy as basis for paleoclimatic inferences is a testament to the close link between leaf form and climate (e.g., Wolf 1971), but this relationship is not without complications (Peppe et al. 2010). For example, leaf traits concentrated in particular biomes or climatic regimes might be, at least in part, a byproduct of select lineages being overrepresented in those areas (Hinojosa et al. 2010; Little et al. 2010). Leaf forms associated with particular climates might also, in some cases, have arisen before the climates themselves, suggesting that new climatic regimes can serve as a filter for preadapted forms (Ackerly 2004). More generally, because leaves possess developmentally integrated suites of traits, it is unrealistic to expect individual traits to respond to climatic changes in simple, predictable ways. Examining changes in the structure of leaf trait integration across climatic shifts offers a basis for understanding the evolutionary underpinnings of environmental transitions, beyond the correlation of individual traits with different climatic variables.

Given that different suites of traits are associated with megathermal (‘tropical’) vs. mesothermal (‘temperate’) climates (Wolfe 1995), we might generally expect the structure of leaf trait covariance to differ between these types of environments. We also might expect the strength of integration to control the extent to which lineages are able to readily shift between such environments, with more relaxed integration creating an opportunity for more frequent tropical-temperate shifts. Vitaceae represent an excellent system for exploring these problems because it has considerable taxonomic diversity in both tropical and temperate environments, as well as broad variation in leaf form with regard to leaf size, complexity (simple vs. compound), lobing, and tooth size, structure, and density. The dataset examined here captures different properties of leaf size and venation and tooth density, traits that have clear relationships with both temperature and precipitation levels (Spicer et al. 2021). Our results, detailed below, broadly show that integration regimes of leaf traits correspond closely with climate, with the strength of integration determining the ease with which lineages can evolutionarily shift into different environments.

*Environmentally-linked shifts in evolutionary covariation.* Our approach uncovered evidence for three distinct evolutionary covariation regimes across Vitaceae leaf traits (Fig. 1). These regimes are largely congruent with broad patterns in climatic shifts (Fig. 2). In particular, regime 0 corresponds closely to the set of Vitaceae lineages that made the most significant transitions into freezing habitats (e.g., *Vitis, Parthenocissus*, etc). The clade encompassing *Cissus*, *Tetrastigma*, and related lineages corresponds to another co-variational regime. *Ampelocissus* also occupies its own regime, perhaps delimiting a phenotypic shift that occurred during the movement of this genus back into tropical biomes. Although we reconstructed the ancestor of Vitaceae, and many early branches in the phylogeny, to be freezing-tolerant, this is perhaps at odds with the prevailing climate during the early diversification of family, ca. 70 to 45 mya. During this time, global temperatures were considerably warmer than the present, and freezing conditions were perhaps only present (if at all) at high elevations or high latitudes, at least until the onset of climatic deterioration in the mid to late Eocene (Zachos et al. 2001). Most Cretaceous to early Cenozoic Vitaceae fossils are known from middle to low latitudes (e.g., Chen and Manchester 2007; Manchester et al. 2013), suggesting that the ancestors of most major lineages likely did not experience freezing conditions. However, early representatives of Vitaceae (or particular lineages) may have possessed traits (e.g., deciduousness) that predisposed them to thrive in freezing conditions. Furthermore, an accurate reconstruction of the climate occupancy of ancestral Vitaceae, although certainly of interest, is not of central importance for the focal questions of this study. The critical finding is that major covariation regimes largely coincide with lineages occupying particular climatic conditions (‘tropical’ vs ‘temperate’), suggesting that evolutionary shifts in leaf trait integration also coincide with (or underlie) major environmental shifts in Vitaceae.

*Habitat shifts and covariation strength.* More climatically variable lineages had weaker integration, on average (Table 1). The lineages under regime 1, which encompasses both tropical and temperate lineages, displayed the lowest overall correlation strengths. There are at least two possible explanations for this pattern: 1) the environmentally disparate lineages encompassed under regime 1 may be more evolutionarily flexible and morphologically diverse because of trait parcellation, or 2) lineages within regime 1 may undergo shifts in the structure of G (the genetic covariance matrix) during these repeated movements into frozen habitats. In the former scenario, G is relaxed across the entire regime, allowing lineages moving into freezing habitats to easily accommodate any resulting shifts in C (the selective covariance matrix). In the latter, G remodels itself during shifts into freezing climates, perhaps in response to shifts in C. In this case, the appearance of relaxed covariation would actually be driven by averaging across several, variable, covariance regimes left undetected due to lineage sampling. Either of these scenarios would be reflective of evolutionary flexibility in the structure of integration and so distinguishing between them is not important here. For our purposes, we are content to accept that the repeated shifts into freezing climates observed in regime 1 are linked with either a covariance structure that is more relaxed overall, facilitating a range of responses to selection, or more evolutionary labile, undergoing rapid change to accommodate the demands of new environments. Either case suggests that evolution of the macroevolutionary covariance structure (and by extension, its constituent parts, G and C) facilitated tolerance of unpredictable and distinct habitats, encountered either by migration into new areas, or in response to long-term climate changes occurring in situ. While we focused on temperate-tropical shifts here (with respect to freezing), we also note that the species included in regime 1 also occupy diverse habitats with respect to precipitation, including shifts into xeric environments in continental Africa, Madagascar, and parts of the Neotropics, which might similarly be influencing patterns of trait integration in this regime.

Comparing the patterns shown by regimes 0 and 1 with that of regime 2 illustrates how ecological specialization can generate covariance patterns that are more conserved at the macroevolutionary level. Regime 2, which is overwhelmingly specialized in tropical climates, displays the phylogenetic covariance matrix with the strongest structure and the strongest evolutionary links between traits, on average. On the surface, this result may appear to be at odds with ecological work on trait covariation within communities along environmental stress gradients (Dwyer and Laughlin 2017). Ecological work has found that more stressful environments host plant communities with stronger covariation between functional traits. This is because environmental stressors induce functional constraints that disadvantage certain trait combinations. Lineages that display unfavorable trait combinations are filtered out of certain areas. In this case, trait covariation actually serves as a catalyst, rather than constraint, for some lineages to move into more challenging environments. Nevertheless, trade-offs imposed by competing environmental stressors appear to create slightly more complex dynamics in covariation patterns (Brown et al. 2022). Along similar lines, we suggest that increased tradeoffs induced by the flexible and sometimes unpredictable climates occupied by lineages in temperate and mixed temperate-tropical regions lead to a more generally volatile structure of evolutionary covariance. In addition, while some climates may filter out lineages based on trait covariation patterns over ecological scales, our work shows how lineages themselves can shift the structure of trait covariation and occupy new habitats over macroevolutionary timescales.

*Diversification rates across covariation regimes*. We grouped regimes 0 and 2 into a single diversification regime and compared against rates within regime 1 to test the hypothesis that the relaxed structure of evolutionary covariation and repeated independent movements from tropical into temperate habitats may have led to distinct macroevolutionary dynamics. We found significant differences in rates of speciation, extinction, net diversification, and turnover when comparing across covariation regimes (Table 2). The strength of leaf integration across Vitaceae is linked with diversification rates. More tightly integrated lineages (regimes 0 and 2) exhibit lower turnover and higher net diversification rates than more weakly integrated lineages (regime 1). This correlation between leaf integration strength and diversification rates also may be linked to differences in climate niche evolution across each regime. The repeated climatic shifts observed in regime 1 correspond to overall higher turnover, and marginally lower net diversification. More climatically stable regimes, which are also more tightly integrated, turnover less and generally have a higher net increase in diversity as a result.

*Leaf evolvability, climate shifts, and macroevolutionary dynamics*. The shifts in macroevolutionary covariance between leaf traits are the consequence of both shifting structures of multivariate selection and genetic constraints. While it was not possible here, given data limitations, to disentangle the relative influence of each of these in shaping patterns emergent at the phylogenetic scale, the coincidence of repeated movements between tropical and freezing environments with shifts in the structure of trait covariation suggests that vitaceous leaves are generally evolvable in response to environmental changes. This is reflected in overall trait disparity, with the most climatically labile lineages occupying the largest spread of morphospace (Fig. 3). In contrast, the less climatically diverse lineages, housed within regimes 0 and 2, display lower disparity overall and tighter mean integration between traits.

The patterns displayed across regime 1, which has undergone repeated movements into freezing habitats from a tropical ancestor, provides a compelling example. While the lineages contained in regimes 0 and 2 are relatively uniform in climate tolerance, regime 1 has repeatedly made the difficult transition from tropical to freezing. The covariation pattern in regime 1 indicates enhanced evolutionary flexibility, i.e., evolvability, as indicated by weakened integration as compared to the other regimes (Table 1). The multivariate vectors of selection on leaf traits likely shift during movement into new climates. The ability for lineages to withstand repeated shifts into freezing habitats suggests that *G* likely does not strongly constrain response of the population means to the new directions imposed by selection in these new environments. If *G* did maintain long-term constraints across these transitions, these migrant lineages, constrained within a maladaptive phenotypic space relative to their new habitats, would likely go extinct because of a decreased efficacy navigating these new habitats and competition from perhaps better-adapted species  (Van Valen 1973). Although we analyzed these patterns in light of only *abiotic* (environmental) factors, we assume that new environments will also contain different biotic contexts. And regardless of the relative importance of biotic and abiotic factors in driving these macroevolutionary patterns, as originally formulated, the Red Queen accommodates both. We favor this interpretation given that the two likely work synchronously. We therefore assume that the environmental shifts we identified, along with the corresponding shifts in phenotype and development, may indicate changes in both abiotic and biotic factors.

We observed a pattern of high leaf evolvability across environmental transitions paired with elevated turnover rates in lineages transitioning into derived habitats. Diversification rate variation has been explained by many possible dynamics, for example, latitudinal correlates with energy input: “the Red Queen runs faster when she is hot” (Brown 2014). Our analyses reflect a somewhat simpler dynamic that unifies several levels of macroevolutionary patterns. The patterns in Vitaceae suggest that *the Red*

*Queen runs faster when she is uncomfortable* (see similar arguments in Stebbins 1974; Vasconcelos et al. 2022b). Encountering new habitats due to migration and/or climate change results in the emergence of a completely new set of biotic and abiotic conditions that may yield a variety of responses that are intrinsic to each individual lineage. These responses may be rooted in developmental and genetic constraints on phenotypes, population-level variation, and extinction dynamics. As a result, while extinction tends to increase when lineages encounter new habitats, this is compensated for by increased speciation among phenotypically flexible lineages. Highly evolvable lineages, and perhaps those preadapted as well (as lianas such as Vitaceae might be for a variety of conditions), are better equipped to keep up with these novel and likely challenging conditions by increasing their evolutionary pace. In the case of Vitaceae, this results in a more flexible phenotypic covariance structure, wider phenotypic disparity, and elevated turnover rates. Further research identifying the specific link between phenotypic innovation, constraint, and speciation rates will help to further refine our understanding of how lineages persist in the face of a shifting evolutionary landscape.

This second layer might explain results in vertebrates that conflict with latitudinal explanations for diversification rate variation (Rabosky et al., 2018), which found “paradoxically” higher speciation rates far from the tropics. These patterns may reflect a more extreme manifestation of the causes we outline here. Movement to more extreme environments may, in some lineages, increase variation in macroevolutionary parameters (lineage diversification, the origin of phenotypic novelties, etc) to a level that overwhelms latitudinal patterns. For example, certain ecological conditions in temperate and arctic regions may be so far from a lineage’s initial capability to accommodate them that it must increase its macroevolutionary activity beyond that displayed at the tropics to outpace extinction. This may manifest itself in higher turnover, faster and wider phenotypic disparification, and macroevolutionary integration patterns that are structured more flexibly. The relative importance of latitudinal vs intrinsic explanations likely varies across clades, environments, and epochs. Deeper understanding of the level(s) at which selection operates and how intrinsic evolvability interacts with movement into new ecological contexts will help to further disentangle the root causes of these dynamics and disparity in pattern across studies and taxa.

It also seems worthwhile to note that the lineages within regime 1 do not cluster according to climate niche in leaf morphospace (Fig. 3). This suggests that, during repeated transitions back into freezing climates, each lineage tends to carve out a unique evolutionary path and ultimately approach similar environmental challenges with different phenotypic solutions. Alternatively, it is possible that variation in other climatic variables is causing these lineages to diffuse into different regions of morphospace. Shifts into arid habitats, which became more widespread during the Neogene, might have influenced leaf evolution and morphospace occupancy in various ways, independently or alongside shifts into freezing conditions.

*Shifts in macroevolutionary integration as a scale-unifying construct*. Our results provide one illustration of the potential for a hierarchically integrated view of biological modularity. The formulation of our model provides a bridge between quantitative and population genetics, macroevolutionary patterns in multivariate trait disparity and lineage diversification, and ecological dynamics. The modularity that emerges from covariation patterns at each level may combine in complex ways to yield the evolutionary behaviors observed at subsequently higher levels. Macroevolutionary integration patterns provide a bridge between these scales and a route through which to more carefully dissect how processes at each scale interact to form the patterns we observe across the tree of life. More broadly, investigating shifts in macroevolutionary integration can generate a more hierarchically cohesive understanding of phenotypic evolution. Examining shifts in integration between evolutionary divergences affords the potential to link the cumulative effects of well-characterized population processes over macroevolutionary time. This framework can be further leveraged to explain how shifts in multivariate complexity mapped to macroevolutionary timescales correspond to major ecological shifts, thereby making the initial steps in a new framework through which to seek a truly cohesive and view of biological complexity across temporal, taxonomic, and spatial scales.

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**Tables**

| Covariation regime | Mean absolute correlation strength |
|---|---|
| Regime 0 | 0.42 |
| Regime 1 | 0.24 |
| Regime 2 | 0.47 |

*Table 1*. Mean correlation strength within each reconstructed regime, calculated as the mean of the absolute value of the correlations present in the correlation matrix reconstructed for each regime.

| Covariation regime | $\lambda$ | $\mu$ | Net diversification ($\lambda$ - $\mu$) | Turnover ($\lambda$+$\mu$) |
|---|---|---|---|---|
| 0 + 2 | 0.442 | 0.376 | 0.059 | 0.818 |
| 1 | 0.569 | 0.500 | 0.043 | 1.070 |

*Table 2*. Mean diversification rates across covariation regimes. Given the similarities in the strength of the correlation, as well as issues related to power, we combined corvariation Regimes 0 and 2 and compared against Regime 1. In all cases the differences shown are significant based on a paired *t*-test.

**Figures**

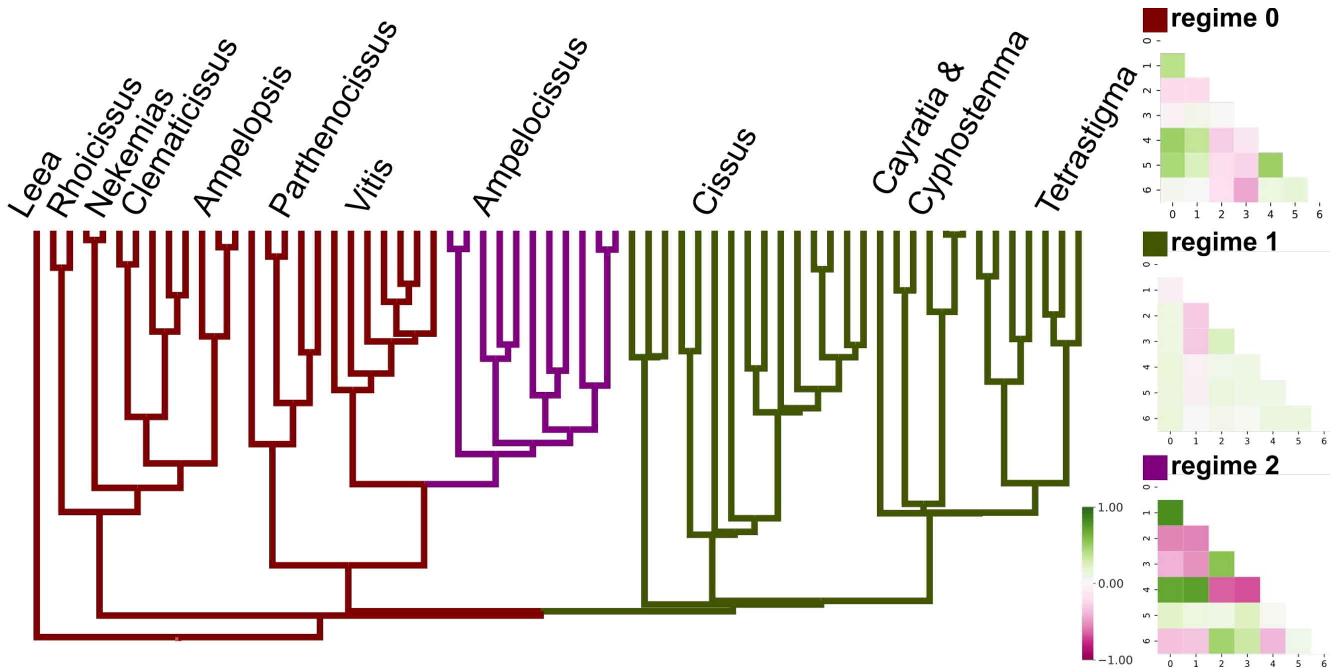


*Figure 1*. Reconstructed macroevolutionary integration regimes mapped to Vitaceae phylogeny and reconstructions of covariation patterns displayed by each regime.

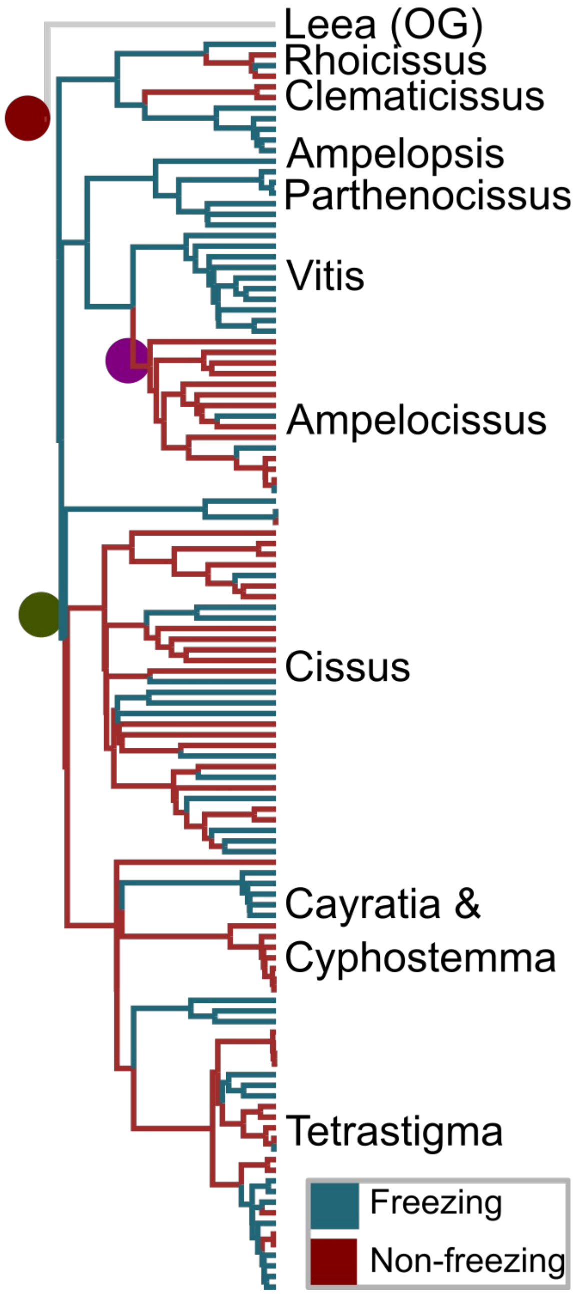


*Figure 2*. Freezing tolerance mapped to Vitaceae phylogeny. Coloured dots correspond to macroevolutionary integration shift points. Reconstruction of climate tolerance was performed on a superset of the taxa available for the morphological analyses. Taxa not included in the morphological analyses were assumed to follow the same integration pattern as their nearest sibling taxa that were present in the morphological analysis.

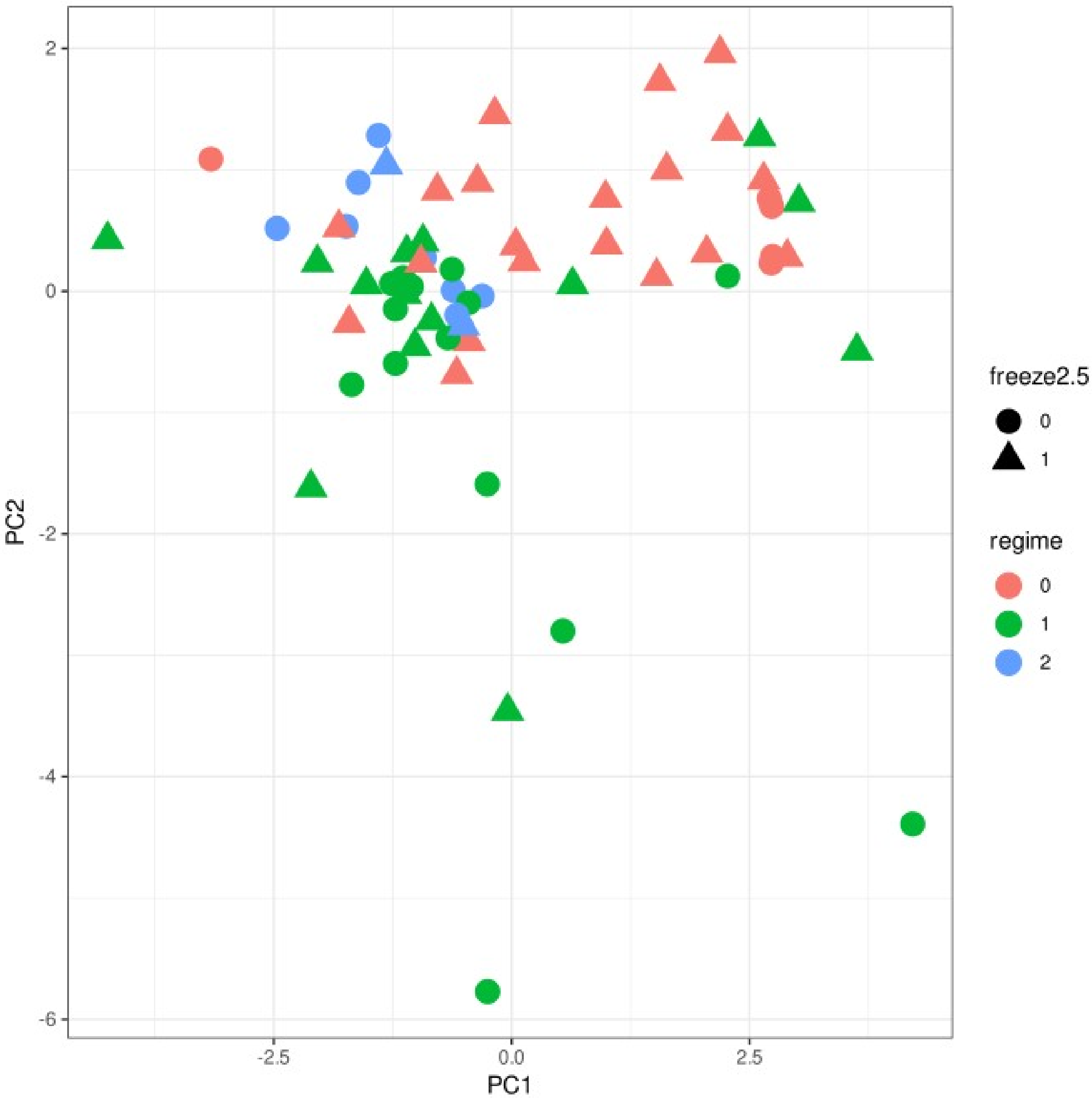


*Figure 3*. Vitaceae leaf trait morphospace.

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