

On the complexity of triggering evolutionary radiations

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Summary

- Recent developments in phylogenetic methods have made it possible to reconstruct evolutionary radiations from extant taxa, but identifying the triggers of radiations is still problematic. Here, we propose a conceptual framework to explore the role of variables that may impact radiations. We classify the variables into extrinsic conditions vs intrinsic traits, whether they provide background conditions, trigger the radiation, or modulate the radiation.
- We used three clades representing angiosperm phylogenetic and structural diversity (Ericaceae, Fagales and Poales) as test groups. We located radiation events, selected variables potentially associated with diversification, and inferred the temporal sequences of evolution.
- We found 13 shifts in diversification regimes in the three clades. We classified the associated variables, and determined whether they originated before the relevant radiation (backgrounds), originated simultaneously with the radiations (triggers), or evolved later (modulators).
- By applying this conceptual framework, we establish that radiations require both extrinsic conditions and intrinsic traits, but that the sequence of these is not important. We also show that diversification drivers can be detected by being more variable within a radiation than conserved traits that only allow occupation of a new habitat. This framework facilitates exploration of the causative factors of evolutionary radiations.

Introduction

Evolutionary radiations usually imply two processes: multiplication of species (or increased taxonomic diversity) and increased phenotypic disparity (Givnish, 1997; Schluter, 2000; Losos, 2009; Glor, 2010; Losos & Mahler, 2010). In this paper, we use the term ‘radiation’ to refer to the proliferation of species only (i.e. ‘explosive speciation’ *sensu* Givnish (2010a)), rather than the accelerated diversification of ecological roles *sensu* Givnish (1997). We therefore understand radiation as a significant increase in the taxonomic diversification rate. Clades that have at least an order of magnitude more species than their sister clades are regarded as having undergone radiations (Sanderson & Donoghue, 1994).

In angiosperms, birds and mammals, and possibly also other clades, much of the current species richness is the result of radiation events (O’Leary *et al.*, 2013; Moen & Morlon, 2014; Zanne *et al.*, 2014). Understanding the causes and constraints of radiations is an important step in the study of the evolution of diversity. Radiations were initially explored by palaeontologists, who demonstrated high diversification rates after mass extinctions, followed by a slowdown in the diversification rate (Stanley, 1979). Molecular phylogenies and recent developments in phylogeny-based macroevolutionary inference have made it possible to infer radiations from extant taxa with greater precision and objectivity

than before. This has stimulated much research to establish a critical protocol for estimating and locating radiation events and their correlates, which can be understood to be potential triggers of these radiations. This led to the development of a tool box for identifying rate shifts and linking these to potential triggers (Pybus & Harvey, 2000; Maddison *et al.*, 2007; Alfaro *et al.*, 2009; FitzJohn *et al.*, 2009; FitzJohn, 2010, 2012; Stadler, 2011; Rabosky, 2014).

Simpson (1953, p. 223) developed a model for radiations that involves ‘more or less simultaneous divergence of multiple lineages [...]’. He suggested that radiation was a consequence of entering a new adaptive zone. The adaptive zone can be understood to be a geographical area or a habitat, in which the radiating lineage could expand taxonomically, structurally and/or ecologically. Typical adaptive zones could be isolated islands or unusual habitats such as epiphytic habitats or those on oligotrophic soils for plants. In order to enter an adaptive zone, three conditions need to be satisfied. First, the lineage needs physical access to this zone, and this is often the result of dispersal. Secondly, access to a new adaptive zone could be facilitated by the evolution of appropriate traits, that is, features that allow the organism to occupy novel environments and to interact with existing environments in a novel way, which may facilitate later ecological divergence. Finally, Simpson (1953) argued that either the zone should be empty, or the occupants should be competitively inferior. These two ideas of ecological opportunity (an adaptive zone *sensu* Simpson (1953)) and evolutionary

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change needed to occupy this zone, and the interplay between them, have dominated recent thinking about and explanations of radiations (Moore & Donoghue, 2007; Drummond *et al.*, 2012a).

As a consequence of the lack of complete fossil records for the vast majority of organisms, interpreting radiations using fossils alone has many limitations and constitutes a difficult task (Kidwell & Holland, 2002; Adrain & Westrop, 2003). At least since the work on the radiation of phytophagous insects by Mitter *et al.* (1988), which was based on phylogenies derived from extant organisms, radiation has been increasingly interpreted as a rapid evolution of species richness. The adaptive zone was now referred to as the 'ecological opportunity', and the evolutionary change that allowed access to the ecological opportunity as a 'key innovation'. However, key innovations can also initiate radiations by decreasing the probability of extinction via increased individual fitness, or by favouring reproductive and/or ecological specialization (Heard & Hauser, 1995). Simplistically, if the key innovation is only understood as enabling access to the ecological opportunity and accelerated generation of diversity constitutes a radiation, then Glor (2010) and Losos (2010) argued that an additional attribute is needed: a driver of diversification. This driver could be intrinsic, such as a set of traits that allow for faster speciation, or extrinsic, such as the occupation of a complex habitat. Both these conditions could affect the speciation rate by accelerating reproductive isolation. Thus, the 'Simpsonian' model, with appropriate modifications, is still useful in the molecular era.

Early phylogenetic work contrasted sister clades and sought correlates of significant differences in the richness of the clades, and these traits were then interpreted to be key innovations (Mitter *et al.*, 1988; Hodges & Arnold, 1995). With more detailed phylogenies becoming available, and especially with the generation of time-calibrated branch lengths, much more detailed analyses became possible, using algorithms such as the binary state speciation and extinction algorithm (BiSSE) (Maddison *et al.*, 2007). Case studies soon revealed that the situation can be quite complex, and that the apparent key innovations may evolve before the ecological opportunities and the initiation of the radiations, or vice versa. The notothenioid fishes, for example, evolved antifreeze glycoproteins 42–22 million yr ago (Ma), some 10 Myr before the Antarctic waters became very cold, and the species radiation was initiated (Near *et al.*, 2012). The BiSSE algorithm can be used to detect evidence for trait-dependent diversification and differential diversification rates, but these do not necessarily coincide with diversification rate shifts or radiations. A radiation constitutes a whole clade with an, on average, accelerated diversification rate, while changes in diversification rate could be scattered across a phylogeny without any specific radiating clade. There seems to be no general protocol for locating triggers of radiations.

The variables used to investigate a correlation with diversification rates are generally simplifications of the much more complex biological and environmental processes affecting diversification rate heterogeneity in a group of organisms. It may not always be easy to establish which variables summarize which complex traits

in the biology of the species, yet it is the full biological syndrome that facilitates the radiation (Verdú & Pausas, 2013). C_4 photosynthesis is an example of such a complex trait because it is coupled with physiological features, specialized leaf anatomy and organelle structure and distribution (Laetsch, 1974). C_4 plants fix CO_2 via the Calvin cycle, leading to a four-carbon compound instead of the three-carbon compound produced by C_3 plants (Sage, 2004), and they also display a unique 'Kranz' anatomy (Hamberlandt, 1904). This whole complex could be quantified by a single variable, such as the $\delta^{12}C : \delta^{13}C$ isotopic ratio. To complicate matters, it has been shown that foliar anatomy (i.e. the proportion of bundle sheath tissue) preceded and facilitated the evolution of C_4 photosynthesis in grasses (Christin *et al.*, 2013). A similar nested arrangement of characteristics is also found in the complex orchid flowers. Here, the evolution of the floral structure involved, more or less in sequence, the evolution of an inferior ovary, zygomorphy in the perianth, a reduction in the number of stamens to one, fusion of pollen into pollinia, fusing of the stamen and style into a gynostemium and development of a viscidium (Rudall & Bateman, 2002). The last trait which makes the syndrome fully functional can therefore be regarded as the key innovation. The traits do not need to be so fully integrated, but could all be responding to the same environmental conditions. The leaf economic spectrum describes the balance of investment in leaves, ranging from cheap, short-lived, high-return leaves to expensive, long-lived, low-return leaves (Wright *et al.*, 2004; Cornwell *et al.*, 2014), and these are linked to the productivity of the environment. Specific leaf area (SLA), the ratio between leaf area and leaf weight ($mm^2 mg^{-1}$), is one of the components of the spectrum, and sometimes used as a proxy for other traits, such as leaf nitrogen, photosynthetic capacity and leaf longevity (Onstein *et al.*, 2014). However, unlike the C_4 photosynthetic pathway, or the orchid flowers, the traits that make up the leaf economic spectrum are not necessarily tightly associated. A further complication, which has recently been disentangled, is the occurrence of cryptic precursor traits, postulated to be necessary for the key innovations to evolve (Marazzi *et al.*, 2012; Werner *et al.*, 2014). For example, Werner *et al.* (2014), in an angiosperm-wide analysis, detected an over 100 Myr old, single and cryptic evolutionary innovation of symbiotic N_2 fixation, followed by multiple gains (e.g. in the Cucurbitales, Fabaceae, Fagales and Rosales) and losses of the symbiosis.

In order to better understand the likely triggers of radiations, we developed an explicit conceptual framework and classification of variables as they relate to radiations. This framework scores variables for three attributes: 'types', 'timings' that can be used to characterize the temporal sequence of variable shifts in relation to the initiation of radiation, and 'roles' that distinguish different functions of variables during diversification.

The first attribute (the 'type') distinguishes between variables that represent extrinsic conditions vs intrinsic traits. Extrinsic conditions refer to all conditions outside the organisms, such as physical space, climate, other organisms, and any form of habitat not yet occupied or not effectively occupied. These describe Simpson's adaptive zone. Intrinsic traits evolve with the organism and thus can be physiological or morphological

‘functional traits’ which affect the fitness of the organism through their effects on growth, survival and reproduction (Violle *et al.*, 2007).

The second attribute (the ‘timing’) divides these variables (both extrinsic and intrinsic) into three groups based on their temporal relationship to the start of the radiation. Background variables (‘backgrounds’ hereafter) are established before the radiation starts and are necessary to create the conditions in which a radiation can start. If only considering extrinsic variables, backgrounds could be regarded as ‘key landscapes’ *sensu* Givnish (1997). If they are intrinsic, Lieberman (2012) refers to these variables as exaptations, a term adopted from Gould & Vrba (1982). The second group consists of the trigger variables (‘triggers’ hereafter) of the radiations, which have to be established contemporaneously with the start of the radiation. The third group consists of modulator variables (‘modulators’ hereafter) of the radiation. These variables become established after the start of the radiation. These could function to fine-tune the radiation to its environmental condition, or could impart an additional impulse to the radiation. Importantly, modulators could therefore also be triggers for nested radiations.

The third attribute (the ‘role’) divides the variables according to their presumed functions as diversification drivers. These include, first, those variables that primarily facilitate survival of the lineage. These variables should therefore be phylogenetically conserved and invariable during the radiation. We refer to them as ‘simple’ variables. The second group includes those variables that stimulate and/or maintain diversification (Glor, 2010; Losos, 2010). Theory predicts that these should be phylogenetically labile in the radiation and thereby provide numerous ways in which closely related species can coexist. We refer to these as ‘polymorphic’ variables. Polymorphic variables may potentially stimulate speciation rates by partitioning the environment or niche of the organism, such as key innovation (Hodges & Arnold, 1995) or traits that cause ecological divergence (Carlson *et al.*, 2011) or, in case of extrinsic conditions, environmental heterogeneity providing multiple niches for ecological adaptation (Rosenzweig, 1995; Hughes & Eastwood, 2006; Antonelli & Sanmartín, 2011).

Here, we tested whether this paradigm can be used to explore evolutionary radiations, using three clades selected from across the phylogenetic and structural diversity of angiosperms. In order to set up a framework for classifying and identifying the variables associated with radiations, we ask whether (1) as suggested by Simpson (1953), extrinsic and intrinsic variables are most common as backgrounds and triggers, respectively; (2) modulators

and backgrounds are more likely to be polymorphic and simple traits, respectively; (3) this framework could be used in radiation studies for selecting and filtering variables that are linked to radiations; and (4) the list of variables is adequate to account for the observed radiations.

Materials and Methods

Protocol and clades

To explore evolutionary radiations, we devised the following protocol. We first selected clades (Table 1) that may have radiations, and then located the radiation events without any *a priori* assumption about the topological position of these radiations. Our focus was on discrete radiations (i.e. radiating clades), not on accelerations in the diversification rates (i.e. radiating lineages), which can be diffuse across a phylogeny. Secondly, we selected the extrinsic and intrinsic variables which could be correlated to the radiations (i.e. backgrounds, triggers or modulators). Thirdly, we tested whether these variables had a significant effect on net diversification rates (speciation rate minus extinction rate) through their effect on speciation and extinction dynamics. We filtered out those variables that did not have a significant effect on the net diversification rate, and that are therefore not likely to be potential causes of radiations. Then, we established the temporal sequence of all the remaining variables in relation to the initiation of the radiation to classify the variables into backgrounds, triggers or modulators, and to evaluate their role within the radiation as simple (conserved) or polymorphic (labile) variables.

Locating radiations

We used published dated phylogenetic hypotheses for Ericaceae (Schwery *et al.*, 2014), Fagales (Xing *et al.*, 2014) and Poales (Bouchenak-Khelladi *et al.*, 2014) (Table 1). For all three clades, maximum clade credibility (MCC) trees were generated using BEAST v1.7 and v1.7.5 (Drummond *et al.*, 2006, 2012b; Drummond & Rambaut, 2007). These three phylogenetic trees represented the widest sampling for each clade to date (Ericaceae: 450 out of 4426 spp.; Fagales: 515 out of 1317 spp.; and Poales: 545 out of 20 000 spp.), and reflect the taxonomical, geographical, ecological and morphological diversity of the relevant clade.

In order to detect significant changes in the diversification dynamics (speciation and extinction rates), we analysed the MCC tree of each of the three clades with BAMM 1.0 (Rabosky, 2014), after excluding the outgroups. BAMM uses reversible-jump

Table 1 Clades used in this analysis, the sampling for the phylogenetic analysis, DNA loci used to infer phylogeny and number of diversification dynamics shifts detected

Clades	Higher group	Species	Species sampled	DNA loci	References	Diversification dynamics shifts
Ericaceae	Ericales	4426	450	<i>rbcl</i> , <i>matK</i>	Schwery <i>et al.</i> (2014)	6
Fagales	Rosids	1317	515	<i>rbcl</i> , <i>matK</i> , <i>trnL-F</i> , <i>ITS</i> , Crabs Claw	Xing <i>et al.</i> (2014)	4
Poales	Monocots	20 000	545	<i>rbcl</i> , <i>ndhF</i>	Bouchenak-Khelladi <i>et al.</i> (2014)	5

matK, Maturase K; ndhF, NADH dehydrogenase F; rbcl, Ribulose biphosphate carboxylase large chain.

Markov chain Monte Carlo (MCMC) to select between models that vary in the number of diversification regimes, thus accounting for rate variation through time and among lineages. We assigned sampling fractions at family and subfamily (Poales), tribal (Ericaceae) and generic (Fagales) levels to include the effect of species not sampled in the phylogeny on diversification heterogeneity. We ran two MCMCs for 100 000 000 generations with a sampling frequency of 1000 for each clade. We checked for convergence for each run by plotting the log-likelihood trace of the MCMC output file and checked that the effective sample sizes of the runs exceeded 200. Using the *BAMMTOOLS* package (Rabosky, 2014) in R, we identified the 95% credible set of distinct shift configurations and the overall best set of rate shifts given the data. These shifts in diversification dynamics are referred to as initiation of radiations, except where there is a slow down in net diversification rate. It is possible that a more detailed sampling, especially in the Poales where the sampling fraction was estimated down to subfamily level, might have led to more detected changes in the diversification dynamics.

Candidate variables affecting radiation and their type

Ideally, all variables that could influence diversification should be investigated. Here, however, we selected those variables for which we had some prior indication that they could be of importance. For the intrinsic variables, we used physiological arguments of improved performance given the ecological opportunities, while extrinsic variables describe the habitats in which the relevant clade is most common. These variables were assigned a 'type' by dividing them into extrinsic conditions and intrinsic traits. We did not explicitly group the variables into syndromes (as in Givnish *et al.*, 2014), as covariation in the variables would be evident when mapped on the phylogeny.

Variable states were assembled in a species \times variable matrix and scored, where possible, for all species present in the phylogenetic trees of Ericaceae, Fagales and Poales. As these variables are often phylogenetically conserved within a genus, scoring followed a phylogenetic top-down approach, in which all species in a genus would be assigned a similar state of a variable if our source of information confirmed this. In some cases (e.g. evergreen/deciduous in *Quercus*) the trait was variable within genera, in which case we scored it for each species. In other cases (e.g. SLA data for Ericaceae), there are missing data in our matrix. For all variable states, we scored presence only (Hardy & Linder, 2005) because states of a variable may not always be exclusive (e.g. a species can have a shrubby as well as a tree-like growth form). This resulted in a binary (presence/absence) data matrix for each state of a variable (see Supporting Information Methods S1).

Filtering variables affecting radiations

BiSSE (Maddison *et al.*, 2007), implemented in *DIVERSITREE* 0.4-5 (FitzJohn *et al.*, 2009; FitzJohn, 2012), was used to test the hypothesis that presence/absence of a variable was correlated with different diversification rates on the MCC tree. BiSSE employs maximum likelihood optimization to estimate absolute rates of

asymmetric character change (q), speciation (λ), and/or extinction (μ) by maximizing the likelihood of these parameters for a given topology with branch lengths (Maddison *et al.*, 2007). We compared the fit of seven diversification models (see Methods S1) in which speciation and/or extinction and/or transition rates were constrained to have similar rates for presence/absence of the variable with a likelihood-ratio test and Akaike Information Criterion (AIC). The model with the least number of parameters which did not perform significantly worse than the full six-parameter model (χ^2 distribution $P < 0.05$; $\Delta\text{AIC} > 2$) was chosen as the best model for each variable. This model was used in an MCMC in BiSSE to estimate the posterior density of the parameters (λ , μ and q) for each state (presence/absence). We ran 10 000 MCMC iterations for every clade and each variable. By examining the 95% credible intervals of the posterior samples for each parameter, we inferred posterior Bayesian support for a difference in diversification rates between states (FitzJohn *et al.*, 2009). If the presence of a variable was associated with increased rates of diversification, it was retained in the set of backgrounds, triggers and modulators.

Separating backgrounds, triggers and modulators

In order to determine the temporal order of the variables in relation to the shifts in diversification dynamics identified by *BAMM*, we optimized the retained variables which may be causing radiations on the MCC tree (without outgroups) using 'asr.marginal' implemented in the R package *DIVERSITREE* (FitzJohn *et al.*, 2009; FitzJohn, 2012). Ancestral reconstructions were made using the estimated parameters (transition rates among character states) of the best model found in BiSSE, which automatically corrects for the influence of diversification rate differences when inferring ancestral states. We obtained the marginal probability of each state (presence/absence) for each variable at each node. In addition, we obtained the 95% highest posterior density (HPD) of the estimated node age from *BEAST*, based on a minimum of 10 000 post burn-in trees.

We divided each study clade (i.e. Ericaceae, Fagales and Poales) into separate partial trees for each of the radiations. These partial trees included the nodes that led up to the respective radiation (i.e. the nodes from the root of the tree towards the radiations), and those included in the radiation (Fig. 1). Nodes that did not play a role in the evolutionary pathway leading to the radiation (for example in sister clades of the radiating group) were excluded. The change in the probability of presence of a variable was assessed over time by plotting this probability against the median age and the 95% HPD of each node (Fig. 1). This allowed us to assign the 'timing' of each variable. Variables that exceeded a probability of 0.75 before the rate shift, at a node that did not overlap with the 95% HPD of the node where the radiation was initiated, were defined to be backgrounds (Fig. 1a). Variables that exceeded a probability of 0.75 at a node that overlapped with the initiation of the radiation were interpreted to be potential triggers (Fig. 1b). Variables that exceeded a probability of 0.75 after the rate shift (i.e. at a node that did not overlap in time with the initiation of the radiation) were interpreted to be

modulators (Fig. 1c). Backgrounds, triggers and modulators were then classified into their ‘role’: ‘simple’ if they were conserved within the radiating clade (i.e. >95% of the nodes having a probability of ≥ 0.75 throughout the radiation) or ‘polymorphic’ if they were labile within the radiating clade (i.e. probabilities of ≥ 0.75 as well as < 0.75) (Fig. 1d).

Results

Radiations

In the three clades, our phylogenetic results were largely congruent with those previously published (e.g. Ericaceae (Kron *et al.*,

2002; McGuire & Kron, 2005; Bush *et al.*, 2009; Gillespie & Kron, 2010), Fagales (Sauquet *et al.*, 2012; Zhang *et al.*, 2013), and Poales (Givnish *et al.*, 2010b, 2014; Briggs, 2011; Bouček-nak-Khelladi *et al.*, 2014; Briggs *et al.*, 2014). Time-calibrated phylogenies are available in the Dryad Digital Repository (<http://www.datadryad.org>; doi: 10.5061/dryad.9pg3r).

We found six radiations in Ericaceae, three in Fagales with one slow-down, and four in Poales with one slow-down (Figs 2–4).

Filtering variables affecting radiations

We found eight out of 20 intrinsic and four out of seven extrinsic variables significantly positively associated with diversification

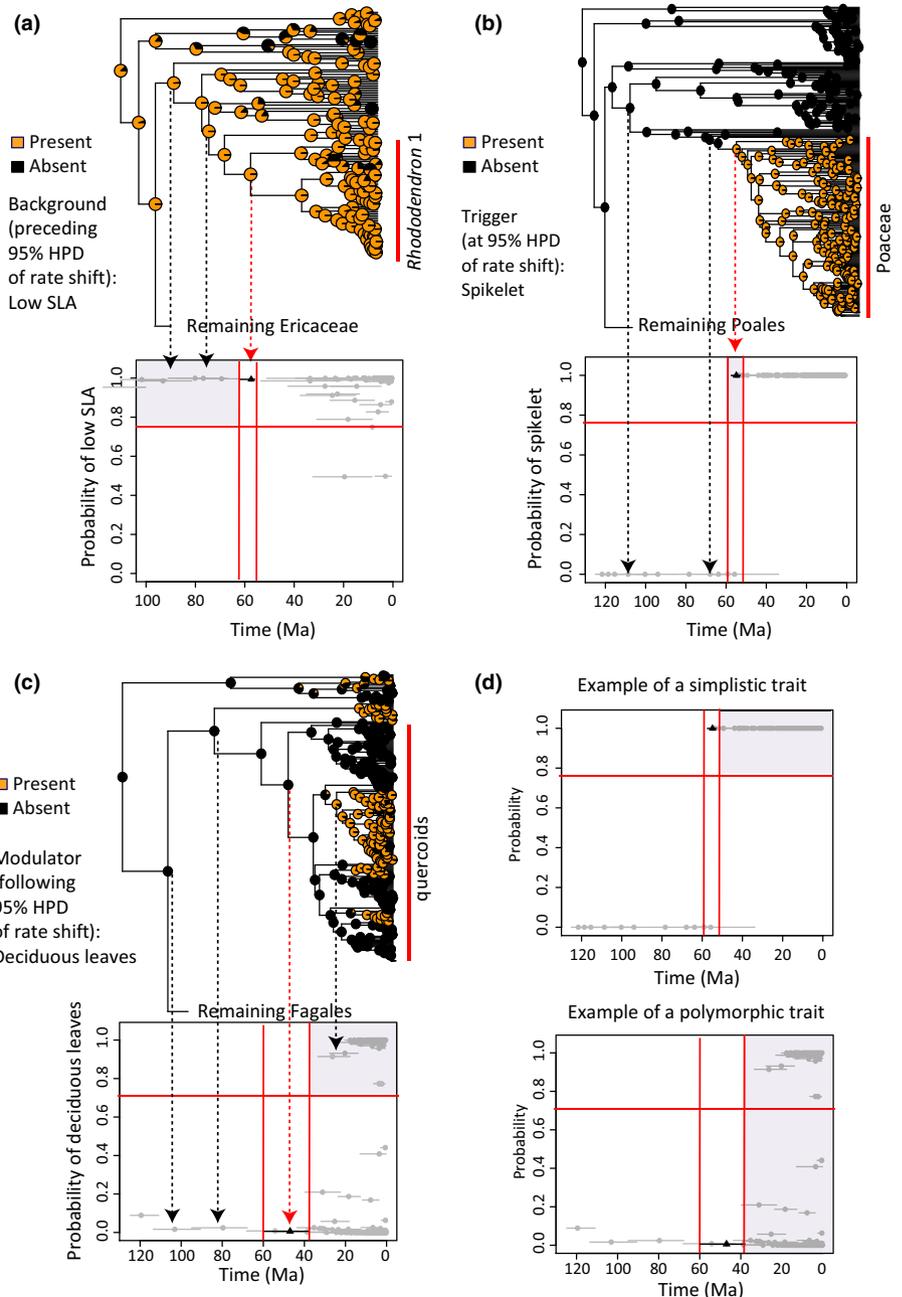


Fig. 1 Diagram explaining how variables have been classified as (a) background, (b) trigger and (c) modulator, and (d) simple (conserved) or polymorphic (labile). All nodes leading and belonging to the radiating clade are plotted onto a graph with the ancestral state probability of a variable, as reconstructed under the BiSSE model, on the y-axis and time in million years ago (Ma) on the x-axis. Dashed arrows illustrate the connection between nodes in the tree and their position on the graph. Black and grey horizontal bars of the nodes represent the 95% highest posterior density (HPD) of the node age. Black triangles represent the node at which a shift in diversification regime was found. Vertical and horizontal red lines on the graphs indicate the threshold (0.75) for significant ancestral reconstructions (horizontal), and the time interval (vertical) in which significantly optimized nodes are considered (a) backgrounds (i.e. when significantly optimized ancestral nodes are preceding the rate shift), (b) triggers (i.e. when the first significantly optimized ancestral node overlaps the 95% HPD of the rate shift) or (c) modulators (i.e. when optimized nodes occur after the rate shift). SLA, specific leaf area.

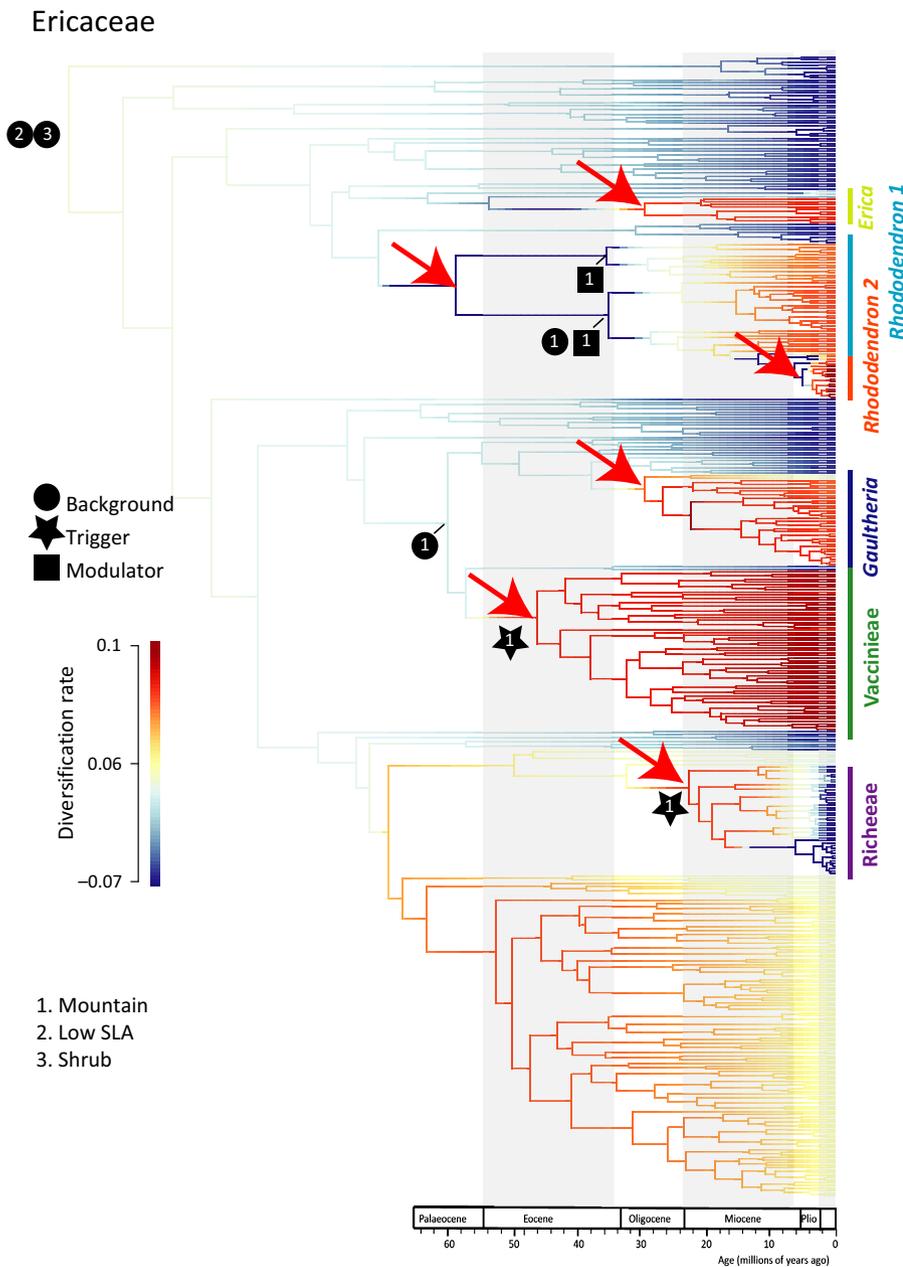


Fig. 2 Maximum clade credibility (MCC) tree from the BEAST analysis for Ericaceae.

Diversification rates are shown along each branch of the phylogeny. Each colour section of a branch represents the mean of the posterior density of net diversification rate. The red (speed up) arrows indicate where shifts in diversification regime occur. Each number represents one of the variables tested. We found six radiations, which correspond to the temperate Afro-European genus *Erica* (c. 860 spp.), Australasian Richeeae (Styphelioideae) (c. 70 spp.), *Rhododendron* 1 (c. 860 spp.) and a nested radiation of some Himalayan members of *Rhododendron* 2 (c. 150 spp.), and two South America–Asian clades: *Gaultheria* (c. 130 spp.) and the tribe Vaccinieae (c. 1564 spp.). SLA, specific leaf area.

rate changes by BiSSE (Tables 2–4; see Fig. S1). In two clades, some variables had no significant effect on net diversification rates, such as deciduous/evergreen leaves, epiphytism and tree growth form in Ericaceae (Table 2) and open tropical biomes, scale leaves and serotiny in Fagales (Table 3). Seven variables had a significant negative effect on the diversification rate; as our coding is presence only, we ignored these (Tables 2–4).

Temporal classification

For each radiation, the retained variables were separated into backgrounds, triggers, and modulators (Fig. S2). Background variables were found to be either only extrinsic (two radiations), only intrinsic (five radiations), or both extrinsic and intrinsic (two radiations). Triggers were found to be only extrinsic (two

radiations), only intrinsic (two radiations), or both (three radiations). Finally, modulators were found to be only extrinsic (one radiation) or only intrinsic (four radiations) (Table 5). Backgrounds were found in all radiations, except in Betulaceae, quercoids, Poaceae and Bromeliaceae (Tables 2, 4). No triggers were found for six radiations: the Cyperoideae in Poales, *Allocasuarina* in Fagales, and *Erica*, *Gaultheria* and *Rhododendron* 1 and 2 in Ericaceae (Tables 2–4). No modulators were found for eight radiations: Cyperaceae and Bromeliaceae in Poales, *Allocasuarina* in Fagales, and *Gaultheria*, *Rhododendron* 2, *Erica*, Richeeae and Vaccinieae in Ericaceae (Tables 2–4). We did not find support for the hypotheses that backgrounds are more likely to be extrinsic variables, and triggers more likely to be intrinsic variables, as the frequency of the timing (background, triggers or modulators) was not significantly different

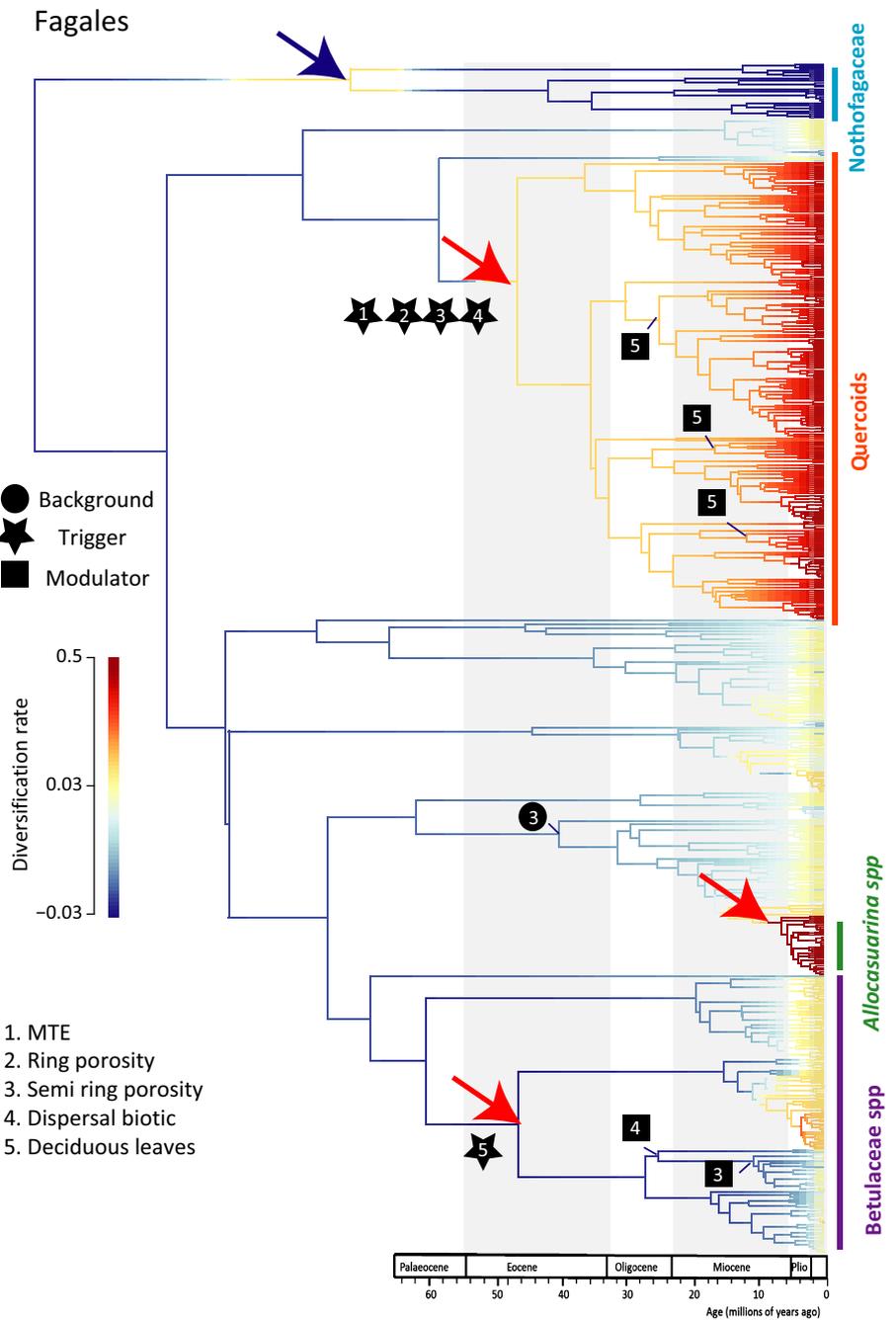


Fig. 3 Maximum clade credibility (MCC) tree from the BEAST analysis for Fagales. Diversification rates are shown along each branch of the phylogeny. Each colour section of a branch represents the mean of the posterior density of net diversification rate. The blue (slow down) and red (speed up) arrows indicate where shifts in diversification regime occur. Each number represents one of the variables tested. Fagales exhibits three radiations: the Northern Hemisphere subtropical-temperate quercoids (*Quercus*, *Lithocarpus* and *Castanopsis*; c. 923 spp.) and the (cold) temperate *Carpinus* (c. 49 spp.) and *Betula* (c. 97 spp.) (i.e. both members of Betulaceae), and a clade of the sclerophyllous Australian *Allocasuarina* (c. 61 spp.). The temperate South American–Australasian *Nothofagus* (c. 36 spp.) shows a slow-down.

between types (intrinsic or extrinsic) (χ^2 test (Preacher, 2001); $\chi^2 = 1.309$; $P = 0.520$).

Diversification drivers

Eleven of the background variables are simple and seven are polymorphic (Table 6). Among the triggers, four are simple and eight are polymorphic, and all six modulators are polymorphic (Table 6). We found support for the hypothesis that modulators are more likely to be diversification drivers (i.e. polymorphic, labile variables) than triggers and backgrounds (Fisher’s exact test: $P = 0.034$). As expected, we also found

support for the hypothesis that background variables are more likely to be simple compared with modulators (Fisher’s exact test: $P = 0.016$).

Discussion

General

In the three clades we investigated, we found 13 radiations and two slow-downs (Figs 2–4). Possibly because of the lack of detailed sampling (especially within Poales), we did not detect significant shifts in diversification regimes in the more recent

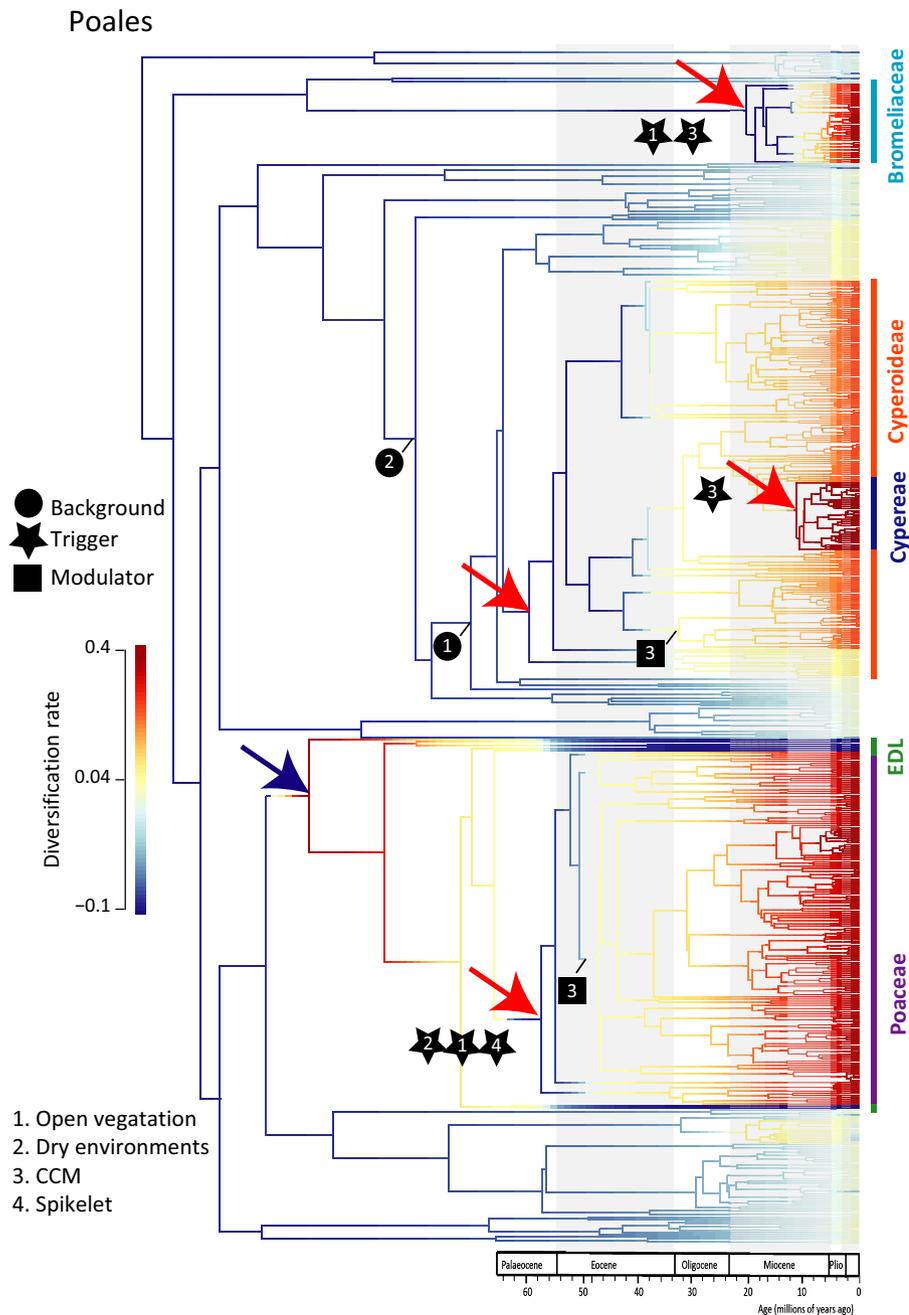


Fig. 4 Maximum clade credibility (MCC) tree from the BEAST analysis for Poales. Diversification rates are shown along each branch of the phylogeny. Each colour section of a branch represents the mean of the posterior density of net diversification rate. The blue (slow down) and red (speed up) arrows indicate where shifts in diversification regime occur. Each number represents one of the variables tested. There are four radiations: the South American Bromeliaceae (3500 spp.), the global Cyperoideae (c. 5250 spp.) followed by a radiation within Cyperaceae, and the global spikelet clade of the Poaceae (c. 10 000 spp.). The latter is preceded by a slow-down at the base of the graminid clade (c. 15 spp.), which includes Ecteiocoleaceae, Flagellariaceae, Joinvilleaceae, and the early-diverging lineages (EDLs) of Poaceae. CCM, CO₂-concentrating mechanisms.

histories of the families. Most radiations (10 out of 13) seem to require both extrinsic conditions and intrinsic traits. For all radiations, except for Betulaceae and the quercoids in Fagales and Poaceae and Bromeliaceae in Poales, we located background changes, but only two of these are extrinsic (Table 5). In six radiations we found no triggers. Two radiations were found to be triggered by intrinsic traits, two by extrinsic traits, and three by a combination of intrinsic and extrinsic variables (Tables 2–4). In only five of the 13 radiations did we find modulators (Table 5). As predicted, variables that arise late relative to the start of the radiation are more likely to be polymorphic, and so are more likely to stimulate diversification (e.g. either by allowing coexistence after speciation or by driving divergence during speciation) rather than

persistence in a particular environment (extrinsic) and/or morphology (intrinsic).

Although BiSSE may currently be the best method to filter out variables not linked to diversification rate shifts, it is possible that some discarded variables, even though not significantly associated with high diversification rates across the whole phylogeny, may still be potential causes of radiation for a specific clade. Therefore, we included the classification of those variables relative to radiations (Tables 2–4; Fig. S2).

There is no biologically objective way of locating radiations. BAMM is probably the best tool, but if the species sampling ratio is given only at nodes that are very deep in the tree it may shift the node of the diversification regime shift further into the past than

Table 2 Traits and their classification, performance in BiSSE, and temporal classification relative to the diversification dynamics shifts in Ericaceae

Variable	Type	BiSSE	SHIFT							Ancestral
			Richeeae	<i>Erica</i>	<i>Rhododendron 1</i>	<i>Rhododendron 2</i>	<i>Gaultheria</i>	Vaccinieae		
Habitat leaf	Mountain	Extrinsic	↑*	Trigger P	x	Post P	Pre P	Pre P	Trigger P	Ambiguous
	Low SLA	Intrinsic	↑*	Pre S	Pre S	Pre P	Pre S	Pre S	Pre P	Low SLA
	Deciduous leaves	Intrinsic	ns	x	x	Post P	x	x	Post P	Nondeciduous
	Evergreen leaves	Intrinsic	ns	Pre S	Pre S	Pre P	Pre S	Pre S	Pre P	Evergreen
Growth form	Shrub	Intrinsic	↑*	Pre S	Pre P	Pre P	Pre S	Pre S	Pre P	Shrub
	Herb	Intrinsic	↓*	x	x	x	x	x	x	Nonherb
	Tree	Intrinsic	ns	x	Post P	Post P	x	x	x	Nontree
	Epiphyte	Intrinsic	ns	x	x	x	x	x	Trigger P	Nonepiphyte

↑ and ↓, positive and negative associations with diversification rates, respectively, from the BiSSE analyses. *, statistical significance; ns, nonsignificant association for the trait. 'Pre', 'trigger' and 'post' refer to the temporal relationship of the trait to the radiation: background, trigger and modulator, respectively (cf. Introduction section). P and S, the function of the trait to the radiation: polymorphic and simple, respectively (cf. Introduction). x, the trait cannot be classified relative to the model shift (i.e. probability below 0.75 for the ancestral reconstructions). Shaded rows indicate the variables selected that are associated with radiations. SLA, specific leaf area.

Table 3 Traits and their classification, performance in BiSSE, and temporal classification relative to the diversification dynamics shifts in Fagales

Variable	Type	BiSSE	SHIFT				Ancestral	
			<i>Allocasuarina</i> spp.	Betulaceae spp.	<i>Nothofagus</i> [¶]	Quercoids		
Habitat	MTE	Extrinsic	↑*	x	x	n/c	Trigger P	Ambiguous
	TEF	Extrinsic	↓*	x	x	n/c	Pre P	TEF
	TDF	Extrinsic	↓*	x	Pre S	n/c	Post P	TDF
	Tropics (open)	Extrinsic	ns	Pre S	x	n/c	Post P	Non-tropics
Wood porosity	Ring porosity	Intrinsic	↑*	x	x	n/c	Trigger P	Non-ring
	Semi-ring porosity	Intrinsic	↑*	Pre S	Post P	n/c	Trigger P	Non-semi-ring porous
	Diffuse	Intrinsic	↓*	Pre S	Pre S	n/c	Pre P	Diffuse
Dispersal	Biotic dispersal	Intrinsic	↑*	x	Post P	n/c	Trigger S	Non-biotic
	Abiotic dispersal	Intrinsic	↓*	Pre S	Pre P	n/c	x	Abiotic
	Passive dispersal	Intrinsic	↓*	x	x	n/c	x	Passive
Leaf	Deciduous leaves	Intrinsic	↑*	x	Trigger S	n/c	Post P	Non-deciduous
	Evergreen leaves	Intrinsic	↓*	Pre S	x	n/c	Pre P	Evergreen
	Scale leaves	Intrinsic	ns	Pre S	x	n/c	x	No scale leaves
Other	Serotiny	Intrinsic	ns	Pre S	x	n/c	x	Non-serotinous
	N ₂ fixation	Intrinsic	↓	x	Post P	n/c	x	Non-N ₂ fixation

MTE, Mediterranean-type ecosystem; TEF, Tropical evergreen forest; TDF, temperate deciduous forest; N₂ fixation, nitrogen fixation. ↑ and ↓, positive and negative associations with diversification rates, respectively, from the BiSSE analyses. *, statistical significance; ns, nonsignificant association for the trait. 'Pre', 'trigger' and 'post' refer to the temporal relationship of the trait to the radiation: background, trigger and modulator, respectively (cf. Introduction section) P and S, the function of the trait to the radiation: polymorphic and simple, respectively (cf. Introduction). x, the trait cannot be classified relative to the model shift (i.e. probability below 0.75 for the ancestral reconstructions). ¶The rate shift found in *Nothofagus* indicates a slow-down. n/c, variables were not considered. Shaded rows indicate the variables selected that are associated with radiations.

Table 4 Traits and their classification, performance in BiSSE, and temporal classification relative to the diversification dynamics shifts in Poales

Variable	Type	BiSSE	SHIFT					Ancestral	
			Poaceae	EDL [¶]	Cyeroideae	Cypereae	Bromeliaceae		
Habitat	Open	Extrinsic	↑*	Trigger P	n/c	Pre S	Pre S	Trigger P	Non-open
	Dry	Extrinsic	↑*	Trigger P	n/c	Pre P	Pre S	Trigger P	Non-dry
Photosynthesis	CCM	Intrinsic	↑*	Post P	n/c	Post P	Trigger S	Trigger P	Non-CCM
Other	Spikelet	Intrinsic	↑*	Trigger S	n/c	x	x	x	Non-spikelet

CCM, CO₂-concentrating mechanisms (i.e. C₃ vs C₄ and CAM). ↑ and ↓, positive and negative associations with diversification rates, respectively, from the BiSSE analyses. *, statistical significance for the association of the trait. 'Pre', 'trigger' and 'post' refer to the temporal relationship of the trait to the radiation: background, trigger and modulator, respectively (cf. Introduction section). P and S, the function of the trait to the radiation: polymorphic and simple, respectively (cf. Introduction). x, the trait cannot be classified relative to the model shift (i.e. probability below 0.75 for the ancestral reconstructions). ¶The rate shift found in early-diverging lineages (EDLs) of the graminid clade (Ecdociaceae, Flagellariaceae and Joinvilleaceae) and the EDLs of Poaceae indicates a slow-down. n/c, variables were not considered. Shaded rows indicate the variables selected that are associated with radiations.

Table 5 The number of radiations where backgrounds, triggers and modulators (in rows) are (1) extrinsic, intrinsic or both, and (2) simple or polymorphic, or both (in columns)

	Only extrinsic			Only intrinsic			Both extrinsic and intrinsic			Total radiations with backgrounds, triggers and modulators
	Simple	Polymorphic	Both	Simple	Polymorphic	Both	Simple	Polymorphic	Both	
Background	1	0	1	2	2	1	0	0	2	10/13
Trigger	0	2	0	2	0	0	0	1*	2	7/13
Modulation	0	1	0	0	4	0	0	0	0	5/13

*This cell includes only one radiation, and we report on the trigger variables associated with the Bromeliaceae radiation, which include open and dry habitats (polymorphic) as an extrinsic variable and CO₂-concentrating mechanism (i.e. C₃ vs C₄ and CAM photosynthesis) (polymorphic) as an intrinsic variable (cf. Table 4).

Table 6 The distribution of retained variables according to their type, timing and roles for the 13 radiations

	Background		Trigger		Modulator	
	Simple	Polymorphic	Simple	Polymorphic	Simple	Polymorphic
Extrinsic	3	2	0	5	0	1
Intrinsic	8	5	4	3	0	5

where it should be, and it could miss nested radiations. Consequently, there may be nested radiations in our study clades that have not been detected because of sparse taxon sampling and the assignment of unsampled diversity to higher taxonomic levels. This could account for the difference between our radiation shifts in Bromeliaceae and those postulated by Givnish *et al.* (2014). Finally, the methods used for molecular dating (fossil assignments, prior distributions and rate correction methods) may impact our results. However, as the temporal classification of variables is relative to rate shifts obtained on the same chronogram, such dating errors will not impact our results.

Intrinsic and extrinsic conditions

Theoretically, a radiation needs both extrinsic conditions and intrinsic traits except when it occurs in a geographically isolated and noncompetitive habitat. Extrinsic conditions and intrinsic traits may be complex in several ways, making it difficult to identify the underlying and effective causes of these conditions.

Across the three clades, we found that shifts in extrinsic conditions often triggered the radiation (shifts to mountains in two out of six Ericaceae radiations, a shift to Mediterranean-type ecosystems in the quercoid, and shifts to open and dry vegetation in the Poaceae and Bromeliaceae radiations), as well as being the background (open and dry vegetation in the Cyperoideae and the Cypereae, and shifts to mountain in *Rhododendron* 2 and *Gaultheria* in the Ericaceae) or the modulator (mountains in *Rhododendron* 1). Thus, ecological opportunities may influence radiations in general even if the initiation of the radiation happens later, or if the opportunity arises after the initiation of the radiation.

Past climate change is often proposed as a trigger for radiations. This is usually based on a three-part argument. The first part of the argument is biome or habitat change optimized over a

phylogeny and modelled to have taken place at a particular node. In the second part, this node, if on a time-calibrated phylogeny, provides an estimate of the time of this change. In the third part, this dating is used to provide a link to global climate changes. This logic has been used to link the radiation of succulent plants (Arakaki *et al.*, 2011), cycads (Nagalingum *et al.*, 2011), and grasslands (Jacobs *et al.*, 1999) to the 'modern planet' climate established *c.* 14 Ma by the Antarctic glaciations (Zachos *et al.*, 2001), which resulted in widespread seasonal dry climates. Such global climate-driven 'turnover events' (Vrba, 1985, 1993) possibly also occurred at the Eocene Optimum and the Eocene–Oligocene transition. In Poales, the radiations of Cyperoideae and the spikelet clade of Poaceae occurred at 50 and 55 Ma, respectively, suggesting that the Eocene Optimum may have acted as an extrinsic factor for these two radiations. More probably, the establishment of seasonal monsoonal climates (Huber & Goldner, 2012) in the Late Eocene might have caused the establishment of more open vegetation into which these two Poalean clades radiated.

In our analyses, extrinsic potential causes of radiations were often associated with intrinsic changes. Across the three clades, we found that shifts in intrinsic traits were also potential triggers for radiations (ring porosity and biotic dispersal in the quercoid radiation, deciduous leaves in the Betulaceae radiation, CAM photosynthesis in Bromeliaceae, and a spikelet in the Poaceae radiation), as well as backgrounds (low SLA and a shrubby growth form in all radiations in Ericaceae, and semi-ring porous wood in Fagales) or modulators (deciduous leaves in quercoids, biotic dispersal and semi-ring porous wood in Betulaceae and C₄ photosynthesis in the Cyperoideae and Poaceae radiations) (Figs 2–4). Our method shows that intrinsic traits are much more important in determining the backgrounds for radiations than suggested by Simpson's model (1953). This model implies that the environment existed, waiting for lineages either to reach it

(physical access or dispersal) or evolve suitable traits to enable them to enter it. Ericaceae present a complex situation. Here, the background is probably the combination of ericoid roots, low-SLA leaves, and a habitat on oligotrophic soils (Read, 1996). Our analysis could not disentangle this set of interactions as the whole family shows this syndrome, and our analysis starts only at family level. However, as the radiations are within the family, this syndrome acts as background for these radiations. A comparable situation was reported in the succulent clades of the Caryophyllales, where succulence evolved before the radiations started (Arakaki *et al.*, 2011); presumably this was in response to locally dry habitats. The actual radiations were triggered by Late Miocene aridification and the evolution of more extensive areas of seasonal climate. Lieberman (2012) suggested that these should be called 'exaptive radiations' as the trait evolved before the extrinsic condition. However, in both Ericaceae and Caryophyllales, the trait syndromes (ericoid mycorrhizae, low SLA leaves, and succulence) evolved in response to the conditions in which they still function. The trigger of the radiation was the expansion of these habitats by orogeny (Ericaceae) or climate change (Caryophyllales). Consequently, the term 'preadaptation' might be more suitable.

The roles of extrinsic and intrinsic variables in a radiation are complex and seem to be intermingled, especially because plants modulate the environment (Linder *et al.*, 2012) and can change the extrinsic conditions not only for themselves but also for other organisms. This complex interaction may well have been of central importance in the Poaceae radiation, and also for the Cyperoidae. Poaceae diversification slowed down significantly in closed vegetation (the graminid diversification dynamics change), and accelerated in open habitats (the spikelet-clade radiation) (Fig. 4). In the latter case, grasses and their intrinsic traits created part of the extrinsic environment, the open vegetation. The evolution of angiosperm forests in the late Cretaceous also acted as a biotic modifier (Linder *et al.*, 2012) of the environment (cool, shady) by providing new food and new chemical resources. This has been shown to be linked to the radiation of epiphytic ferns (Schneider *et al.*, 2004) and ants (Moreau *et al.*, 2006). The radiations in forest understory plants such as *Impatiens* (Janssens *et al.*, 2009) and *Begonia* (Thomas *et al.*, 2012) were only possible in the cool shade of the tropical forests. The transformation of forests to savannas may have been facilitated by grasses fuelling fire, and the expansion of grassland is associated with an increase in fire frequency (Bond *et al.*, 2003, 2005; Beerling & Osborne, 2006; Hoetzel *et al.*, 2013). This modulation complicates the simplistic distinction between intrinsic and extrinsic variables.

Although radiations occur in a context in which multiple variables are involved, theoretically, each radiation should be triggered by a single variable. This may be the last element of a trait syndrome, or the actual colonization event of a new biome. However, we cannot resolve a singular trigger in three cases (Tables 2–4), and there could be several reasons for this. First, where the radiation is subtended by a long branch (such as the stem of Bromeliaceae; Fig. 4) the sequence of changes along these branches cannot be resolved, and so background changes cannot be separated from triggers. Secondly, because we start with the assumption that all variables are triggers, if the dating has a wide

variance this assumption cannot be rejected. In this case, variables that should be background or modulators are still listed as triggers. Finally, the lack of resolution could also be real. Roquet *et al.* (2013) show that the cushion growth form is not only an adaptation to alpine conditions, but is linked to the radiation in *Androsace* (Primulaceae). They suggest that this growth form change occurs as the populations are elevated by orogeny selecting for adaptation to the increasingly harsh alpine conditions. In this case, it is possible that the extrinsic condition (mountains) and intrinsic trait (cushion growth form) evolve at the same time, as 'compound triggers'.

Diversification drivers

Several of the intrinsic and extrinsic traits identified as triggers or as modulators might function as diversification drivers, particularly if they are polymorphic within the radiation. These may include mountains in the Ericaceae radiations (triggers in the Richeae, the Himalayan *Rhododendron* 2, and the Vaccinieae radiations; Fig. 2; Table 2; see Schwery *et al.*, 2014). In the Fagales, these include biotic dispersal and semi-ring porosity in the Betulaceae radiation, and Mediterranean-type ecosystems, ring porosity and leaf deciduousness in the quercoid radiation (Fig. 3; Table 3). Open and dry vegetation can be included for the Poaceae and the Bromeliaceae radiations, and C_4 and CAM photosynthesis for the Cyperoidae, the Poaceae and the Bromeliaceae radiations in Poales (Fig. 4; Table 4). The predominance of polymorphic variables among the modulators, and their total absence from the background variables, is consistent with polymorphic variables functioning as diversification drivers.

Radiation modulators, which arise after the start of the radiation, include one extrinsic condition and four intrinsic traits (Table 5). The shift to mountain habitats in *Rhododendron* 1 functions as a modulator (Table 2), which implies that the ancestrally low-SLA leaves and the shrub habit may have been preadaptations for colonizing mountain systems. Some of the traits might constitute further adaptations to the environment, and as such can be seen to 'fine-tune' an adaptive radiation. This is illustrated by the CAM photosynthesis and absorptive trichomes in Bromeliaceae, allowing the plants to deal with short-term dry habitats (Givnish *et al.*, 2014; Silvestro *et al.*, 2014). A comparable situation is found in the Poaceae. Both cold tolerance, which was possibly the key to the north-temperate radiation of the Pooideae (Edwards & Smith, 2010), and C_4 photosynthesis (Edwards & Smith, 2010; Spriggs *et al.*, 2014) evolved as modulators after the radiation of the spikelet clade. The evolution of such modulators is important to maintain the radiation, and generate a set of nested radiations which give the impression of a single large radiation.

We did not take into account the complexity of traits. Traits with many different states, such as the different shapes of floral spurs, may also be effective in stimulating diversification. Other traits that may stimulate diversification could include mechanisms that allow more precise mate recognition, such as the dichromatism in cichlids (Wagner *et al.*, 2012), and so limit gene flow. Polyploidy in plants (Soltis *et al.*, 2009) and chromosomal

evolution caused by diffuse centromeres in Cyperaceae (Givnish *et al.*, 1999) may act as drivers of post-mating reproductive barriers and therefore may stimulate diversification. Flowers and their rich diversity of form are often linked to more precise pollen transfer and so indirect specific mate recognition (van der Niet & Johnson, 2012). Diversification may simply be a side effect of more space being available. If this is the case, then climate change creating extensive open habitat may also have been the speciation mechanism in many of the radiations we documented – for example the *Betula* radiation into the extensive cold northern Late Miocene habitats and the grass and Cyperoideae radiations into the new open seasonally dry pyrophytic Late Miocene habitats. The montane radiations in Ericaceae may have been the result of the fragmented montane habitats and the steeper divergent selective gradients. Testing such hypotheses remains quite challenging.

It would be difficult to assemble the complete list of traits and environmental conditions that influence radiations, as this requires an exhaustive search through trait-space. The method we used here starts with prior hypotheses of potential traits, which are then tested. Consequently, it is not surprising if more detailed studies, such as that of Givnish *et al.* (2014), find additional traits.

Conclusions

Classifying the variables into three attributes allowed us to explicitly distinguish the roles of extrinsic conditions and intrinsic traits in a radiation. First, we were able to test the validity of the Simpsonian model (extrinsic variables set up the ecological opportunity and intrinsic traits start and modulate the radiation). Secondly, this framework allowed us to infer diversification scenarios. Finally, this protocol may be relevant for identifying variables (extrinsic vs intrinsic) and testing whether backgrounds, triggers and modulators were selected. It is very likely that not all variables linked to the radiations were sampled in our study and this could, for example, account for the lack of extrinsic conditions acting as backgrounds in Fagales. Our novel classification permits the *a posteriori* selection of variables that may play roles as backgrounds, triggers and/or modulators in the radiation of a group of organisms.

Using the paradigm that variables linked to radiations can be sorted into three classes leads to a much better insight into the conditions and traits that might be contributing to radiations. Contrary to expectations, we show that both extrinsic conditions and intrinsic traits are involved in setting up the background conditions for radiations to take place as well as triggering them, and possibly modulating ongoing radiations. Finally, diversification drivers can be recognized by always being polymorphic. The triggers of radiations can be decomposed into types, timing and roles and this could enhance our understanding of the processes that lead to the generation of the extraordinary diversity of organisms.

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Supporting Information

Additional supporting information may be found in the online version of this article.

Fig. S1 Posterior probability distributions for Ericaceae, Fagales and Poales in the best BiSSE model.

Fig. S2 Temporal classification of the variables for Ericaceae, Fagales and Poales following the protocol described in the Materials and Methods section and in Fig. 1.

Methods S1 Variable (extrinsic and intrinsic) coding and BiSSE diversification models tested for Ericaceae, Fagales and Poales.

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