Rates of niche and phenotype evolution lag behind diversification in a temperate radiation

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Environmental change can create opportunities for increased rates of lineage diversification, but continued species accumulation has been hypothesized to lead to slowdowns via competitive exclusion and niche partitioning. Such density-dependent models imply tight linkages between diversification and trait evolution, but there are plausible alternative models. Little is known about the association between diversification and key ecological and phenotypic traits at broad phylogenetic and spatial scales. Do trait evolutionary rates coincide with rates of diversification, are there lags among these rates, or is diversification niche-neutral? To address these questions, we combine a deeply sampled phylogeny for a major flowering plant clade—Saxifragales—with phenotype and niche data to examine temporal patterns of evolutionary rates. The considerable phenotypic and habitat diversity of Saxifragales is greatest in temperate biomes. Global expansion of these habitats since the mid-Miocene provided ecological opportunities that, with density-dependent adaptive radiation, should result in simultaneous rate increases for diversification, niche, and phenotype, followed by decreases with habitat saturation. Instead, we find that these rates have significantly different timings, with increases in diversification occurring at the mid-Miocene Climatic Optimum (~15 Mya), followed by increases in niche and phenotypic evolutionary rates by ~5 Mya; all rates increase exponentially to the present. We attribute this surprising lack of temporal coincidence to initial niche-neutral diversification followed by ecological and phenotypic divergence coincident with more extreme cold and dry habitats that proliferated into the Pleistocene. A lack of density-dependence contrasts with investigations of other cosmopolitan lineages, suggesting alternative patterns may be common in the diversification of temperate lineages.

Significance

Alternative models of evolutionary processes suggest different associations between species diversification and trait evolution, but limited empirical evidence is available to test these models across large clades at global extents. Here we investigate the relative timing of species diversification and niche and phenotypic evolution across a global plant radiation (Saxifragales) with enormous phenotypic and habitat variation. We demonstrate strong temporal lags among rates, with increased diversification occurring first, followed by niche and phenotype. Accelerated diversification rates are coincident with mid-Miocene expansion of temperate biomes. Later increases in niche and phenotypic evolutionary rates argue against density-dependent diversification alone, indicating a major role for ecological opportunity. These results have broad implications for understanding diversification processes and the origin of present-day temperate biotas.


The authors declare no conflict of interest.

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Data deposition: The sequences reported in this paper have been deposited in the Sequence Read Archive (Bioproject accession no. PRJNA492276). Scripts to run analyses are available at GitHub (https://github.com/ryanafolk/Saxifragalespatial_scripts). Analysis products are available at Dryad (doi:10.5061/dryad.d8q24).

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possible associations among rates of species diversification and niche and phenotypic evolution to a few well-known clades of life [e.g., birds (9) and mammals (2)].

Here we compare the overall timing and rates of diversification and of niche and phenotypic evolution (i.e., niche and phenotypic lability) for a globally distributed plant clade, Saxifragales (approximately 2,400 species). This group is highly diverse in niche and phenotype, encompassing annual and perennial herbs, succulents, parasites, aquatics, shrubs, vines, lianas, epiphytes, and large trees. It harbors most of its diversity in temperate conditions (including montane and arid biomes) that expanded globally during cooling and drying trends in the last 15 My (15, 29, 30). In the context of both expanding and novel habitat, cold- and arid-adapted clades should experience rate increases for speciation, niche, and phenotypic traits, because the shift into new niches (via new niche-delimiting morphological traits) would provide opportunities for diversification outside of the ancestral niche. Continuing ecological opportunity may therefore provide a mechanism to escape density dependence (5–8). Saxifragales serve as an exemplar clade to examine macroevolutionary responses of lineages to the transition from a warmer, wetter Earth in the Eocene to early Miocene to colder, drier conditions that predominated afterward. Furthermore, because subclades of Saxifragales occupy diverse habitats, analysis of Saxifragales as a whole can serve as a useful multifaceted case study of the processes associated with diversification.

We built deeply sampled datasets comprising a phylogenetic backbone tree, a supermatrix covering most species in the clade, and ecological niche and phenotypic trait variables to estimate rates of species diversification and lability of ecological niche and phenotypic traits using macroevolutionary trait models. With this framework, we address the following key questions: (i) Is the macroevolutionary timing of diversification of a major, primarily temperate clade coincident with global cooling, aridification, and the proliferation of temperate habitats beginning in the late Miocene? (ii) Given this recent origin and continued expansion of major temperate biomes, have Saxifragales escaped density-dependent diversification downturns? (iii) Are shifts in niche and phenotypic lability tightly correlated with shifts in diversification rates, and, if not, in what sequence do these rate shifts occur?

Results

Biodiversity Data Assembly. Using a phylogeny for Saxifragales inferred from a dataset of 301 genes and 627 species covering all major lineages (SI Appendix, Fig. S1) as a backbone constraint for a supermatrix analysis, we generated a tree comprising 1,736 species (72% of species diversity for Saxifragales; SI Appendix, Fig. S2), of

![Fig. 1. Ancestral reconstruction across Saxifragales for PC1 of our dataset of 35 environmental variables; branches are colored in a rainbow scale from low ordinated values (red and yellow; hotter and to some extent wetter habitats, as well as the hottest arid habitats) to high ordinated values (green and blue; mostly colder and drier habitats). Black dots at nodes represent major ecological niche shifts (the upper 95th percentile of node–parent node differences). Red dots at nodes represent diversification shifts in the maximum credibility set. The inset density curves show tip rates for diversification (green), niche (orange), and phenotype (blue), all scaled from the minimum to the maximum reconstructed value. Around the edge are photographs of major representative habitats. Family codes are as follows: (a) Peridiscaceae, (b) Paeoniaceae, (c) Daphniphyllaceae, (d) Cercidiphyllaceae, (e) Altingiaceae, (f) Hamamelidaceae, (g) Iteaceae, (h) Grossulariaceae, (i) Saxifragaceae, (j) Cynomoriaceae, (k) Tetracarpaeaceae, (l) Aphanopetalaceae, (m) Penthoraceae, (n) Haloragaceae, and (o) Crassulaceae.](image-url)
which 1,455 (61%; SI Appendix, Fig. S3) were matched with occurrence data (736,703 occurrence records, an average of 317 occurrences per species). Major relationships recovered were consistent with recent phylogenetic work (31), including monophyly for all clades recognized as families. For the 23 phylogenetic traits collected, 1,388 species (58%) had the minimum of 20% trait coverage we imposed for downstream analysis.

**Niche and Phenotype Ordination.** The primary loadings on ordinated niche data captured temperature (SI Appendix, Table S1). Low values (Fig. 1, red and yellow) are associated with hotter and, to some extent, wetter habitats, as well as the hottest arid habitats; high values (Fig. 1, blue and green) are associated with cooler and drier habitats. Ordinated phenotype data (SI Appendix, Table S2) primarily captured a complex set of traits including plant height; woodiness; inflorescence, sepal, and petal shape descriptors; seed length; and several meristic traits (stamen, petal, and sepal number). Low ordinated phenotype values captured primarily low herbaceous rosette plants, and high values captured trees and shrubs.

**Niche and Phenotype: Conservatism and Correlated Evolution.** Although niche shifts are numerous throughout Saxifragales (Fig. 1), we found strong evidence for phylogenetic constraints on niche space, consistent with some degree of conservatism of both niche and phenotypic traits over >100 My of evolutionary time (λ test; niche: $P = 8.3e-222$; phenotype: $p \sim 0$; SI Appendix, Figs. S4 and S5). To discern whether the phenotypic data captured potential niche-delimiting phenotypes, we asked whether the niche and phenotypic predictors were tightly correlated; we found strong evidence for correlated evolution between niche and phenotype ($P = 4.1e-36$) and their pairwise disparity ($P < 2.2e-16$; SI Appendix, Fig. S6).

**Timing of Macroevolutionary Rates.** Net species diversification and rates of niche and phenotypic evolution (lability) as reconstructed by BAMM (Bayesian Analysis of Macroevolutionary Mixtures) all experienced strong increases toward the present, beginning in approximately the mid-Miocene (Fig. 2A and SI Appendix, Fig. S7), contemporaneous with the mid-Miocene Climatic Optimum (32–34). However, surprisingly, diversification rates through time show significantly earlier increases than for phenotypic and niche lability. Comparing the time to 50% of contemporaneous rates, we found diversification, niche, and phenotypic rates had significantly different timing [Fig. 2B; ANOVA $P < 2e-16$; all pairwise rate differences significant for Tukey honestly significant difference (HSD)], with niche and phenotypic rates lagging behind diversification. Phenotype also experienced a smaller lag either before, or more often after, niche rates for this and other analyses, although this ordering was sensitive to two alternative time calibration methods (Methods) and more often niche and phenotype timings were indistinguishable. Consistent with our rate estimates through time, shifts in diversification regimes were generally phylogenetically deeper than major ancestral shifts (upper 95th percentile) in niche and phenotype space (Fig. 2C; ANOVA $P = 0.00036$; all pairwise differences significant for Tukey HSD except niche vs. phenotype). Most major niche shifts (88.1% of the upper 95th percentile) and major phenotype shifts (84.6% of the upper 95th percentile) postdated 15 Mya.

We also calculated the MS diversification statistic (35) and Ornstein–Uhlenbeck (OU) $\sigma^2$ trait rate parameters (36) for the entire tree and every subclade. In addition to the pure-birth MS statistic ($\zeta = 0$), we used a series of extinction proportions ($\zeta = 0.1, 0.5, 0.9$) to assess the impact of extinction on the MS results. We identified lags in evolutionary rates with early diversification increases followed by niche and then phenotype (Fig. 2D and SI Appendix, Figs. S8–S13), which was significant for all pairwise comparisons (Tukey HSD; rates scaled to a proportion of their contemporary rate for comparability; $P < 0.002$ for all comparisons).

Higher extinction proportions reduced overall diversification rate scaling but pairwise comparisons remained significant.

**Correlation Between Macroevolutionary Rates and Climate/Climatic Variation.** After the mid-Miocene Climatic Optimum, the time frame in which we observed major increases in macroevolutionary rates (for diversification, niche, and phenotype), there was a very strong correlation between historical global temperature data (37) and macroevolutionary rates reconstructed by

*Fig. 2. (A) Median rates and rate distributions for net diversification, niche, and phenotypic macroevolutionary rates (colors shown in legend). The unit of diversification is speciation events per million years; niche and phenotypic rates are unitless. For relative comparability, the y axis is scaled from zero to the maximum median rate for all datasets. The gray curve in the background is a global temperature dataset (37). High niche evolutionary rates result from large scaling of the PCA ordination of environmental data (SI Appendix, Fig. S41). (B) Box plot showing the distributions of times to 50% of contemporaneous evolutionary rates from the mid-Miocene (15 Mya to present). (C) Box plot showing the distributions of times to present for either major shifts in ancestral reconstructions (environment, phenotype; 95th percentile of node–parent node differences) or shifts in the best shift configuration (diversification). (D) Box plot of evolutionary rates for MS (diversification) and $\sigma^2$ (niche and phenotype) as proportionally scaled to the maximum (contemporary) rates. Higher clade values for, for example, diversification indicate earlier rate increases. MS here parameterizes extinction as $\zeta = 0.5$; see SI Appendix, Figs. S8–S11 for evaluation of extinction fractions.*
BAMM (fit using an exponential model; net diversification; F-test $P < 2.2e-16$; $R^2 = 0.8352$; niche lability: $P < 2.2e-16$, $R^2 = 0.845$; phenotypic lability: $P < 2.2e-16$, $R^2 = 0.779$). We also observed a weaker, but significant, relationship between rates and variation in temperature (measured as the SD over moving 0.1-My intervals, fit using an exponential model; net diversification: $F$-test $P < 2.2e-16$, $R^2 = 0.2003$; niche lability: $P = 3.23e-2$, $R^2 = 0.0323$; phenotypic lability: $P = 6.25e-8$, $R^2 = 0.05527$; SI Appendix, Figs. S14–S19). A combined model incorporating both temperature and temperature variability best explained the data (mean adjusted $R^2$ increase 0.0163, Aikake weight 0.705 for diversification, ~1 for niche and phenotypes), suggesting that temperature and temperature variability have independent explanatory power.

We fit likelihood models of temperature ($n = 9$) and time dependence ($n = 9$) in RPANDA [refs. 38–40; see Fig. 4 and SI Appendix, Tables S3–S5] to evaluate the association of diversification, niche, and phenotypic rates with historical temperature. For niche and phenotypic rates, support for temperature dependence was decisive (combined Aikake weights of linear and exponential models ~1); Aikake weights moderately supported an exponential temperature relationship with niche lability (0.84), but support for either linear or exponential dependence was equivocal for phenotypic lability. For diversification rates, RPANDA was unable to distinguish a single best model. The best model (exponential speciation constant extinction with respect to time) did not include climate, but $\Delta$AIC (Aikake information criterion) was only 0.325 and the $\Delta$Aike weight 0.025 compared with the second-best model (linear speciation and extinction with respect to temperature). The sum of Aikake weights for models with temperature was 0.6837 vs. 0.3163 for those without it (Fig. 4 and SI Appendix, Tables S3–S5 and S8–S10), indicating that the majority of the likelihood was in temperature-dependent models.

Robustness to Priors. Concern has been raised about the sensitivity of BAMM to prior specification [41]; to assess this issue, we explored a series of extreme prior formulations on expected rate events and rate priors, varied over two orders of magnitude compared with the recommended BAMM priors (for further details see SI Appendix, Supporting Information Text and Figs. S20–S22). Expected event priors did not produce noticeable effects on rate curves. Rate priors had an effect particularly on rate curves after 15 My, but other examples clearly show that diversification rates predate shifts in other rates, with the timing consistent with that reported for the whole tree. Likewise, while niche and phenotype have a diverse set of timing patterns, patterns in each of two large clades (Saxifragaceae alliance and Crassulaceae alliance; cf. Fig. 3B where these taxa are defined, together comprising 97% of species diversity) show a precedence of niche rates consistent with our global analyses. Subclade plots also suggest a negative relationship between niche lability and clade median temperature (measured as the most recent common ancestor ancestral reconstruction of mean annual temperature; SI Appendix, Fig. S26). Consistent with this finding, we found that niche lability had a moderately significant association with habitat (STRAPP: $P = 0.033$). Other cladewise plots did not reveal a clear pattern for net diversification and phenotypic lability (SI Appendix, Figs. S27 and S28).

Discussion

Our results demonstrate in a clade of flowering plants that rates of species diversification are not coincident with ecological and rates (STRAPP: $P = 0.879$), niche lability (STRAPP: $P = 0.056$), or phenotypic lability (STRAPP: $P = 0.751$). These results are consistent with a worldwide response of Saxifragales to climatic cooling and aridification, without a clear association with temperate biomes alone (but see below). Ancestral reconstructions of climate space suggest that Saxifragales were already present in temperate habitats before Miocene cooling; the majority of the shifts into the most extreme areas of niche space (the upper 97.5th and lower 2.5th percentiles of contemporary values representing, respectively, the hottest desert and equatorial habitats and the coldest polar and montane habitats) are within the last 5 My (niche: 56.72%; phenotype: 61.54%; Fig. 1 and SI Appendix, Figs. S24 and S25).

Subclade Patterns. We generated a series of subclade rate plots for all families with more than 15 sampled species (Fig. 3A) to assess whether the global pattern we observed was general or driven by particular subclades. Diversification timing was equivocal for Hamamelidaceae and Grossulariaceae, with similar curves to niche rates after 15 My, but other examples clearly show that diversification rates predate shifts in other rates, with the timing consistent with that reported for the whole tree. Likewise, while niche and phenotype have a diverse set of timing patterns, patterns in each of two large clades (Saxifragaceae alliance and Crassulaceae alliance; cf. Fig. 3B) show a precedent of niche rates consistent with our global analyses. Subclade plots also suggest a negative relationship between niche lability and clade median temperature (measured as the most recent common ancestor ancestral reconstruction of mean annual temperature; SI Appendix, Fig. S26). Consistent with this finding, we found that niche lability had a moderately significant association with habitat (STRAPP: $P = 0.033$). Other cladewise plots did not reveal a clear pattern for net diversification and phenotypic lability (SI Appendix, Figs. S27 and S28).

Niche- and Phenotype-Associated Diversification. To assess whether observed rate patterns were associated with spatial variation in climate, we ran a series of trait-associated diversification tests with summary geographic data, using both the BAMM-based STRAPP (Structured Rate Permutations on Phylogenies) statistic and the semiparametric es-SIM statistic. We did not see significant associations of elevation ($\text{STRAPP}: P = 0.868$; es-SIM: $P = 0.849$) or latitude ($\text{STRAPP}: P = 0.818$; es-SIM: $P = 0.194$) with net diversification. Using STRAPP, we also did not find relationships of elevation and latitude with niche lability (elevation: $P = 0.816$; latitude: $P = 0.101$) or phenotypic lability (elevation: $P = 0.386$; latitude: $P = 0.193$). We also did not see a relationship between continental biogeography and diversification.

![Figure 3](image-url)

Fig. 3. (A) Clade rates for major clades (all families with greater than 15 sampled taxa). Rate colors are shown in the legend; the yellow bars give the date of 15 My representing the Mid-Miocene Climatic Optimum. (B) Box plots showing the distribution of rate shifts for two large subclades of similar size, Crassulaceae alliance (Aphanopetalaceae + Crassulaceae + Haloragaceae + Penthoraceae + Tetracarpaeaceae) and the Saxifragaceae alliance (Grossulariaceae + Iteaceae + Saxifragaceae). For phylogenetic distribution of these shifts see SI Appendix, Supporting Information Text and Figs. S29–S31 and S37–S39.)
phenotypic evolution, which themselves are correlated. While rate parameters on niche, phenotype, and diversification all show strong mid-Miocene increases, we document a clear lag, where increases in diversification rates were followed by later increases in niche and phenotypic lability. This finding is robust when utilizing alternative methods and across sensitivity analyses and is based on one of the most densely sampled flowering plant clades to date, with a majority coverage of species for each of the phylogenetic, niche, and trait attributes. Given the sensitivity of diversification methods to species sampling, missing species have been accounted for in all diversification analyses we implemented. At smaller phylogenetic scales, diversification without associated morphological trait change has been reported in tetrapods [lizards (25) and salamanders (26)]. We know of no similar examples documenting not only a lack of association but also a significant lag in timing, in a large, globally distributed clade representing any lineage of life. However, lags between species diversification and subsequent niche divergence have been reported in several bird lineages using sister comparison methods at smaller temporal and spatial scales (refs. 42 and 43, but see ref. 44).

**The Timing of Opportunity.** Given the uncertainty in exactly defining rate shifts in rate-through-time plots alone, we analyzed rate analyses with a plurality of approaches. These included determination of the timing of significant rate shifts and of major shifts in ancestral reconstructions, with congruent results. Strong increases in rates of diversification and of niche and phenotypic evolution in Saxifragales were all dated to occur after the mid-Miocene Climatic Optimum. This timing is particularly significant given that from the late Cretaceous to the mid-Miocene the Earth was primarily covered in warm-tropical and subtropical habitats, with cool-temperate biomes limited to polar regions (33). After the mid-Miocene Climatic Optimum, a global cooling event marked the beginning of modern-day climate regimes and biomes, including a worldwide expansion of arid and cold-temperate biomes where Saxifragales are now most diverse (32, 34, 45–48). The observed coincidence of higher macroevolutionary rates with temperate habitat proliferation is consistent with increased ecological opportunities unavailable throughout much of the history of Saxifragales.

Given this increased ecological opportunity, we found strong evidence that continuing climatic cooling and climatic variability had a significant positive correlation with all three macroevolutionary rates we tested. This relationship is also in line with simulation-based theoretical results (49), where diversification driven by niche conservatism is expected under directional climate change scenarios, and where climatic oscillation primarily drives diversification of taxa with higher niche lability. Further simulation results (13) support a causal relationship between environmental opportunities and both high diversification rates and rapid phenotypic evolution. Both processes act in Saxifragales at different timeframes, with a greater role in deeper diversifications for niche conservatism (that is, lower niche macroevolutionary rates). While our quantitative analyses focused on paleotemperature data, aridification also increased during this period (50), which points to a combined role for decreasing temperature and precipitation in generating contemporary high evolutionary rates.

**An Alternative to Density Dependence.** Our findings of (i) a lag between diversification rates and niche/phenotypic rates across major clades within Saxifragales and (ii) no clear downturn in diversification rates across any major clade within Saxifragales do not support simple models of density-dependent diversification. Rather, they are consistent with a model of primarily niche-neutral diversification, followed by greater levels of niche structuring as interspecific competition increases (13). Because most major niche and phenotypic shifts (Fig. 2C) as well as rate shifts (Fig. 3) postdate diversification shifts, diversification events at first likely occurred primarily in ancestral habitats. As habitats began to be more densely filled, this likely created competitive pressure. Coincident with this pressure was the increased availability of novel habitats through which to escape competition [also seen in simulated diversification events (13)]. Continued availability of novel habitats hence provides a potential explanation for the observed lag of niche and phenotypic macroevolutionary rates behind diversification. Both adaption to novel environments and saturation of established ones should eventually replace initially niche-neutral diversification, as niche partitioning and divergence begin to predominate, resulting in accelerated evolution of niche and of niche-associated phenotypic traits. While the overall phylogenetic pattern showed no evidence of density dependence, we did reconstruct falling diversification rates in BAMM for some lineages (SI Appendix, Figs. S29–S31), consistent with certain species of Saxifragales experiencing a density-dependent pattern not evident in the overall phylogeny.

We found limited evidence that global cooling disproportionately affected lineages in the most extreme temperate habitats, although Saxifragales are highly diverse and prominent members of these contemporary florals. We also did not see evidence of latitudinal or elevational associations with any of the macroevolutionary rate parameters we estimated, nor did we find evidence for any association with specific species. These results may be consistent with the diversity of global responses to climatic cooling across clades despite similar rate patterns; substantial palaeontological and modeling work suggests profound effects of Pleistocene conditions on many tropical biotas, as well as those closer in proximity to glaciated regions (51–55). However, we did see a significant relationship between occupied niche and niche lability; clades that originated in cooler climates generally showed greater increases in lability (SI Appendix, Fig. S21).

**Habitat Patterns Through Time.** Much of the species diversity of Saxifragales in cold and arid habitats is in the large sister families Saxifragaceae and Grossulariaceae (primarily cold-adapted) and Crassulaceae (both arid- and cold-adapted, and largely succulent). Habitat ancestral reconstructions suggest that these groups were already present in cold and arid habitats before their mid-Miocene proliferation. The earliest divergence events in Crassulaceae are accompanied by ecological shifts and succulent specialization that largely occurred before the Miocene. These include parallel major shifts into desert and shrubland habitat in the African clades *Crassula* (50 Mya), *Kalanchoe* (12 Mya), the broader *Kalanchoe* group (13 Mya), the Macaronesian clade (24 Mya), and the New-World *Acre* clade (39 Mya). Likewise, a major shift toward temperate habitat occurs in the ancestor of Saxifragaceae and Grossulariaceae—temperate herbs and shrubs now diverse across the Northern Hemisphere in Arctic-alpine biomes—by 81 Mya, with a further shift into colder habitats in Grossulariaceae by 17 Mya, indicating these lineages had long been in cold habitats before the mid-Miocene. Finally, a shift toward temperate biomes in Hamamelidaceae, Altingiaceae, and Cercidiphyllaceae, comprising most of the remaining temperate shrub diversity, dates to at least 90 Mya. Because these dates for habitat shifts represent crown ages, the dates we report here are minimum age constraints on habitat shifts that could be considerably older. Two lineages—Peridiscaceae and Pierostemon, among the few tropical members of the clade—each experienced a habitat shift into more tropical environments >22 Mya, yet both are characterized by poor species diversity (~0.6% of Saxifragales; see also ref. 56).

Beyond these early shifts, the majority of shifts into the most extreme habitats occurred within the last 5 My (SI Appendix, Fig. S24), consistent with the strong increases in habitat evolutionary
rates in this timeframe. While not all species moved into colder, drier habitats, most shifts were into the most extreme parts of cold and dry habitats in which Saxifragales were ancestrally present (in extant species these represent, for instance, arid shrubland, Arctic tundra, and montane biomes). In most cases these frequent habitat shifts were preceded by diversification shifts. For example, a single diversification increase was reconstructed by BAMM in the *Hedera* group (temperate and Arctic-alpine herbs of Saxifragales; 10 Mya), which was followed by one further diversification shift at 8 Mya and 12 major habitat shifts in descendant lineages. An increase in diversification rate at 43 Mya was followed by six major habitat shifts after 5 Mya in arid-adapted, primarily Australian Haloragaceae. Similar patterns occur in subclades of Grossulariaceae, Crassulaceae, Itaceae, and Saxifragaceae. Hence, the phylogenetic placement of diversification shifts and habitat shifts across major clades was consistent with the pattern we constructed by time-averaged rate curves.

**Assessment of Evolutionary Rates.** The robustness of methods to estimate diversification rates has seen significant discussion, with concerns raised by Moore et al. (41) and Meyer et al. (57) as well as methodological criticisms of these benchmarking methods by Ribes et al. (58) and Rabosky (59). In the context of uncertainty about optimal methods for assessing macroevolutionary patterns, we applied a diversity of available methods, finding overall consistent results. Comparing clade-level estimates of the MS statistic (35) and \( \sigma^2 \) estimates from OU models (36), we found the same lag pattern as that seen in BAMM analyses. Likelihood model choice implemented in RPANDA (38–40) also favored temperature-dependent trait rates we identified based on BAMM rate curves. Likewise, we assessed a series of BAMM prior formulations and found our primary results robust. Across a diversity of methods, robustness analyses, and major subclades of Saxifragales, we found patterns that suggest a strong signal of diversification preceding trait evolution and related to historical climatic changes.

**Conclusion.** Despite the widespread occurrence of rapid radiations across major clades of life (60) and a long interest in such patterns (e.g., ref. 61), the evolutionary processes driving such radiations remain poorly understood (62, 63). As a largely temperate group of Saxifragales, using whole-genome and transcriptome data (74, 75), (chosen based on a minimum taxon occupancy cutoff across Saxifragales; see SI Appendix, Supporting Information Text for a list and details) in aTRAM using off-target sequencing reads. A supermatrix comprising these contigs, as well as all Saxifragales GenBank data for the 24 loci, was assembled using PHLAWD (76). We conducted a likelihood search on this supermatrix using RAxML (77); given the topological similarity and few well-supported incongruences between trees obtained via concatenation and coalescent approaches (SI Appendix, Figs. S1 and S32), the backbone from ExaML was enforced as a constraint. For more details on data assembly and phylogenetics reconstruction see SI Appendix, Supporting Information Text.

**Time Calibration.** We used two approaches to time calibration of our phylogeny. First, we generated a ultrametric dated tree from the result of the supermatrix analysis using a secondary dating approach and the penalized likelihood method as implemented in treePL (78), hereafter referred to as the “treePL tree” (SI Appendix, Fig. S30). Node calibrations were based on median highest posterior density values reported from a recent comprehensive Bayesian analysis of angiosperm divergence times (79). We used the following constraints: crown Saxifragales (i.e., root age) = 112.99, stem Saxifragaceae = 85.69, stem Itaceae = 91.03, crown woody clade (sensu ref. 80) = 95.93, and stem Haloragaceae = 72.09. The initial smoothing parameter was 100, and a thorough search was performed with “priming” to determine optimal run parameters and cross-validation. Second, we downloaded a dataset to RAxML of 20 phylogenetically representative taxa and the 50 most phylogenetically informative loci (based on Robinson–Foulds distance from the concatenated phylogeny; cf. ref. 81) to enable running a more computationally intensive Bayesian dating approach in BEAST (82, 83). We used a “congruificatory” approach (84, 85) in treePL to interpolate dates for nodes only sampled in the complete supermatrix phylogeny. Hereafter we refer to this BEAST tree as the “congruificatory BEAST tree” (SI Appendix, Fig. S36). The constraints we used are derived from vetted fossils in ref. 79: Altingiaceae, stem constraint 89.3 Mya; Haloragaceae, stem constraint 70.6 Mya; Itaceae, stem constraint 89.0 Mya; Ribes, stem constraint 48.9 Mya; and the root age constraint above. For details on priors and the congruificatory approach, see SI Appendix, Supporting Information Text. Because dates and downstream results were similar for these two dating approaches, the BEAST approach is used for further statistical tests in the main text; both are provided for comparison (Fig. 2; see SI Appendix, Figs. S23 and S33–S35; and Fig. 4; see SI Appendix, Tables S3–S5, and S7–S9).

**Acquisition of Locality Records.** All locality data from each Saxifragales family were downloaded as DarwinCore archives from iDigBio and GBIF on January 16, 2017. Using Python csv tools, we removed records lacking geographic coordinates and disaggregated the data into individual species files (and into variety/subspecies files where these DarwinCore fields were provided). We manually examined the species files for the presence of fossil taxa, cultivars, and stray taxa erroneously placed in Saxifragales, and such records were removed. For more details on processing occurrence records see SI Appendix, Supporting Information Text.

**Niche Predictor Assembly.** We assembled 35 environmental layers at 30-s resolution that capture features of climate, soil, landcover, and topography. These comprised 19 BioClim temperature and precipitation variables (http://www.worldclim.org/bioclim), seven soil layers (SoilGrids250m; ref. 86), six landcover classes (https://www.earthenv.org/landcover), and three topographical layers (elevation, GTOP030; https://fsta.cr.usgs.gov/GTOP030; aspect and slope calculated from these in qGIS). The SoilGrids250m product was aggregated to 30-s resolution via averaging, resampled to the extent of other layers by nearest neighbor, and finally averaged across 5-, 15-, and 30-cm sampling depths (all steps in GDAL; https://www.gdal.org). We implemented custom high-throughput Python methods based on GDAL to extract environmental conditions from observed points. Median values for each predictor were used in downstream analyses.

**Morphological Trait Assembly.** We built upon a previous matrix (87) by extracting records from major global floras and primary literature to build a dataset of phenotypic traits broadly scorable across Saxifragales. These traits were (i) categorical: perennial/annual, woody/herbaceous, flowering season, leaf shape, inflorescence type, sepal number, sepal color, sepal shape, petal number, petal color, petal shape, and stamen number and (ii) continuous: plant height, petiole length, leaf length, leaf width, sepal length, sepal width, petal length, petal width, stamen length, style length, and seed length. We built an automated Python pipeline to standardize verbatim measurements and qualitative descriptors for downstream analysis. For continuous traits, after outlier measurements were discarded, the midpoint...
was taken [e.g., {1–}12–14 cm would yield 7 cm]. For categorical data, each text descriptor was standardized with a term synonym list and coded se-
quentially from zero, and (for those taxa with multiple trait descriptors) a \( \sim \) likelihood. Blue colors represent temperature-neutral models. While all legend colors were plotted, not all are visible in the figure because some models have

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\begin{align*}
\mu &= \text{speciation and } \\
\beta &= \text{rate parameter. For instance, \( \text{"time-dependent" exponential } \lambda; \text{constant } \mu \) means that speciation is ex-
ponentially related to time and extinction is constant. Overall, red to orange colors represent temperature-dependent models, which occupy most of the
\end{align*}
\]

likelihood. Blue colors represent temperature-neutral models. While all legend colors were plotted, not all are visible in the figure because some models have

\[
\begin{align*}
\mu &= \text{speciation and } \\
\beta &= \text{rate parameter. For instance, \( \text{"time-dependent" exponential } \lambda; \text{constant } \mu \) means that speciation is ex-
ponentially related to time and extinction is constant. Overall, red to orange colors represent temperature-dependent models, which occupy most of the
\end{align*}
\]

recommendations in BAMM documentation. We additionally set seg\( \text{length} \) (likelihood grain) as 0.02 and the minimum clade size for shift inference for
two to constrain estimates of rate shifts to internal branches. To account for non-random taxon sampling in diversification analysis we used family-
level sampling statistics (SI Appendix, Table S10) to specify clade sampling prob-
abilities. The first 10% of generations were discarded as burn-in.

To understand evolutionary rates in niche and phenotypic traits (evolu-
tionary liability) throughout Saxifragales, we also implemented comple-
mentary runs using the BAMM trait model on ecological and phenotypic data. For niche lability, we used the first axis of the phylogenetic PCA of niche
predictors and ran the analysis for 250 million generations with a 10% burn-
in. For lability in phenotypic trait data, we used the first axis of the phylo-

genetic multidimensional scaling of phenotypic traits and ran the analysis for
two billion generations with a 75% burn-in. Settings followed those in the
diversification analysis, with the exception that we did not adjust for family-
level sampling, given that this is not implemented in the BAMM trait model. All
of these analyses were run on both the congruified BEAST tree (SI Appendix, Figs. S29–S31) and the treePL tree (SI Appendix, Figs. S37–S39). For further
details on the rate analyses see SI Appendix, Supporting Information Text.

We also assessed macroevolutionary rate timing by recursively calculating
two simpler rate-invariant models across all tree subclades. We used the \( R \) package geiger (90, 91) to calculate (i) the MS statistic (ref. 35; cf. refs. 59 and
92), calculated using crown ages and corrected for missing taxa in each clade
by the overall tree sampling percentage) and (ii) \( \sigma^2 \) rates from the favored OU
model. To render these rates comparable, we scaled them to a proportion of
the maximum (i.e., contemporary) rates, after removing the upper 99th per-
centile to discard a small number of outlier rates. For these relative rates,
which were approximately exponentially distributed (SI Appendix, Figs. SB–
S13), higher values mean rates closer to their contemporary maxima; hence,
for each clade a higher relative rate implies an earlier rate increase.

Macroevolutionary Rates and Global Climate. We used a regression approach
to investigate relationships between global temperature and macroevolution-
ary rates estimated in BAMM. We used the 5-point mean \( \delta^{18}O \) of the Zachos
deep-sea isotope dataset (37) extrapolated to global temperature (93). To measure temperature variability, we calculated the SD in a moving window
analytic; on the basis of initial plots, we used a window width of 0.1 My. After
comparing linear, quadratic, and exponential models using the AIC (94), we
selected exponential models, treating each macroevolutionary rate as a re-

response variable. To evaluate whether temperature and temperature variability had independent explanatory power, we built a combined exponential model
with both treated as predictors and assessed both the increase in adjusted \( R^2 
\text{ and AIC and Akaike weights for independent models and the combined model.}

We also fit a series of explicit temperature-dependent likelihood models for
traits and diversification (38, 39) as implemented in RPANDA (40). Because
these models require complete temperature coverage for the nearly 113-My
history of the clade, we used a longer \( T \) dataset (95), calculating a 5-point
mean and applying the temperature conversion as above. For diversification,
we fit nine time-dependent models: constant, linear, and exponential pure
birth as well as all possible combinations of linear and exponential birth with

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\begin{align*}
\text{Yield} &= \text{a composite distance metric on the interval [0, 1], that is,}
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\end{align*}
\]
constant, linear, and exponential extinction. We also fit nine temperature-dependent models with the same birth–death functions as the time-dependent models. For temperature-dependent trait models, in both niche and phenotypic data we compared the fit of the first ordination axis on Brownian and OU trait models with both linear and exponentially temperature-dependent trait models. The niche data were scaled by 1,000 to avoid likelihood optimization errors. We calculated AIC, and Akaake weights to assess the best models and related model fits, considering a model as decisively supported if it had a majority of the relative likelihood (Akaake weight >0.5).

Correlation of Rates with Niche, Traits, and Geography. We tested whether diversification, niche, or phenotypic rates are related to biogeography (i.e., certain parts of geographic space have high evolutionary rates) using a series of geographic summary data (continental biogeography, latitude, and elevation). Using STRAPP (96) and ES-im (97), we specifically tested for a relationship of macroevolutionary rates to the first axis of niche and trait ordinations, species mean latitude and elevation, and continent. For further details see SI Appendix, Supporting Information Text.

Data Availability. Sequence data have been deposited at the Sequence Read Archive (ref. 98; for individual accession numbers see SI Appendix, Table S1), Alignments, trees, and other analysis products have been deposited at Dryad (99). Code for spatial analyses has been deposited at GitHub (100).

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5. Rabosky DL, Donnellan SC, Grundler M, Lovette IJ (2014) Analysis and visualization of macroevolutionary rates to the first axis of niche and trait ordinations, species mean latitude and elevation, and continent. For further details see SI Appendix, Supporting Information Text.


