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5 **Title**

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

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23 **Abstract**

24 Understanding how the intrinsic ability of populations and species to meet shifting selective demands
25 shapes evolutionary patterns over both short and long timescales is a major question in biology. One
26 major axis of evolutionary flexibility can be measured by phenotypic integration and modularity. The
27 strength, scale, and structure of integration may constrain or catalyze evolution in the face of new
28 selective pressures. We analyze a dataset of seven leaf measurements across Vitaceae to examine
29 whether the structure of macroevolutionary integration is linked to transitions between temperate and
30 tropical habitats by examining how the structure of integration shifts at discrete points along a
31 phylogeny. We also examine these patterns in light of lineage diversification rates to understand how
32 and whether patterns in the evolvability of complex multivariate phenotypes are linked to higher-level
33 macroevolutionary dynamics. We found that shifts in the structure of macroevolutionary integration in
34 leaves coincide or precipitate colonization events into temperate climates. Lineages that underwent
35 associated shifts in leaf trait integration and subsequent movement into temperate habitats also
36 displayed lower turnover and higher net diversification, suggesting a link between shifting vectors of
37 selection, internal constraint, and lineage persistence in the face of changing environments.
38

39 **Introduction**

40 Phenotypic traits often covary. The causes, consequences, and biological significance of trait
41 covariation are complex and manifest distinct patterns across levels of temporal and biological scale.
42 Trait covariation provides a numeric basis for partitioning the phenotype into semi-autonomous
43 regions, where suites of traits internally covary, but are independent from one another. This is referred
44 to as modularity (Wagner et al., 2007). The evolution of modularity and its relationship to major
45 unanswered questions in evolutionary theory has long been intuited, but few empirical links have been
46 drawn between the modular patterns that emerge at different levels of the biological hierarchy.
47 Examples at a handful of these levels follow.
48

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

69

70 Expanding temporal scale outward, the evolution of covariation patterns has repeatedly come up in
71 paleontological and macroevolutionary studies. In these studies, covariation is measured using a
72 diversity of approaches and data sources and so may perhaps be best considered more broadly as
73 reflecting the general structure of modularity that emerges over long evolutionary timescales. It is
74 possible that the origin of new morphologies is facilitated by shifts in the structure of modularity.
75 Qualitative morphological analysis (Vermeij 1973), shifts in patterns displayed by discrete traits
76 (Wagner 2018), and coordinated patterns in evolutionary disparity and rate among suites of quantitative
77 traits (Parins-Fukuchi 2020) have all been used to reach this conclusion. Paleontological work also
78 suggests that shifts in the strength of covariation may mediate long-term trends in phenotypic evolution
79 (Goswami et al. 2015). A parallel but distinct avenue of research has also shown that changes in the
80 strength of correlation between pairs of traits may underlie ecological transitions (Revell and Collar
81 2009, Revell et al. 2022). All of these diverse contexts are consistent with the population genetic notion
82 that phenotypic innovations may correspond to changes in ‘parcellation’ and ‘integration’ (i.e.,
83 separating and joining together, respectively) of traits (Wagner and Altenberg 1996), but no explicit
84 links have been drawn. The impact of constraint induced by integration patterns on macroevolutionary
85 patterns, such as lineage survival, are also very poorly known.

86

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

98

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

114

115 **Methods and Materials**

116 *Data and code.* Leaf measurements across Vitaceae were obtained from Chen (2009). The following
117 seven traits were included: leaf width, leaf length, petiole length, petiole width, distance between
118 secondary veins, tooth location (distance from leaf base), and petiolule length (of lateral leaflets)
119 These morphological data were cleaned and log-transformed prior to analysis. Data files and code
120 associated with this study are available on figshare
121 (https://figshare.com/articles/dataset/vitaceae_data/21754205).

122

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

128

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

135

136 We will start our explanation of the method using the simplest version of the model: one where the
137 structure of covariation is shared across the entire phylogeny. We first perform an ancestral state
138 reconstruction (ASR) under Brownian motion on each of the traits (Maddison 1991). From here, we
139 estimate directional vectors of change along each branch by subtracting the value at each node from
140 that of its parent. At this point, each trait has been transformed into a set of $2n-2$ (n is the number of
141 lineages included in the phylogeny) vectors of edgewise evolutionary divergence. We then construct a
142 correlation matrix using the vectors for each trait. This measures the magnitude with which each trait
143 pair undergoes coordinated evolutionary changes and the direction of the association (positive or
144 negative). In other words, it gives the covariance between changes in population means. The precise
145 evolutionary interpretation of this matrix, typically referred to as V , has been outlined by Felsenstein
146 (1988) using the equation:

147
 148 $V = GCG$ (eqn. 1)

149
 150 G is the genetic covariance matrix, while C represents the set of covariances in the selection vectors for
 151 each pair of traits. Taken together, V is then defined through a combination of the set of genetic
 152 constraints and the effects of coordinated selection regimes. As a side note, Felsenstein (1988) gave an
 153 explicit critique of the method through which we construct V . Specifically, he pointed out that ASRs
 154 are not true data, but instead inferences drawn from data. While we, of course, agree on a basic level,
 155 we believe the method is sufficient for our aim of reconstructing broad shifts in evolutionary
 156 covariation. Practically speaking, while ASRs are often rife with error, we believe that our own
 157 questions can be adequately tackled with estimates containing relatively high error. The most important
 158 aspect is identifying positive and negative correlations that particularly stand out and how that structure
 159 changes across a tree. It is also worth noting that many phylogenetic comparative methods have arisen
 160 since the time of Felsenstein's writing that use essentially the same information we use here – traits
 161 mapped to a phylogeny – to derive robust historical inferences. We are therefore confident in our
 162 approach given our purposes. Detailed examination of each pairwise trait relationship, or a full
 163 breakdown and interpretation of the G and C components, may benefit from more careful
 164 methodological consideration, or at least further validation that the resulting covariances are
 165 numerically robust to this approach. However, such fine-scaled analysis is not included among our
 166 goals at present.

167
 168 Estimating V is fundamental to our approach. It provides a natural link between the population genetic
 169 conceptualization of covariation and modularity, defined ultimately by G , and the patterns observed
 170 over deeper timescales, including those explored by paleontologists and macroevolutionists. If we
 171 observe shifts in V , we are forced to acknowledge the likely reality that those shifts are at least partially
 172 facilitated by shifts in G . This is because we know, over shorter timescales, that selection tends to be
 173 inhibited if it is misaligned with G . Of course, the reality is that C also likely shifts during major
 174 ecological transitions. Any heterogeneity must therefore be considered as the sum of these population
 175 processes.

176
 177 To consider the possibility that the structure of modularity has shifted across the phylogeny, we defined
 178 a heterogeneous model structured as a phylogenetic mixture of multivariate normal distributions. In the
 179 single V model, we model V as a single multivariate normal distribution with a mean vector of expected
 180 changes equal to zero (this is the expectation under Brownian motion) and covariance matrix equal to
 181 V . In order to more fairly compare V across the tree, we placed all traits on the same scale and rescaled
 182 all estimated covariance matrices to correlation matrices. Examining covariance matrices instead may
 183 also be a useful application of the method, by searching simultaneously for shifts in both evolutionary
 184 rate and covariation patterns, but was not our goal here. The probability density function of this
 185 distribution gives us a likelihood function with which to evaluate the evidence in favor of our model.
 186 To find the best-supported set of covariance regimes, we employed an automated search algorithm
 187 based on that implemented by Smith et al. (2022). A summary of the algorithm follows.

188
 189 *The search algorithm.* The algorithm requires a specified minimum size threshold, defined by the
 190 number of tips, for clades to be considered. For example, if we specify 10, clades with 10 or more tips
 191 will be considered as possible shift points. This greatly improves computational efficiency and also
 192 helps protect against estimating poorly defined covariance matrices on very small clades. For every
 193 clade that meets this size criterion, a covariance matrix is then estimated using *only* the edges
 194 subtending the clade root. The algorithm then proceeds as follows: evaluate the likelihood of a
 195 combined model, whereby the data are characterized using two multivariate normal distributions, one

196 encompassing the proposed shift and the other encompassing the rest of the taxa in the tree. Calculate
197 the Akaike information criterion (AIC) value using this combined likelihood. If the AIC indicates an
198 improvement in fit, save the estimated parameters and AIC scores; if not, discard them. Rank all of the
199 shifts according to their improvement in AIC over the base (single regime) model. Proceed through this
200 ranked list. Retain each model that, when combined with the previously retained models in the ranked
201 list, yields an AIC improvement over the base model. This procedure has the benefit of naturally
202 identifying the optimal shift point in the case where several adjacent nodes all represent possible shift
203 locations. The ranking ensures that the best-supported location will be added first; others will have to
204 add significantly to the explanatory power of the model if they are to be included as a nested shift.

205

206 *Environmental habit.* We constructed a dataset characterizing the environmental occupancy of each
207 lineage, as defined by freezing tolerance. First, we generated a dataset of spatial occurrences across
208 Vitaceae by gathering data from the Global Biodiversity Information Facility (GBIF–
209 <https://www.gbif.org/>). We then extracted climate data across these locales using the Bioclim dataset
210 (<https://www.worldclim.org/data/bioclim.html>). We defined any lineage as freezing tolerant for which
211 2.5% or more of occurrences across their sampled geographic range experience minimum temperatures
212 at or below zero degrees celsius during the coldest month of the year. We then performed a parsimony
213 analysis to map freezing tolerance to the phylogeny in order to compare the location of shifts in
214 covariation structure to those in the environment.

215

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

234

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

242

243

244

245 **Results and Discussion**

246

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

248 A close relationship between leaf form and climate has long been recognized (e.g., Givnish 1987;
 249 Spicer et al. 2021). This is reflected by the repeated, independent evolution of particular leaf syndromes
 250 and functional traits in similar climates—e.g., more rounded, toothed and lobed leaves in temperate
 251 environments (e.g., Schmerler et al. 2012). The widespread use of leaf physiognomy as basis for
 252 paleoclimatic inferences is a testament to the close link between leaf form and climate (e.g., Wolfe
 253 1971), but this relationship is not without complications (Peppe et al. 2010). For example, leaf traits
 254 concentrated in particular biomes or climatic regimes might be, at least in part, a byproduct of select
 255 lineages being overrepresented in those areas (Hinojosa et al. 2010; Little et al. 2010). Leaf forms
 256 associated with particular climates might also, in some cases, have arisen before the climates
 257 themselves, suggesting that new climatic regimes can serve as a filter for preadapted forms (Ackerly
 258 2004). More generally, because leaves possess developmentally integrated suites of traits, it is
 259 unrealistic to expect individual traits to respond to climatic changes in simple, predictable ways.
 260 Examining changes in the structure of leaf trait integration across climatic shifts offers a basis for
 261 understanding the evolutionary underpinnings of environmental transitions, beyond the correlation of
 262 individual traits with different climatic variables.

263

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

277

278 *Shifts in evolutionary integration.* Our approach uncovered evidence for five distinct evolutionary
 279 covariation regimes across Vitaceous leaf traits (Fig. 1). The ancestral regime is broadly distributed
 280 across the tree, encapsulating *Rhoicissus*, *Cayratia*, *Cyphostemma*, *Tetrastigma*, and several *Cissus*
 281 lineages. Several shifts in the evolutionary correlational structure are nested throughout the tree.

282

283 *Climate niche evolution.* Vitaceae displays two distinct modes of climate niche evolution, as it relates
 284 to freezing (Fig. 2). We reconstructed the most recent common ancestor as tropical. The clade
 285 composed of *Cissus*, *Cayratia*, *Cyphostemma*, and *Tetrastigma* was largely tropical. Climate niche was
 286 relatively labile in this clade, with several lineages having transitioned into freezing habitats. Despite
 287 this flexibility, none of the freezing lineages are very speciose, suggesting that their occupation of
 288 temperate regions may be fairly transient. Contrastingly, three Vitaceae clades independently made
 289 significant and stable transitions into freezing habitats from their tropical ancestor. The three clades
 290 defined by *Vitis*, *Parthenocissus*, and *Ampelopsis*, respectively, each made their own transition into
 291 freezing habitats. These lineages maintained their newly derived habits, diversifying only after
 292 encountering these new environments.

293

294 *Habitat shifts, integration, and preadaptation.* The shifts in evolutionary leaf integration across
295 Vitaceae were largely congruent with the reconstructed shifts in climate niche. We observed this
296 associated pattern particularly strongly in regimes 2, 3, and 4. The emergence of each of these regimes
297 coincides with, or immediately precedes the movement of each of these clades into freezing habitats
298 from the temperate ancestor. During this time, global temperatures were considerably warmer than the
299 present, and freezing conditions were perhaps only present (if at all) at high elevations or high latitudes,
300 at least until the onset of climatic deterioration in the mid to late Eocene (Zachos et al. 2001). Most
301 Cretaceous to early Cenozoic Vitaceae fossils are known from middle to low latitudes (e.g., Chen and
302 Manchester 2007; Manchester et al. 2013), suggesting that the ancestors of most major lineages likely
303 did not experience freezing conditions. However, early representatives of Vitaceae (or particular
304 lineages) may have possessed traits (e.g., deciduousness) that predisposed them to thrive in freezing
305 conditions. Our analysis suggests that evolutionary shifts in leaf trait integration may have facilitated
306 major environmental shifts in Vitaceae, by emerging earlier, predisposing the lineages encompassed
307 within 2, 3, and 4 to changing environmental conditions, perhaps enabling rapid adaptation and
308 radiation into these new niches. This scenario illustrates how evolution of the macroevolutionary
309 integration structure (and by extension, its constituent parts, *G* and *C*) likely facilitated tolerance of
310 unpredictable and distinct habitats, encountered either by migration into new areas, or in response to
311 long-term climate changes occurring *in situ*.

312
313 The sole shift that was not associated with a major temperate-tropical shift was regime 1, encompassing
314 only *Cissus* lineages. Overall, lineages contained within both regimes 0 and 1 can be characterized as
315 predominantly tropical, with several excursions into temperate space. As a result, it is likely that this
316 shift reflects some other aspect of leaf functional biology. While we focused on temperate-tropical
317 shifts here (with respect to freezing), we also note that the species included in regime 0 and 1 also
318 occupy diverse habitats with respect to precipitation, including shifts into xeric environments in
319 continental Africa, Madagascar, and parts of the Neotropics, which might similarly be influencing
320 patterns of trait integration in this regime. It is thus possible that the phenotypic shifts undergone in
321 regime 1 are functionally related to environmental changes, but future work will be needed to better
322 understand the surrounding context.

323
324 We found that the overall correlation strength was fairly uniform across the regimes (Table 1). This
325 suggests that, at the macroevolutionary scale, the functional impacts of trait correlations may differ
326 from those at the ecological scale. Ecological work on trait covariation within communities along
327 environmental stress gradients (Dwyer and Laughlin 2017). Ecological work has found that more
328 stressful environments host plant communities with stronger covariation between functional traits. This
329 is because environmental stressors induce functional constraints that disadvantage certain trait
330 combinations. Lineages that display unfavorable trait combinations are filtered out of certain areas. In
331 this case, trait covariation actually serves as a catalyst, rather than constraint, for some lineages to
332 move into more challenging environments. Nevertheless, trade-offs imposed by competing
333 environmental stressors appear to create slightly more complex dynamics in covariation patterns
334 (Brown et al. 2022). We found that increased tradeoffs induced by the more stressful climates occupied
335 by lineages diversifying in temperate regions lead to a more generally volatile structure of evolutionary
336 integration. This is highlighted by the repeated shifts in correlation structure in clades that have made
337 decisive shifts into freezing environments. Nevertheless, there remains a key difference at the
338 macroevolutionary scale as compared to ecological scale. While some climates may filter out lineages
339 based on trait covariation patterns over ecological scales, our work shows how lineages themselves can
340 shift the structure of trait covariation and occupy new habitats over macroevolutionary timescales.
341 Thus, instead of observing a weakened correlation structure as lineages radiate into more challenging
342 environments, we found instead that the *structure* underlying those correlations evolves.

343

344 *Diversification rates across covariation regimes.* We grouped regimes 0 and 1 into a single
345 diversification regime and compared against rates within a separate grouping of regimes 2, 3, and 4 to
346 test the hypothesis that the repeated independent movements from tropical into temperate habitats
347 undertaken by regimes 0 and 1 may have led to distinct macroevolutionary dynamics. We found
348 significant differences in rates of speciation, extinction, net diversification, and turnover when
349 comparing across covariation regimes (Table 2). We found that the lineages that underwent shifts in
350 leaf integration as a precursor to temperate diversification (regimes 2, 3, and 4) exhibit lower turnover
351 and higher net diversification rates than predominantly tropical lineages making ephemeral movements
352 into freezing habitats (regimes 0 and 1). The repeated climatic shifts observed across regimes 0 and 1
353 correspond to overall higher turnover, and marginally lower net diversification. More climatically
354 stable regimes, which are also more tightly integrated, turnover less and generally have a higher net
355 increase in diversity as a result.

356

357 *Leaf evolvability, climate shifts, and macroevolutionary dynamics.* The shifts in macroevolutionary
358 integration we observed between leaf traits are the consequence of both shifting structures of
359 multivariate selection and genetic constraints. While it was not possible here, given data limitations, to
360 disentangle the relative influence of each of these in shaping patterns emergent at the phylogenetic
361 scale, the coincidence of major shifts from tropical and into freezing environments with shifts in the
362 structure of trait covariation suggests that vitaceous leaves are generally evolvable in response to
363 environmental changes. Nevertheless, we also observed a complex pattern in leaf morphospace as it
364 relates to climate evolution and evolutionary integration. Regimes 0 and 1 occupy the largest spread of
365 morphospace (Fig. 3). In contrast, the lineages that have become more stereotyped as temperate,
366 housed within regimes 2, 3, and 4, display lower disparity overall and tighter mean integration between
367 traits. This might suggest that what allowed these lineages to thrive in newly temperate habitats was a
368 structure of evolutionary covariation compatible with the new selection vectors imposed by these
369 environments. This would allow these lineages to thrive while occupying a relatively small portion of
370 morphospace. On the other hand, the frequent ephemeral movements into freezing environments
371 undertaken by the lineages within regimes 0 and 1 may have stimulated broader diffusion throughout
372 morphospace. This increased disparity may reflect the less-specialized nature of these lineages.

373

374 Lineages that have colonized and subsequently diversified in newly temperate habitats display
375 quantitatively and qualitatively different macroevolutionary dynamics than those that have remained
376 primarily in the tropics. The integration patterns deployed by regimes 2, 3 and 4 were derived from the
377 ancestral regime, precipitating shifts into temperate habitats. These lineages became fairly canalized
378 both morphologically and climatically. The lineages contained within regimes 0 and 1 displayed
379 different climate niche evolution dynamics, repeatedly making the difficult transition from tropical to
380 freezing. This shows that the ancestral regime displayed a high degree of flexibility by both undergoing
381 rearrangement into new, derived integration patterns that facilitated diversification in new habitats, and
382 by facilitating the labile climate changes and broad morphospace diffusion displayed by the remaining
383 lineages. We can therefore characterize Vitaceae macroevolutionary dynamics on two complementary
384 axes as reflecting the ability of lineages to: 1) withstand repeated, but ephemeral, shifts into freezing
385 habitats under a static structure of leaf evolutionary integration (regimes 0 and 1) and 2) modify the
386 structure of leaf evolutionary integration before colonizing and diversifying in new environments
387 (regimes 2, 3, and 4).

388

389 The multivariate vectors of selection on leaf traits likely shift during movement into new climates. The
390 ability for lineages to withstand repeated shifts into freezing habitats suggests that G likely does not
391 strongly constrain response of the population means to the new directions imposed by selection in these

392 new environments. If G did maintain long-term constraints across these transitions, these migrant
393 lineages, constrained within a maladaptive phenotypic space relative to their new habitats, would likely
394 go extinct because of a decreased efficacy navigating these new habitats and competition from perhaps
395 better-adapted species (Van Valen 1973). Although we analyzed these patterns in light of only *abiotic*
396 (environmental) factors, we assume that new environments will also contain different biotic contexts.
397 And regardless of the relative importance of biotic and abiotic factors in driving these
398 macroevolutionary patterns, as originally formulated, the Red Queen accommodates both. We favor
399 this interpretation given that the two likely work synchronously. We therefore assume that the
400 environmental shifts we identified, along with the corresponding shifts in phenotype and development,
401 may indicate changes in both abiotic and biotic factors.

402
403 We observed a pattern of high leaf evolvability across environmental transitions paired with elevated
404 turnover rates in lineages transitioning into derived habitats. Diversification rate variation has been
405 explained by many possible dynamics, for example, latitudinal correlates with energy input: “the Red
406 Queen runs faster when she is hot” (Brown 2014). Our analyses reflect a somewhat simpler dynamic
407 that unifies several levels of macroevolutionary patterns. The patterns in Vitaceae suggest that *the Red*
408 *Queen runs faster when she is uncomfortable* (see similar arguments in Stebbins 1974; Vasconcelos et
409 al. 2022b). Repeatedly encountering new habitats due to migration and/or climate change results in the
410 emergence of a completely new set of biotic and abiotic conditions that may yield a variety of
411 responses that are intrinsic to each individual lineage. These responses may be rooted in developmental
412 and genetic constraints on phenotypes, population-level variation, and extinction dynamics. As a result,
413 while extinction tends to increase when lineages encounter new habitats, this is compensated for by
414 increased speciation among phenotypically flexible lineages.

415
416 Vitaceae has navigated these dynamics in two contrasting ways. The lineages in regimes 2, 3, and 4
417 may have thrived and diversified in new, freezing, habitats by inheriting a modified structure of
418 macroevolutionary integration. The lineages in regimes 0 and 1 appear to withstand repeated
419 encounters with freezing habitats by increasing their macroevolutionary pace, as measured by elevated
420 turnover rates. It is possible that these lineages are more likely to go extinct when they encounter
421 freezing habitats than lineages within regimes 2, 3, and 4, but are able to persist over long timescales
422 by maintaining elevated speciation rates. Regimes 2, 3, and 4, which have experienced diversification
423 events in freezing habitats, are thus perhaps more ‘comfortable’ in these new environments due to their
424 derived patterns in genetic and selective constraints. Nevertheless, there may be some morphological
425 bet-hedging happening. While regimes 3 and 4 are predominantly freezing, and regimes 0 and 1 are
426 predominantly tropical, regime 2 (*Vitis-Ampelocissus*) is evenly balanced between both. We found that
427 regime 2 was the least integrated lineage overall (Table 1), raising the possibility that this simultaneous
428 diversification in both temperate and tropical areas may have been facilitated by an overall more
429 flexible structure of genetic and functional constraint. This would distinguish the macroevolutionary
430 strategy of regime 2 from regimes 3 and 4, which appear to be more specialized and canalized in
431 freezing habitats. These three regimes are further distinguished from regimes 0 and 1 in that they may
432 persist in freezing environments due to possessing modified morphological structures, as opposed to
433 increased rates of speciation. More work mapping the links between phenotypic innovation, constraint,
434 and speciation rates in different lineages will help to further refine our understanding of how lineages
435 persist in the face of a shifting evolutionary landscape.

436
437 This second layer might explain results in vertebrates that conflict with latitudinal explanations for
438 diversification rate variation (Rabosky et al., 2018), which found “paradoxically” higher speciation
439 rates far from the tropics. These patterns may reflect a more extreme manifestation of the causes we
440 outline here. Movement to more extreme environments may, in some lineages, increase variation in

441 macroevolutionary parameters (lineage diversification, the origin of phenotypic novelties, etc) to a
442 level that overwhelms latitudinal patterns. For example, certain ecological conditions in temperate and
443 arctic regions may be so far from a lineage's initial capability to accommodate them that it must
444 increase its macroevolutionary activity beyond that displayed at the tropics to outpace extinction. This
445 may manifest itself in higher turnover, faster and wider phenotypic disparification, and
446 macroevolutionary integration patterns that are structured more flexibly. The relative importance of
447 latitudinal vs intrinsic explanations likely varies across clades, environments, and epochs. Deeper
448 understanding of the level(s) at which selection operates and how intrinsic evolvability interacts with
449 movement into new ecological contexts will help to further disentangle the root causes of these
450 dynamics and disparity in pattern across studies and taxa.

451
452 It also seems worthwhile to note that the lineages within regime 0 do not cluster according to climate
453 niche in leaf morphospace (Fig. 3). This suggests that, during repeated transitions back into freezing
454 climates, each lineage tends to carve out a unique evolutionary path and ultimately approach similar
455 environmental challenges with different phenotypic solutions. Alternatively, it is possible that variation
456 in other climatic variables is causing these lineages to diffuse into different regions of morphospace.
457 Shifts into arid habitats, which became more widespread during the Neogene, might have influenced
458 leaf evolution and morphospace occupancy in various ways, independently or alongside shifts into
459 freezing conditions.

460
461 *Shifts in macroevolutionary integration as a scale-unifying construct.* Our results provide one
462 illustration of the potential for a hierarchically integrated view of biological modularity. The
463 formulation of our model provides a bridge between quantitative and population genetics,
464 macroevolutionary patterns in multivariate trait disparity and lineage diversification, and ecological
465 dynamics. The modularity that emerges from covariation patterns at each level may combine in
466 complex ways to yield the evolutionary behaviors observed at subsequently higher levels.
467 Macroevolutionary integration patterns provide a bridge between these scales and a route through
468 which to more carefully dissect how processes at each scale interact to form the patterns we observe
469 across the tree of life. More broadly, investigating shifts in macroevolutionary integration can generate
470 a more hierarchically cohesive understanding of phenotypic evolution. Examining shifts in integration
471 between evolutionary divergences affords the potential to link the cumulative effects of well-
472 characterized population processes over macroevolutionary time. This framework can be further
473 leveraged to explain how shifts in multivariate complexity mapped to macroevolutionary timescales
474 correspond to major ecological shifts, thereby making the initial steps in a new framework through
475 which to seek a truly cohesive and view of biological complexity across temporal, taxonomic, and
476 spatial scales.

477

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489

490 **Tables**

491

Covariation regime	Mean absolute correlation strength
0	0.51
1	0.50
2	0.45
3	0.55
4	0.54

492

493 *Table 1.* Mean overall strength of correlation for each regime.

494

495

Covariation regime	λ	μ	Net diversification ($\lambda - \mu$)	Turnover ($\lambda + \mu$)
0+1	0.553 (± 0.168)	0.483 (± 0.190)	0.045 (± 0.009)	1.037 (± 0.178)
2+3+4	0.450 (± 0.142)	0.384 (± 0.165)	0.058 (± 0.006)	0.834 (± 0.153)

496

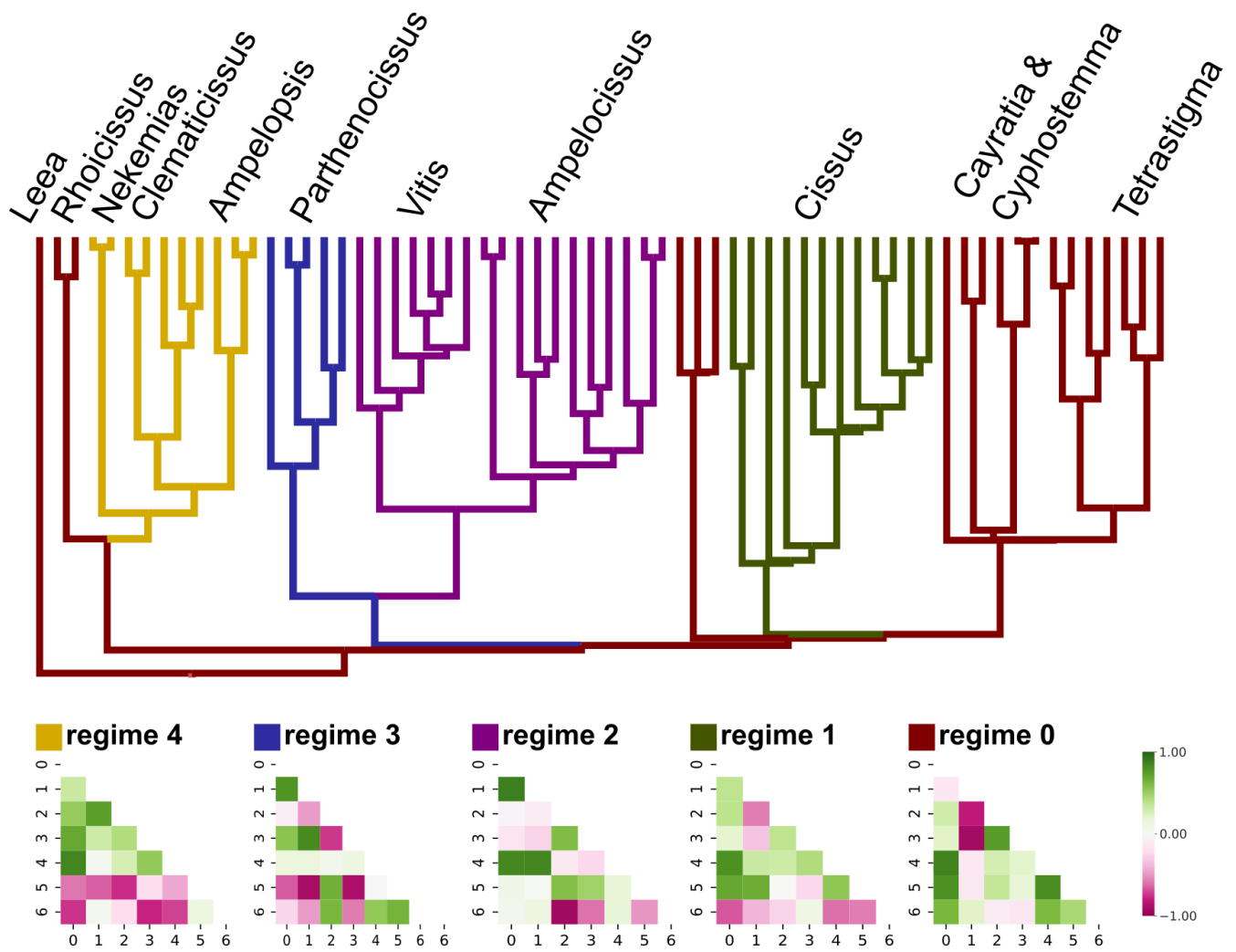
497 *Table 2.* Mean diversification rates across covariation regimes. We partitioned the tree into the clade
 498 composed of regimes 0 and 1 and regimes 2, 3 and 4 because of the distinct patterns in climate niche
 499 evolution in each clade and to improve statistical power. In all cases the differences shown are
 500 significant based on a paired *t*-test ($P < 0.10$).

501

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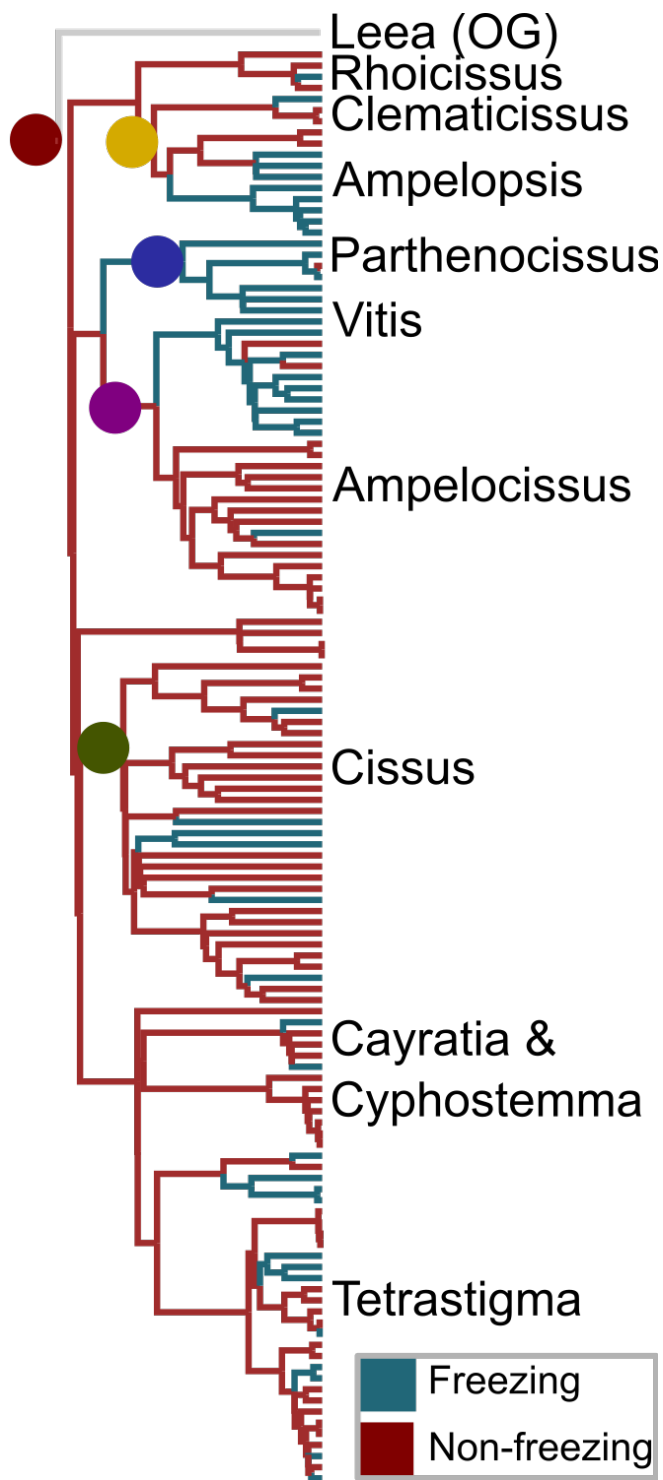
503 **Figures**

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



511

512

513 *Figure 2.* Freezing tolerance mapped to Vitaceae phylogeny. Coloured dots correspond to

514 macroevolutionary integration shift points. Reconstruction of climate tolerance was performed on a

515 superset of the taxa available for the morphological analyses. Taxa not included in the morphological

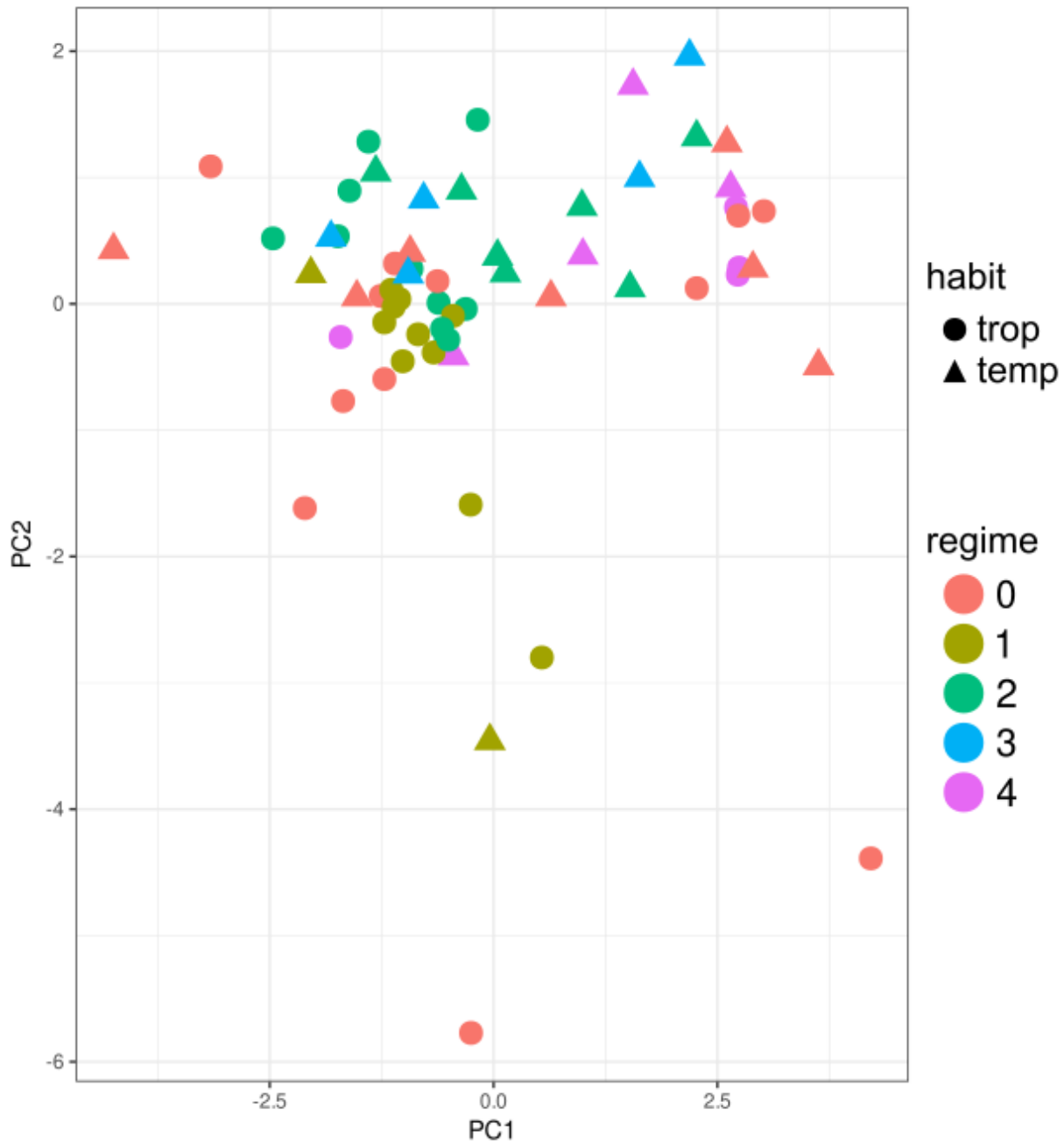
516 analyses were assumed to follow the same integration pattern as their nearest sibling taxa that were

517 present in the morphological analysis.

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521
522 *Figure 3.* Vitaceae leaf trait morphospace.
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