

PHYLOGENETIC RELATIONSHIPS AND CHARACTER EVOLUTION ANALYSIS OF SAXIFRAGALES USING A SUPERMATRIX APPROACH¹

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- *Premise of the study:* We sought novel evolutionary insights for the highly diverse Saxifragales by constructing a large phylogenetic tree encompassing 36.8% of the species-level biodiversity.
- *Methods:* We built a phylogenetic tree for 909 species of Saxifragales and used this hypothesis to examine character evolution for annual or perennial habit, woody or herbaceous habit, ovary position, petal number, carpel number, and stamen to petal ratio. We employed likelihood approaches to investigate the effect of habit and life history on speciation and extinction within this clade.
- *Key results:* Two major shifts occurred from a woody ancestor to the herbaceous habit, with multiple secondary changes from herbaceous to woody. Transitions among superior, subinferior, and inferior ovaries appear equiprobable. A major increase in petal number is correlated with a large increase in carpel number; these increases have co-occurred multiple times in Crassulaceae. Perennial or woody lineages have higher rates of speciation than annual or herbaceous ones, but higher probabilities of extinction offset these differences. Hence, net diversification rates are highest for annual, herbaceous lineages and lowest for woody perennials. The shift from annuality to perenniality in herbaceous taxa is frequent. Conversely, woody perennial lineages to woody annual transitions are infrequent; if they occur, the woody annual state is left immediately.
- *Conclusions:* The large tree provides new insights into character evolution that are not obvious with smaller trees. Our results indicate that in some cases the evolution of angiosperms might be conditioned by constraints that have been so far overlooked.

Key words: ancestral state reconstruction; diversification analyses; large phylogenies; Saxifragales.

Recent years have seen the increasing use of large phylogenetic trees to investigate general patterns of character evolution and to test specific hypotheses within a more exhaustive phylogenetic context (see Smith et al., 2011). The use of large phylogenies with near comprehensive, species-level sampling has provided novel evolutionary insights into the relationship between rates of molecular evolution and growth form in plants (Smith and Donoghue, 2008), the diversification of the mammalian crown group (Bininda-Emonds et al., 2007), the evolution of plant lineages possessing C₄ photosynthesis (Edwards and Smith, 2010), and clade-specific responses to climate change (Edwards et al., 2007; Willis et al., 2008). Large phylogenetic

trees representing plant communities also have been used to identify biodiversity hotspots (Forest et al., 2007) and to examine factors driving community assembly (Cavender-Bares et al., 2006). The use of trees with many hundreds to thousands of exemplars contrasts with long-used approaches that rely on a much smaller framework of representative taxa for the study of particular groups of interest.

The eudicots (= *Eudicotyledonae*; sensu Cantino et al., 2007) comprise over 70% of all angiosperm species (Drinnan et al., 1994). Whereas early-diverging or basal eudicots exhibit high variability in floral form, the origin of the core eudicots (*Gunneridae*, and in particular the *Pentapetalae*) corresponds with the appearance of highly synorganized flowers (Endress, 1987, 1994; Soltis et al., 2005). For example, floral merosity becomes largely fixed in the core eudicots (four- or five-merous; reviewed by Soltis et al., 2005). In fact, major core eudicot clades show largely invariant patterns in floral form (e.g., *Asteridae* with two fused carpels, inferior ovary, five-merous flowers. However, some core eudicot subclades exhibit extensive floral variation, as well as considerable variation in other characters. This pattern of variation raises the question of the extent to which floral morphology is modifiable and contingent on the variation of other traits.

Besides the evolution of the floral plan, another longstanding question in the evolution of the angiosperms is the influence of life form components such as longevity and woodiness on the speciation and extinction of plant lineages. The most widespread

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hypotheses are that growth form changes usually involve the evolution of herbaceousness from woodiness (Dodd et al., 1999) and that short-lived lineages are more diverse, partly due to their shorter generation time (Givnish, 2010). However, these hypotheses remain to be tested within a group that is sufficiently diverse, both morphologically and taxonomically. One such clade is Saxifragales.

Although Saxifragales, with an estimated 2470 species, is a relatively small angiosperm order, it is highly diverse morphologically, including trees, shrubs, lianas, annual and perennial herbs, succulents, and aquatics. Flowers also show considerable variation in sepal, petal, stamen, and carpel number, as well as ovary position (Cronquist, 1981; Takhtajan, 1997; Gustafsson and Albert, 1999; reviewed in Soltis et al., 2005). General patterns of character evolution in Saxifragales have been examined using summary phylogenetic trees with one or a few exemplars per family (e.g., Gustafsson and Albert, 1999; Soltis et al., 2005; Carlswald et al., 2011). However, these reconstructions do not capture the extent and patterns of variation within diverse families and most genera. To examine better the patterns of variation in Saxifragales, we constructed a phylogenetic hypothesis of 950 taxa by synthesizing the available sequence data from Saxifragales and several closely related taxa.

As background, the order Saxifragales is a member of the large *Superrosidae* clade, where it is sister to *Rosidae* and represents part of a well-documented early diversification of the angiosperms (Magallón et al., 1999; Moore et al., 2007, 2010; Smith et al., 2010; Soltis et al., 2011; Wang et al., 2009). Saxifragales sensu APG III (2009) include 16 families: Altingiaceae, Cercidiphyllaceae, Crassulaceae, Daphniphyllaceae, Grossulariaceae, Haloragaceae sensu lato (including Tetracarpaceae, Penthoraceae, and Aphanopetalaceae), Hamamelidaceae, Iteaceae, Paeoniaceae, Pterostemonaceae (sometimes included in Iteaceae), Saxifragaceae, and Peridiscaceae (including Medusandraceae) (Davis and Chase, 2004; Soltis et al., 2007; Wurdack and Davis, 2009). The composition of Saxifragales was a major surprise of molecular systematics, uniting families that were previously circumscribed into three or four traditional subclasses based on morphology. The difficulty of classifying these families together highlights the great morphological diversity encompassed by the clade.

Some molecular analyses have placed the nonphotosynthetic genus *Cynomorium* (Cynomoriaceae) in Saxifragales, albeit with low bootstrap support (Nickrent et al., 2005). However, other analyses have suggested that the genus belongs in Santalales (Jian et al., 2008) or Rosales (Zhang et al., 2009; Moore et al., 2010). Given the uncertain placement of *Cynomorium*, we excluded the genus from this study.

The monophyly of Saxifragales has been well supported by many studies (e.g., Morgan and Soltis, 1993; Soltis et al., 1997a, b, 2000, 2011; Soltis and Soltis, 1997; Hoot et al., 1999). However, resolution of the deep-level relationships within the clade has, until recently, been confounded due to an ancient, rapid radiation. Using 50845 bp of sequence data, Jian et al. (2008) elucidated the framework of relationships in Saxifragales. Peridiscaceae are sister to the rest of Saxifragales. Paeoniaceae are sister to a “woody clade” composed of Cercidiphyllaceae, Daphniphyllaceae, Altingiaceae and Hamamelidaceae. This clade (Paeoniaceae + woody clade) is sister to the rest of Saxifragales, which comprises a well-supported “core Saxifragales”. Two subclades compose core Saxifragales: Crassulaceae + Haloragaceae s.l. (including *Aphanopetalum*, Tetracarpaceae, Penthoraceae, and Haloragaceae), and the Saxifragaceae alliance,

in which Saxifragaceae + Grossulariaceae are sister to Iteaceae + Pterostemonaceae.

Saxifragales provide a good case study to investigate diversification patterns in the angiosperms. Both molecular data and the fossil record suggest that the diversification of Saxifragales was rapid and provide a well-defined time frame for the evolution of the group. Fossils indicate that Saxifragales was once more diverse and widespread than the extant members suggest and that the group encompassed wide phenotypic diversity early in its history (Crane, 1989; Endress, 1989; Hernández-Castillo and Cevallos-Ferriz, 1999; Hermsen et al., 2003, 2006; Pigg et al., 2004). The earliest fossil evidence of Saxifragales is within the Turonian-Campanian, providing a minimum age of 89.5 Myr for the group (Magallón et al., 1999; Hermsen et al., 2006). Molecular estimates based on various angiosperm-wide divergence time estimation approaches, however, suggest older ages of 111 to 121 Myr (Wikström et al., 2001), 114 Myr (stem group; Magallón and Castillo, 2009), and 96–111 Myr or 106–107 Myr (crown group) (Bell et al., 2010). These ages are comparable with those provided by Anderson et al. (2005), with 102–108 Myr for the crown and stem groups, respectively. Molecular dating studies within Saxifragales confirmed that the initial diversification was extremely rapid (Fishbein et al., 2001; Jian et al., 2008). Jian et al. (2008) estimated the origin and subsequent diversification of Saxifragales between 112 (± 9.7) to 120 (± 10.2) Ma, with the major lineages within the clade appearing in as little as 3–6 Ma.

Using a supermatrix approach, we present a phylogenetic tree comprising 909 species of the angiosperm clade Saxifragales (and 41 outgroup taxa), which is a much greater representation of the total biodiversity of this clade than included in previous studies, which had only 40 or fewer exemplars (Fishbein et al., 2001; Fishbein and Soltis, 2004; Jian et al., 2008). We then use this phylogenetic hypothesis to examine character evolution for several traits: life history (annual/perennial); habit (woody/herbaceous); ovary position, petal number, carpel number, and stamen to petal ratio. Additionally, we use the data to investigate how life history and habit influence the tempo evolution in Saxifragales. We explore whether the duration of the life cycle influences diversification, i.e., if annuality is correlated to higher net diversification and whether putative differences in diversification between annual and perennial plants are due to variation in extinction or speciation rates. We also investigate the directionality in the transitions between the different life forms to determine whether the evolution of annual lineages from woody perennials is indeed the predominant trend.

MATERIALS AND METHODS

Nucleotide supermatrix data assembly and gene tree construction—Several families of Saxifragales have been the focus of more targeted phylogenetic analysis; hence, there are many available sequences from Saxifragales taxa (e.g., Sang et al., 1995, 1997; Soltis and Kuzoff, 1995; Mes and Hart, 1996; Soltis et al., 1996a, b, 2001; Mes et al., 1997; Li et al., 1999; Mort et al., 2001, 2005, 2009; Senter and Soltis, 2003; Schultheis and Donoghue, 2004; Goncharova et al., 2006; Ickert-Bond and Wen, 2006; Magallón, 2007; Moody and Les, 2007; Pan et al., 2007; Carrillo-Reyes et al., 2008). We downloaded all available core nucleotide sequences for Saxifragales and selected outgroups (*Gunnera*, *Platanus*, *Trochodendron*, and *Vitaceae*) from GenBank. We performed an all-by-all pairwise BLAST search for the sequences using *blastn*, with significant BLAST hits defined as those having a maximum e-value of 1.0×10^{-10} , with $\geq 50\%$ overlap of both the target and query sequences. We then identified groups of putative homologs by forming clusters of sequences that all had a significant BLAST hit with at least one other sequence in the cluster. We

later identified the clusters based on notation in GenBank and kept those from plastid, mitochondrial, and nuclear loci that had been used for phylogenetic analyses (e.g., ITS and 18S), and discarded any others. From each remaining cluster, we pruned sequences that did not have a proper species name, removed any subspecies designations, and removed all but one sequence per species (keeping the longest sequence, or, if the sequences were the same length, randomly selecting a sequence).

Next, we discarded any clusters that had sequences from fewer than 10 species. The remaining clusters were aligned using the program MUSCLE (Edgar, 2004), and the alignments were manually checked and adjusted. Clusters lacking parsimony informative sites were discarded. We checked for erroneous sequences by building trees from each of these clusters using maximum likelihood (ML), implemented with the program RAXML (Stamatakis, 2006). These topologies were visually examined and sequences resulting in unambiguously spurious placement of taxa were removed. We repeated this error-checking protocol until no anomalies could be identified. We stress, however, that these removed sequences are not necessarily “bad” or erroneous, but that these unusual placements could instead represent alignment issues, gene/taxon sampling issues, and also that these preliminary tree searches were not thorough.

Supermatrix analysis—We concatenated the pruned gene clusters into a character matrix (Appendix S1, see Supplemental Data with the online version of this article), making sure there was taxonomic overlap between each cluster and at least one other cluster. In total, the supermatrix has 950 species (909 Saxifragales and 41 outgroup species) and is 48 465 characters in length. The supermatrix contains 2 379 996 nucleotides; thus, it is 5.17% filled. Each species contains between 227 and 26 846 nucleotides (mean = 2505, median = 1472). The supermatrix was made by concatenating 51 gene alignments with a total of 2762 sequences; the number of sequences per gene alignment ranges from 10 to 423 sequences. The matrix is available in the Dryad Repository (doi:10.5061/dryad.h4070) and TreeBase (http://treebase.org, submission 13934).

Phylogenetic analysis—We performed ML phylogenetic analysis on the full supermatrix alignment using the program RAXML-VI-Light 1.0.5 (Stamatakis, 2006). The starting tree for the ML search was obtained using a fast maximum parsimony heuristic implemented in RAXML 7.2.8. The ML analysis used the GTRCAT nucleotide substitution model, with the default settings for the optimization of individual per-site substitution rates. To assess uncertainty in the topology and branch length estimates, we also ran 200 nonparametric bootstrap replicates on the original data set. Bootstrap data sets were generated with a Perl script, and the ML analyses of the bootstrap data sets followed the same protocol as the original data set. The ML and bootstrap trees are available on Dryad (doi:10.5061/dryad.h4070) and TreeBase (http://treebase.org, submission 13934).

The ML and Bayesian character reconstruction methods used here require ultrametric trees. Thus, we transformed the optimal ML estimate and all bootstrap trees to chronograms using penalized likelihood (PL; Sanderson, 2002), implemented in the program r8s version 1.71 (Sanderson, 2003). We used a smoothing parameter of 10 000, which was chosen based on cross-validation of the fossil constraints. For the PL analysis, we used most of the same time constraints as Jian et al. (2008). Briefly, crown group Hamamelidaceae were assigned a minimum age of 84 Ma and a maximum age of 86 Ma (Zhou et al., 2001); *Divisestylus* was constrained between 89.5 and 93.5 Ma; *Cercidiphyllum* was constrained between 65 and 71 Ma (Crane, 1984; reviewed in Magallón et al., 1999); and crown Altingiaceae was constrained between 88.5 and 90.4 Ma (Zhou et al., 2001).

Character state coding—Carlsward et al. (2011) conducted the first analysis explicitly to assess the evolution of 65 morphological characters across Saxifragales. However, that study relied on the Jian et al. (2008) tree, which was well resolved and supported, but included only 28 taxa with a few exemplars per family.

In the present study, six features were scored: (1) habit (woody/herbaceous), (2) life history (annual, biennial, perennial), (3) ovary position (superior, subinferior, inferior), (4) carpel number, (5) petal number, and (6) stamen to petal ratio. The states for these and other background information are provided below (see also data sets in Dryad, doi:10.5061/dryad.h4070). States were scored from the literature. Prominent data sources included floras: Flora of North America (www.efloras.org/flora_page.aspx?flora_id=1), Flora of China (www.efloras.org/flora_page.aspx?flora_id=2), Flora of New Zealand (Moore and Edgar, 1970), Flora of Australia (www.environment.gov.au/biodiversity/

abrs/online-resources/flora/main/), and Flora of Japan (www.foj.c.u-tokyo.ac.jp/gbif/; Ohwi, 1984). Additional sources of information included family treatments in Kubitzki (1990 onward), Carlsward et al. (2011), Magallón (2007), and Eggli (2005).

The scoring for most characters, including life history and stamen, sepal, and petal number, was generally straightforward. However, scoring other characters such as ovary position was more complex, as discussed later. Information on life history was difficult to obtain for some members of Haloragaceae (e.g., *Haloragis*), in which both annual and perennial species are reported and the boundary between these states may not be sharp. Also, petal and stamen number were both variable and difficult to score for Paeoniaceae. Different sources sometimes reported different numbers, again reflecting the variability in floral organ number that is well known in the family (e.g., Kubitzki, 1990 onward; Angiosperm Phylogeny Website, http://www.mobot.org/MOBOT/research/APweb/; Stevens, 2001 onward). Both petal and carpel number also varied in some Crassulaceae, with some taxa having higher values for these characters than observed in many other members of Saxifragales (e.g., over 10 petals or 5 carpels).

Habit was scored as either woody (0) or herbaceous (1). Woody here is used in a functional sense. As a working definition, “woody” can be defined as exhibiting conspicuous secondary growth. Hence, trees and shrubs were considered woody. However, for many taxa, this information is not available. We realize that differentiating between these two states (woody/herbaceous) is sometimes not easy. Many herbaceous plants can become woody if given proper conditions. Generally, habit was easy to score for most species, but a few taxa were problematic. For example, some species of *Haloragis* have been variously considered herbaceous or woody, and they were scored as polymorphic. Several species of *Saxifraga* (a largely herbaceous genus) are large plants that develop very thick stems that are considered woody near the base (Webb and Gornall, 1989). For our purposes, to be considered woody, a plant had to be considered in the literature as a shrub or tree, or it had to be stated clearly in the literature that a stem had a vascular cambium and secondary growth. Hence, the several *Saxifraga* species with large basal rosettes and a woody stem apex (e.g., “stems woody at base,” p. 111, or “Stem very short, erect, woody...,” p. 114 [Webb and Gornall, 1989]) were scored as woody. Similarly, some Crassulaceae (a largely herbaceous family of typically rosette-forming plants) have secondary growth and are considered woody. For example, *Cotyledon* consists exclusively of woody perennials (Van Jaarsveld, 2003), and the woody condition is quite common in *Aeonium* (Mes and Hart, 1996; Mort et al., 2007), *Tylecodon* (Van Jaarsveld, 2003), *Crassula* (Tölken, 1985), *Kalanchoe* (Descoings, 2003), and *Graptopetalum* (Van Thiede, 2003). Several other genera, such as *Aichryson* (Bramwell and Bramwell, 1990) and *Sedum* also contain several woody species.

Whereas ovary position is stable in some Saxifragales (e.g., Crassulaceae), it is highly variable in other members of the clade and is especially labile in Saxifragaceae (see Gustafsson and Albert, 1999; Mort and Soltis, 1999; Kuzoff et al., 2001; Soltis et al., 2005). Ovary position was scored as superior (0), inferior (1), and subinferior (2), but we acknowledge that is an oversimplification of states for several reasons. There is in Saxifragales, particularly in Saxifragaceae, a continuum of ovary positions from what appears to the eye to be completely superior to those that are completely inferior. Inferior was used here to describe ovaries considered completely inferior, and superior was used for those that appear to the eye to be completely superior; the remaining ovaries having various degrees of “inferiority” (e.g., 1/4, 1/2, 3/4) were all considered subinferior (2).

Adding complexity to the consideration of ovary position is the fact that detailed developmental studies reveal that all ovaries in Saxifragaceae are ontogenetically “inferior” in that they develop from what is termed an appendicular ground plan (Kuzoff et al., 2001; Soltis et al., 2003). From this ground plan, various degrees of ovary inferiority as well as ovaries that appear completely superior (termed “pseudosuperior”) can be generated via differential expansion of that portion of the ovary that is above the point of attachment of the hypanthium vs. that part that is below (see Kuzoff et al., 2001; Soltis et al., 2003). This same developmental mechanism also operates elsewhere in the Saxifragales (e.g., Hamamelidaceae, Penthoraceae; M. Fishbein, D. Soltis, L. Hufford, unpublished data). However, for our purposes only the degree of apparent ovary “superiority” vs. “inferiority” is important—we are trying to examine the morphological variation that is present across a large natural group regardless of the developmental mechanism that has generated this variation.

Stamen to petal ratio has long been used as a character in systematics (see Stevens, 1991; Penneys and Judd, 2005). We also scored stamen to petal ratio as either less than 1 (0), equal to 1 (1), equal to 2 (2), or greater than 2 (3). Most taxa have ratios = 1 or 2. Some taxa have clearly lost all petals, and in those

cases, we used a stamen to sepal ratio (sepal number typically = petal number) so as not to use a petal number of 0 and bias the results (with rare exceptions, petal number equals sepal number when both are present).

The given characters were used to construct a character matrix. Two initial versions of this matrix were produced. In one version, polymorphisms were scored and retained (file deposited in Dryad, doi:10.5061/dryad.h4070). In another version of this data set, rather than including polymorphisms, only the most frequent state was chosen and employed (file deposited in Dryad). In one analysis, taxa that were polymorphic were simply removed for that character.

Character state reconstruction—We examined character evolution using the supermatrix-based tree described above. Ancestral character state reconstruction was employed using maximum parsimony (MP), maximum likelihood (ML), and stochastic (Bayesian) mapping options in the program Mesquite (Maddison and Maddison, 2011).

Maximum likelihood reconstructions were also conducted using the ace (or ACE; ancestral character estimations) function of the ape R package (Paradis et al., 2004; R Development Core Team, 2010). The r8s analysis used to make the ML tree ultrametric collapsed some branches that were estimated to have zero length, but ace requires dichotomous trees to reconstruct the ancestral states. Therefore, we generated 100 alternative topologies by transforming all polytomies present in the best ultrametric ML tree into randomly resolved dichotomies with branches of length zero using the multi2di function in ape. Ancestral character estimations (ace) were then performed on all of these 100 alternative trees. For every character, we tested two alternative models, the ER (equal rates; assuming equal transition rates among the different character states) and the ARD (all-rates-different; transitions among the different character states allowed to vary freely) models. In every case, we selected the model that exhibited a better fit to the data based on a likelihood ratio test. When likelihood difference between models was not significant, we selected the ER model to avoid over-parameterization.

On the basis of the results of previous focused analyses of subclades within Saxifragaceae, several species did not appear in the expected phylogenetic position in the ML supermatrix analyses (based on the results of previous focused analyses of subclades within Saxifragaceae). For example, *Boykinia intermedia*, *Heuchera hirsutissima*, and *Saxifraga stolonifera* were not placed with congeneric taxa. We conducted reconstructions with these taxa as they appeared in the ML tree, and we also moved the branches of these terminals in Mesquite and attached each at position with close relatives based on previous analyses (i.e., with *Boykinia elata*, *Heuchera rubescens*, and *Saxifraga fortunei*, respectively) and repeated the ML reconstructions (trees not shown).

BayesTraits analyses—To test for a correlation between woodiness/herbaceousness and perenniality/annuality, we used the discrete model in Bayes-Traits (Pagel, 1994; Pagel and Meade, 2006). We used a reversible jump Markov chain Monte Carlo (MCMC) approach to integrate across transition models, including ones that treated the two traits as dependent or independent. Taxa missing a state for any of the characters were removed, leaving a tree of 945 taxa. This was done on both the set of bootstrap trees and the single best likelihood tree in separate runs. All runs used an exponential hyper-prior seeded from a (0, 30) uniform distribution. The size of attempted moves in a Bayes-Traits run is adjusted by a “rate deviation” parameter. This can affect how well a search mixes. We tried values of 0.1, 1, 2, 5, 10, 20, and 40. The runs were sampled every thousandth generation. Multiple runs were tried with each rate deviation parameter and ran from 10 million to 100 million generations. We used the heidel.diag function of coda (Plummer et al., 2006), which implements the test of Heidelberger and Welch (1983), to assess the stationarity of the transition parameters. The burn-in for a given run was taken as the maximum of 5 million generations or the maximum estimated burn-in from this function for each parameter (and in practice, was always 5 million, though the heidel.diag function returned an error in 11% of cases—note that this was a numerical issue, not the same as a diagnosis of lack of stationarity).

Trait-dependent diversification analyses—We also conducted analyses to determine whether any of several life form characteristics (woodiness or herbaceousness, perenniality or annuality) is associated with higher speciation or extinction rates. We realize that estimating extinction rates from phylogenetic trees of only extant taxa may be problematic (Rabosky, 2010); these analyses must therefore be viewed with caution. We first treated the traits as binary characters (woody vs. herbaceous; annual vs. perennial). If binary character states are associated with different rates of diversification, this can affect estimates of the transition rates between character states and also ancestral state

reconstructions (Maddison et al., 2007). The character-associated diversification rates and the transition rates between the different states were jointly estimated with the BiSSE model (Binary State Speciation and Extinction; Maddison et al., 2007). BiSSE estimates six parameters: the speciation rates of lineages in states 0 and 1 (λ_0 and λ_1 , respectively), the extinction rates of lineages in states 0 and 1 (μ_0 and μ_1 , respectively), and the transition rates from 0 to 1 ($q_0 \rightarrow q_1$) and 1 to 0 ($q_1 \rightarrow q_0$). Given that the tree did not have complete taxonomic sampling, these analyses used the skeletal tree approach (FitzJohn et al., 2009), implemented in the R package diversitree (version 0.9-1; FitzJohn et al., 2009; FitzJohn et al., 2012) using both ML and Bayesian inference. We accounted for uncertainty in the Bayesian approach by estimating posterior distributions separately for each of the 100 dichotomized trees. MCMC chains were started at the mean of the prior distribution and run for 10000 steps with exponential prior distributions. Chain convergence was verified using the R package coda (Plummer et al., 2006). All chains converged within the first 1000 generations. However, to be conservative, we discarded the first 2500 steps of every chain and concatenated the last 7500 steps for each tree together to form the posterior probability distributions.

After performing the initial BiSSE analyses, we tested several hypotheses regarding the evolution of perenniality and woodiness in the Saxifragales by comparing the likelihood and AIC (Akaike information criterion; Akaike, 1974) of the full model with those of models in which certain parameters were constrained to specific values. The different hypotheses to test were determined based on the values of the full model and the results of the ace analyses. For instance, we tested whether a certain trait state was associated with higher extinction rates by constraining a model to have equal extinction rates ($\mu_1 = \mu_0$). Models were compared based on their fit to the data (as indicated by the AIC values) using the function anova in diversitree, which also indicates whether the difference in fit is significant or not. Using this approach, we tested whether: (1) the transition from perenniality to annuality was rare or insignificant; (2) different habits are associated with different speciation and/or extinction rates.

Since the states of the two traits are not independent (i.e., annual woody plants are absent in our data set), we also combined the two traits in a single three-state character with values 1 = woody & perennial; 2 = herbaceous & perennial; 3 = herbaceous & annual. We used MuSSE (Multiple State Speciation and Extinction, the generalization of BiSSE for characters with more than two states available in diversitree; FitzJohn et al., 2012) to test (1) if the direct transition rate between perennial woody and annual life forms is greater than zero and (2) the most likely habit state of the Saxifragales most recent common ancestor (MRCA). Because all the dichotomized trees resulted in identical parameters in the initial BiSSE full model analyses, all hypotheses were contrasted using a single randomly selected dichotomous topology.

RESULTS

Supermatrix analyses: Phylogenetic relationships—The relationships revealed in the 950-taxon supermatrix ML phylogenetic tree (Appendix S1; see Supplemental Data with the online version of this article) largely agree with those obtained in previous analyses employing far fewer taxa (e.g., Fishbein et al., 2001; Jian et al., 2008). Peridiscaceae are sister to all other Saxifragales. Within the remaining Saxifragales, Paeniaceae + the woody clade are then sister to “core Saxifragales.” Core Saxifragales comprise Grossulariaceae, Iteaceae, Pterostemonaceae, and Saxifragaceae (the Saxifragaceae alliance) as sister to Crassulaceae + Haloragaceae s.l. Within the woody clade, relationships also agree with those of Jian et al. (2008), with Altingiaceae sister to Hamamelidaceae + Cercidiphyllaceae + Daphniphyllaceae. Similarly, within Saxifragaceae alliance, we also recovered Grossulariaceae + Saxifragaceae sister to Pterostemonaceae + Iteaceae.

Fine-scale relationships also match closely those reported in studies focused on individual families, or subclades within families, such as Altingiaceae (Ickert-Bond and Wen, 2006), Crassulaceae (Mes and Hart, 1996; Mes et al., 1997; Mort et al., 2001, 2005, 2009; Carrillo-Reyes et al., 2008), Grossulariaceae (Senters and Soltis, 2003; Schultheis and Donoghue, 2004), Haloragaceae (Moody and Les, 2007), Hamamelidaceae

(Magallón, 2007), Peridiscaceae (Soltis et al., 2007), Paeoniaceae (Sang et al., 1995, 1997), and Saxifragaceae (Soltis and Kuzoff, 1995; Soltis et al., 1996a, b, 2001). As noted already, however, several species of Saxifragaceae did not appear in the expected phylogenetic position in the ML supermatrix analyses based on the results of previous focused analyses: *Boykinia intermedia*, *Heuchera hirsutissima*, and *Saxifraga stolonifera*.

Overall, bootstrap support for most relationships was generally strong, but lower than the values for the ML tree in Jian et al. (2008; most relationships received 100% bootstrap [BS] support in that study). This is not surprising, however, given the large number of taxa included here compared to Jian et al. (2008), which employed only 28 exemplars. Furthermore, deep-level relationships within Saxifragales had been uncertain due to rapid radiation, and numerous base pairs (>20 000) were ultimately required to resolve those relationships with strong internal support (Jian et al., 2008).

In the present study, the deepest relationships were only weakly supported. Examples include the monophyly of Saxifragales (BS = 74%), all Saxifragaceae following Peridiscaceae (BS = 56%), the woody clade (Altingiaceae, CERCIDIOPHYLLACEAE, DAPHNIPHYLLACEAE, and Hamamelidaceae) + Paeoniaceae (BS = 70%), and core Saxifragales (BS = 71.5%). Most other deep-level relationships were moderately or well supported, including the monophyly of the woody clade (BS = 94.5%), Saxifragaceae alliance (BS = 90.5%), and Crassulaceae + Haloragaceae s.l. (BS = 94%). The monophyly of most families of Saxifragales was also moderately or well supported: Crassulaceae (BS = 96.5%), Hamamelidaceae (BS = 66%), Grossulariaceae (BS = 90.5%), Haloragaceae s.l. (BS = 98%), Saxifragaceae (BS = 86%). The monophyly of many major subclades within families was also generally well-supported, including those within Saxifragaceae: *Saxifraga* s.s. (BS = 76%), *Heuchera* group (BS = 95%), and *Micranthes* (BS = 100%), and those within Crassulaceae: *Kalanchoe* (BS = 90%), the GAMA clade (i.e., *Greenovia*, *Aeonium*, *Monanthes*, *Aichryson*; we have retained use of this clade name, although *Greenovia* has been included in *Aeonium*, Mes and Hart, 1996; BS = 68%), and the clade of *Aeonium*, *Greenovia*, and *Monanthes* (BS = 79%).

Character state reconstruction—Because of space limitations in displaying these large trees, one simplified ML reconstruction for one character (ovary position) is shown in Fig. 1 as an example of what we did. Additional more detailed ML reconstructions for all characters are provided in Appendix S2 (a–f). The MP, ML, and Bayesian character state reconstructions generally yielded very similar results. We provide examples of the types of differences observed. For habit, the MP reconstruction shows the ancestor of Haloragaceae, as well as the ancestor of Paeoniaceae to be equivocal, but with ML the ancestral state for each is herbaceous. For ovary position, the MP reconstructions indicate that the ancestor of two large clades (Saxifragaceae + Grossulariaceae) and also ((Saxifragaceae + Grossulariaceae) + Iteaceae) is equivocal, but with ML the ancestor is reconstructed in each case as superior. Similarly (for ovary position), with MP the ancestor of the (Penthoraceae + Haloragaceae) clade had a superior ovary, but an inferior ovary is more probable based on the ML reconstruction. For carpel number, the MP reconstruction shows the ancestral state for the clade (Crassulaceae + Haloragaceae), as well as for several subclades within Crassulaceae, as equivocal, whereas in all cases, the ML reconstruction indicated that the

ancestors had five carpels. A complete list of differences can be obtained from the authors (Appendix S3).

In Appendix S4 (a–f) we compare reconstructions using MP with polymorphisms, MP with no polymorphisms (with the most common state scored), ML, and Bayesian, in a zoomable format. Appendix S5 (a–f) provides the MP reconstruction (no polymorphisms, most common state scored) in a zoomable circle tree format with states in different colors. Modifying the topology to place the several taxa that had surprising placements in the supermatrix analysis (as noted earlier) in the phylogenetic position that would be expected based on studies focused on individual subclades also had little influence on the character state reconstructions and no impact on the major conclusions (figures not shown; available on request).

To assess the impact of polymorphic character states, we compared MP reconstructions with polymorphisms included and excluded (picking the most common state), and this also had little impact on the overall reconstruction. When polymorphisms were removed there was less ambiguity in the reconstruction of some ancestral states, but our major inferences were not impacted. We also deleted those taxa with polymorphic characters and conducted another ML reconstruction; that too had no impact on the overall results summarized below.

BayesTraits analyses—The analyses to test for a correlation between woodiness/herbaceousness and perenniality/annuality using the bootstrap trees spent 94% of the time on just one tree, and after the first few thousand generations, had extremely low acceptance probabilities, despite the range of rate deviations attempted. The analyses based on the single ML tree had far better mixing (usually lower than the ideal 20%, but all with a median acceptance probability >5%). The 355 017 samples after burn-in represent over 355 million generations from 17 independent runs. The posterior probability of the dependent model was 100.0%: no independent models were sampled after burn-in. There were 1629 models sampled in the search, but one made up over 41% of the posterior probability and had just two free transition parameters. Models with two free transition parameters made up 93.5% of the posterior probability, and models with three free transition parameters made up 5.9% of the posterior probability; no post burn-in model had more than four free transition parameters even though up to eight were possible.

The models showed a high transition rate from the woody + annual combination to other trait combinations (up to 7400 times the slowest transition rate between other traits). This strong transition bias against the woody + annual trait is expected given the absence of woody + annual species. The model deals with lack of observed taxa in this state by inferring that the transition rate from this state is extremely high. Rather than absolute rates, one can focus on how long the process spends in each state once it arrives and where it goes next. The expected amount of time spent in each of the three observed state combinations (woody + perennial, herbaceous + perennial, and herbaceous + annual) before leaving were 2882, 2210, and 1883 times, respectively, the amount of time spent in woody + annual. Transition rates from herbaceous + perennial to herbaceous + annual and to woody + perennial are similar (0.487 vs. 0.513); transitions from either of those states to the herbaceous + perennial rather than to woody + annual happened about 2/3 of the time based on the rates (Appendix S6 and S7). Together, these suggest that the predominant pattern of evolution involves woody + perennial ↔ herbaceous + perennial ↔ herbaceous + annual

ACE of Ovary position
Different Transition Rates

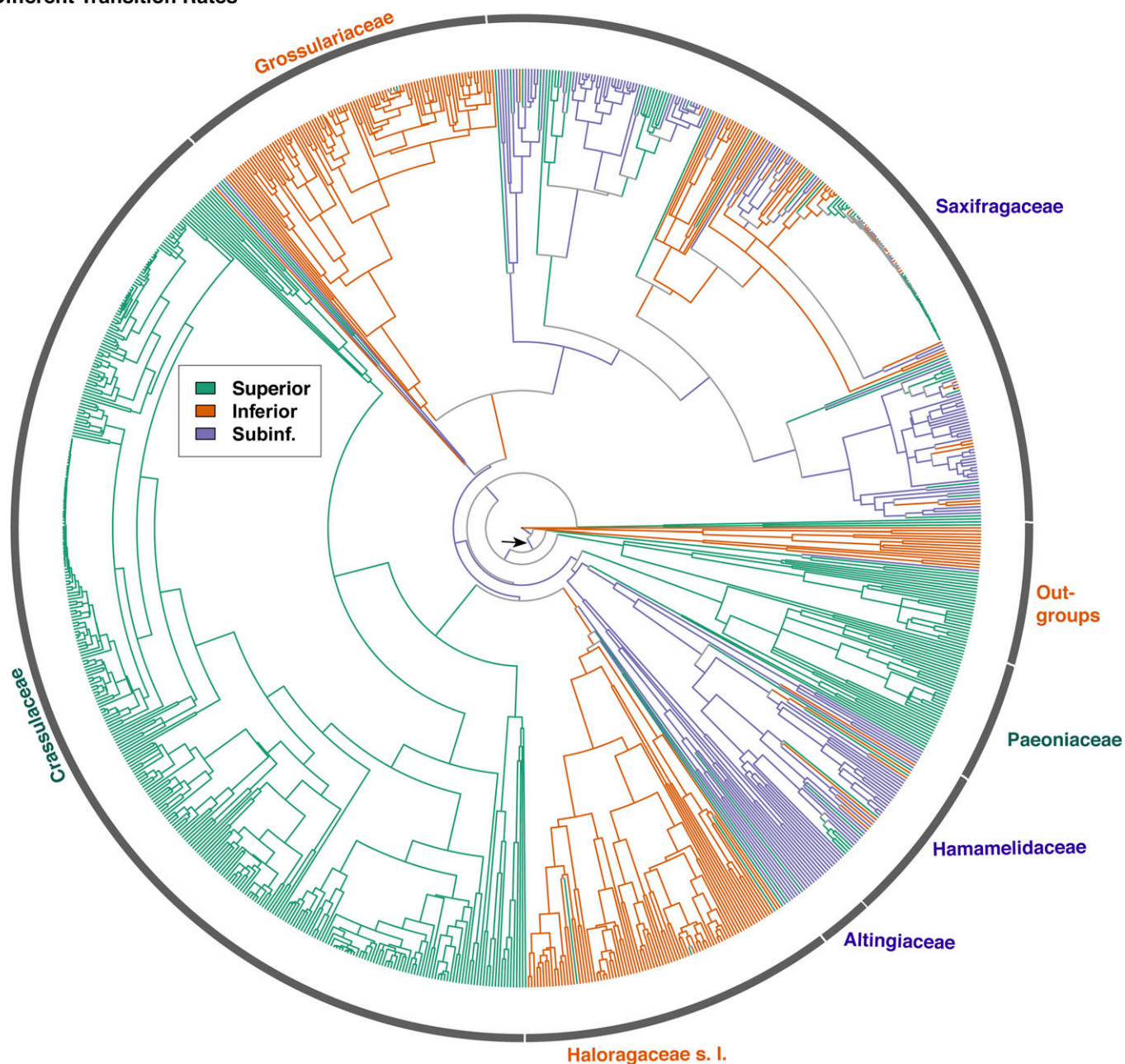


Fig. 1. Simplified maximum likelihood reconstruction of alternative ancestral states for ovary position (see Appendix S2 for a more detailed reconstruction of this and the other characters examined). Arrow designates branch to ingroup. Note that while ovary position is very stable in many families (e.g., Crassulaceae, Grossulariaceae, Haloragaceae), it is highly labile in others (Hamamelidaceae, Saxifragaceae).

transitions, with few or no transitions to woody + annual, and any possible woody + annual taxa changing states almost immediately (Fig. 2).

Diversification analyses—Both life history and growth form influenced speciation and diversification rates. Full-model BiSSE analyses (i.e., models in which all parameters were allowed to vary) indicated that woody lineages had lower speciation (λ) and extinction (μ) rates than herbaceous taxa, which

resulted in lower net diversification ($r = \lambda - \mu$) rates (Fig. 3, Table 1). Models with equal speciation or extinction rates for woody and herbaceous lineages had a significantly worse fit to the data than the full model (Table 2). Conversely, annuals had significantly higher net diversification (r) than perennials due to their significantly lower extinction rates; models with equal extinction rates (μ) for annuals and perennials had a significantly poorer fit to the data than models in which μ was allowed to vary freely, which always resulted in μ annual \ll μ perennial.

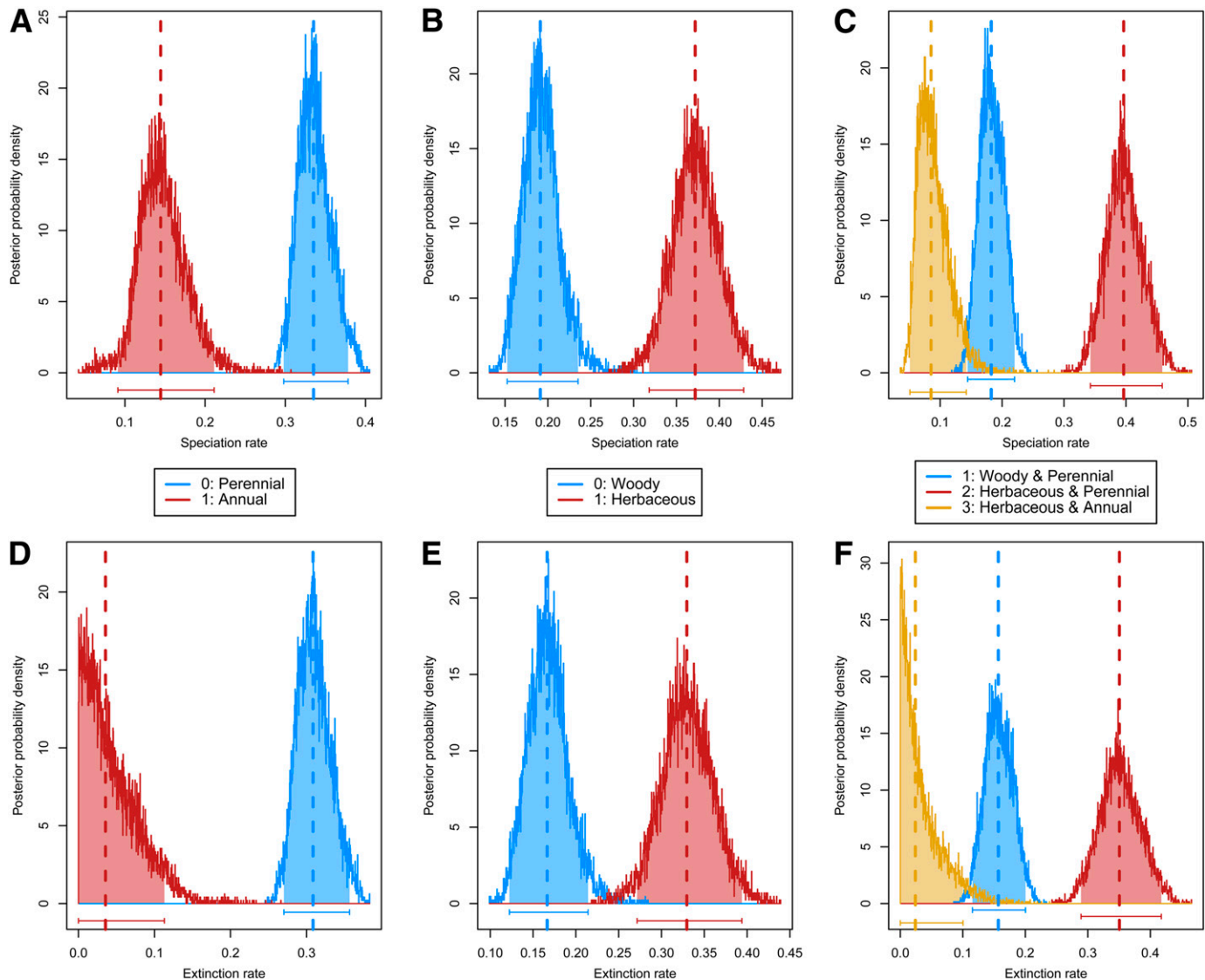


Fig. 2. Diversification rates in annual, perennial, herbaceous, and woody lineages. The plots represent posterior probability distributions for rates of speciation (λ) and extinction (μ). Each distribution represents the posterior distribution obtained from the final 7500 steps in MCMC BiSSE or MuSSE analyses. Dashed lines represent the median value of each distribution. Ninety-five percent credibility intervals are shown as whiskered lines beneath each curve. All rates represent the number of transitions expected along a branch equal in length to the depth of the tree. (A) and (D) annual vs. perennial lineages; (B) and (E) herbaceous vs. woody lineages; (C) and (F) MuSSE results comparing the rates of herbaceous perennials, woody perennial, and herbaceous annuals.

Additionally, BiSSE analyses showed that transitions to perenniality appear to be unidirectional; the transition rate from perennial to annual \ll the rate from annual to perennial (Fig. 3; Table 1A, single trait analysis). Moreover, when the transition rate from perennial to annual was set to 0, the resulting model had a significantly better fit than the full unconstrained model (i.e., allowing for all transitions), as indicated by the AIC and a likelihood ratio test (Table 2A, single trait analysis for perenniality).

The analyses that combined woodiness and annuality in a single three-state character (1: woody & perennial; 2: herbaceous & perennial; 3: annual) indicated that herbaceous annuals are indeed significantly more prone to have higher net diversification rates, which is mostly attributable to a significantly lower extinction rate (Fig. 2; Table 1A, combined trait analysis). The

direct transition between a woody perennial and an annual habit was further ruled out by the multistate analysis performed with MuSSE. The direct transition rate between woody and perennial and annual was ~ 0 (Table 1B) and models in which either or both of the transitions from woody and perennial \rightarrow annual (q_{13}) and from annual \rightarrow woody and perennial (q_{31}) were forced to be 0 had similar fit to the data (as indicated by the AIC value) as the full model (Table 2B). The multistate composite trait was also used to try to elucidate the most likely state of the Saxifragales MRCA (Table 2B). These analyses indicated that an annual, herbaceous MRCA had the lowest likelihood, but it could not be rejected. Moreover, the fit of models with root = woody was almost identical to the fit of models with root = perennial herbaceous (Table 2B, combined trait analysis).

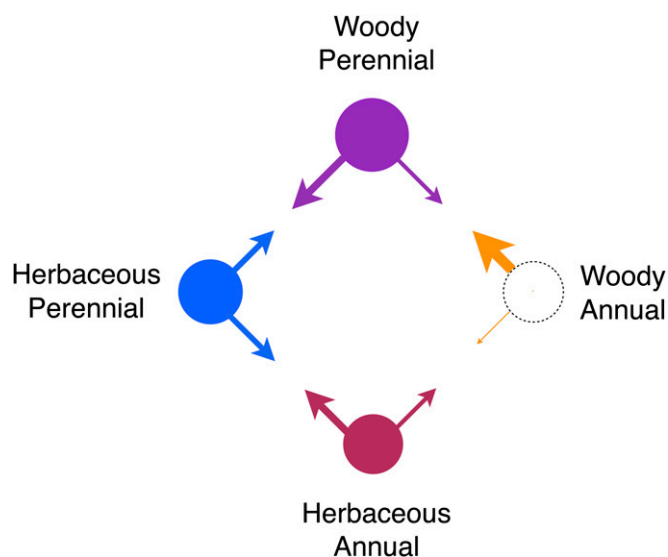


Fig. 3. Summary of transitions among woody + perennial, herbaceous + perennial, herbaceous + annual, and woody + annual based on BayesTraits analyses. The circle area represents the “stickiness” of each state combination—that is, the amount of time spent in that area before going to another state. The actual circle for woody + annual is actually very small (only one pixel wide, so it is surrounded by a dotted line); taxa going into that state (woody + annual) transition out very quickly. Arrow thickness represents the frequency of a given transition. For example, woody + perennial goes toward herbaceous + perennial about twice as often as it goes toward woody + annual (but if it does that, it bounces back almost immediately). The basic conclusion is that transitions have mostly gone between the three observed combinations, but there are not dramatic differences in rates between those, but that woody + annual is at best a transient state. Horizontal and vertical arrows are left out because we forbid two traits to change in the exact same instant of time. This is the standard assumption for such methods but it also makes sense, at least for traits that are not linked strictly together by epistasis.

DISCUSSION

Supermatrix analyses: Phylogenetic relationships—The framework of relationships agrees with those reported by Jian et al. (2008). Peridiscaceae are sister to all other Saxifragales. Paeoniaceae plus a woody clade (comprising Cercidiphyllaceae, Daphniphyllaceae, Hamamelidaceae, and Altingiaceae) are then sister to remaining, or “core Saxifragales.” Core Saxifragales consist of Grossulariaceae, Iteaceae, Pterostemonaceae, and Saxifragaceae (the Saxifragaceae alliance) as sister to Crassulaceae plus Haloragaceae s.l. Within the woody clade, Altingiaceae is sister to Hamamelidaceae plus Cercidiphyllaceae + Daphniphyllaceae. Within the Saxifragaceae alliance, we also recovered Grossulariaceae + Saxifragaceae as sister to Pterostemonaceae + Iteaceae. Relationships within families of Saxifragales also closely agree with earlier studies focused on those clades.

Character state reconstruction—We realize that our results are only as good as the data and models that we have applied. The models applied here likely greatly simplify the true processes of evolution (e.g., Wickett et al., 2011), and we also may be limited by the information contained in single, discrete characters sampled only from extant taxa. By employing these models and applying them to very large phylogenetic trees, we have obtained a series of well-supported results (hypotheses) that we

TABLE 1. Diversification and transition rates for life form characters. (A) Values for the mean and 95% credibility interval of diversification rates for annual, perennial, herbaceous, and woody lineages and rates of evolutionary transitions between the different states. All values obtained with BiSSE. (B) Values for the mean and 95% credibility interval of diversification and transition rates obtained with MuSSE after combining both sets of traits as a single multistate character. rx : diversification rate associated with character state x , computed as $rx = \lambda x - \mu x$, where λx is the speciation rate and μx the extinction rate associated with character state x ; qxy : transition rate from character state x to character state y .

A) Single trait analysis	Woodiness (0: woody; 1: herbaceous)		Annuality (0: perennial; 1: annual)	
	Rate	Mean CI	Mean CI	CI
$r0$	0.025	$\pm 7.15 \times 10^{-05}$	0.027	$\pm 6.03 \times 10^{-05}$
$r1$	0.042	$\pm 8.08 \times 10^{-05}$	0.103	$\pm 3.33 \times 10^{-04}$
$q01$	0.006	$\pm 2.67 \times 10^{-05}$	<0.001	$\pm 5.77 \times 10^{-06}$
$q10$	0.005	$\pm 1.62 \times 10^{-05}$	0.101	$\pm 3.08 \times 10^{-04}$
B) Combined trait analysis (1: woody & perennial; 2: herbaceous & perennial; 3: annual)				
Rate	Mean	CI		
$r1$	0.026	$\pm 7.32 \times 10^{-05}$		
$r2$	0.046	$\pm 9.71 \times 10^{-05}$		
$r3$	0.056	$\pm 2.92 \times 10^{-04}$		
$q12$	0.004	$\pm 2.71 \times 10^{-05}$		
$q13$	<0.0001	$\pm 2.78 \times 10^{-06}$		
$q23$	0.003	$\pm 1.49 \times 10^{-05}$		
$q31$	0.002	$\pm 4.66 \times 10^{-05}$		
$q32$	0.068	$\pm 2.75 \times 10^{-04}$		

hope will be further tested by other researchers. Investigators have noted the importance of nonrandom taxon sampling when using trees to make evolutionary inference (see Heath et al., 2008), but that impact of taxon sampling remains unclear (e.g., Li et al., 2008). While nonrandom sampling may be a concern, we note that in our broad analysis of Saxifragales, our sampling of major subclades is fairly even—we have sampled roughly half of each of the major families in Saxifragales. We hope that these efforts to examine character state reconstruction over large trees for Saxifragales will stimulate other investigators to apply similar studies of character state evolution using large trees in other clades.

Habit—On the basis of the MP reconstructions, the ancestral state for Saxifragales is woody. Maximum likelihood as implemented in Mesquite also suggests a woody ancestor (98%), and the Bayesian inference also indicates a woody ancestor. The woody state is found in outgroups (e.g., Vitaceae, Platanaceae) and in Peridiscaceae, which are sister to the rest of the Saxifragales. In addition, the woody clade (Altingiaceae, Cercidiphyllaceae, Daphniphyllaceae, and Hamamelidaceae) is reconstructed as ancestrally woody. The woody habit also appears to be the ancestral state for Grossulariaceae.

However, the analysis that accounted for character-specific diversification rates did not support this result unambiguously. According to the results obtained with MuSSE, both a woody and a herbaceous perennial MRCA are possible for the Saxifragales. This ambiguity is probably caused by some diverse lineages, in which several transitions between woodiness and herbaceousness appear to have occurred early in the history of the group. For instance, Paeoniaceae (which are primarily herbaceous) are sister to the woody clade, and the ancestral state

TABLE 2. Model comparison for different diversification scenarios. The Akaike information criterion (AIC) of every model is shown. Lower AIC values indicate a better fit of the model to the data. *Model with a fit significantly different from that of the full model.

Model	Model comparison (AIC values)	
	Woodiness (0: woody; 1: herbaceous)	Perenniality (0 Perennial; 1 Annual)
A) Single trait analysis		
Full model	7509.8	7073
No transition ($q_{01} \sim 0$)	7641*	7013.6*
Equal speciation rates	7530.6*	7076.9*
Equal extinction rates	7523.9*	7078.3*
B) Combined trait analysis		
	1: woody & perennial; 2: herbaceous & perennial; 3: annual	
Full model	7690.1	
Root state = 1	7690.3	
Root state = 2	7689.6	
Root state = 3	7690.4	
$q_{13} \sim 0$	7688.2	
$q_{31} \sim 0$	7689.4	
$q_{13} \sim 0 + q_{31} \sim 0$	7687.5	
Equal speciation for perennials ($\lambda_1 = \lambda_2$)	7718.3*	
Equal speciation for herbaceous ($\lambda_2 = \lambda_3$)	7700.2*	

Notes: Constraints included the impossibility of certain character transitions ($q_{xy} \sim 0$; q_{xy} : transition rate from character state x to character state y) and given character states at the root node (root state = x). (A) Single trait and (B) combined trait analyses are presented. *Model with a fit significantly different from that of the full model.

for the family is unclear. A woody subclade of *Paeonia* species was recovered (*P. rockii*, *P. lutea*, *P. delavayi*, *P. suffruticosa*), but this subclade is an early-branching lineage that is not sister to the rest of Paeoniaceae. *Paeonia kavachensis* is derived from within an herbaceous clade and is therefore shown here to be secondarily woody.

On the basis of our MP and some ML reconstructions, we propose the following scenario. Starting with a woody ancestor for Saxifragales, there have been several major changes to the herbaceous habit—the ancestors of Crassulaceae and Saxifragaceae were herbaceous, and these represent independent transitions to the herbaceous habit. A shift to the herbaceous habit also occurred at some point close to the base of the Haloragaceae-Penthoraceae clade, although the exact position of this shift is unclear. *Tetracarpaea* and *Aphanopetalum* are woody and sister to the rest of that clade; the next branching members include taxa that are herbaceous (*Penthorum*) and some that are woody (e.g., *Haloragodendron* species). Starting with *Haloragis* and *Proserpinaca*, Haloragaceae are ancestrally herbaceous.

There were also multiple reversions to the woody habit in herbaceous clades (Crassulaceae, Saxifragaceae). There are numerous cases of these reversions in Crassulaceae. Some are individual, scattered cases (e.g., *Thompsonella*, *Sedum corynephyllum*), but others involve small clades, and the reversions could be considered potential synapomorphies (e.g., *Aichryson tortuosum* and *A. bethencourtianum*; a subclade of *Graptopetalum paraguayense*, *G. bernalense*, *G. mendozae*, *G. amethystinum*). In other cases, the evolution of habit is more complex. Within *Aeonium*, there have been multiple transitions from herbaceous ancestors to small clades that are woody, but with some

transitions back to the herbaceous habit. Distinct (separate) examples of secondary transitions to herbaceous habit include *A. vestitum*, *A. simsii*, and *A. nobile*.

Life history—Beginning from a perennial ancestor, there have been multiple shifts to the annual habit. Results obtained with the diversification analyses indicate that transitions from perenniality to the annual habit are rare, but that annual lineages diversify at a greater rate, mostly due to the higher propensity of extinction of perennial lineages. This pattern results in few but speciose annual groups among extant taxa, in some cases with a seemingly long history of annuality (e.g., *Crassula* spp.). The reverse transition (from annual to perennial) seems to be relatively more frequent, but leaves a weaker signal among extant taxa because perennial lineages tend to go extinct at a higher rate than annuals. Therefore, transitions to annuality tend to lead to the radiation of annual lineages that only rarely produce perennial descendants. For example, in *Aichryson*, from an ancestor that was perennial there has been a shift to the annual habit and then in a few cases a return to the perennial habit.

Ovary position—Much of the Saxifragales is stable in terms of ovary position (superior for many clades), but both Saxifragaceae and Hamamelidaceae are more labile with frequent transitions among the three positions scored (inferior, subinferior, inferior) (Fig. 1). Attesting to this dynamic pattern, it is noteworthy that nearly all ovary position transitions occur at an equal frequency (that is, from inferior to subinferior and the reverse; from superior to subinferior and the reverse; and inferior directly to superior) (Appendix S8), with only direct transitions from superior to inferior half as frequent as the above.

Ovary position evolution is particularly dynamic in Saxifragaceae. For example, the *Heuchera* group had a subinferior ancestor with multiple transitions to a superior and inferior ovary. In contrast, *Micranthes* appears to have had a superior ovary as the ancestral state, but with transitions to both subinferior and completely inferior ovaries. *Saxifraga* also exhibits highly dynamic ovary position evolution with numerous transitions. Some genera are largely invariant and highly stable. For example, *Chrysosplenium* species mostly have inferior ovaries, but with a few transitions to superior. Hamamelidaceae are similar to Saxifragaceae, but with less extensive variation; our reconstructions indicate that from a subinferior ancestor both inferior and superior ovaries have evolved. Even other families that are highly stable show some transitions in ovary position. Almost all species of *Ribes* (Grossulariaceae) possess an inferior ovary, but there are several transitions to a superior ovary.

Carpel number—The ancestral carpel number in Saxifragales is reconstructed as two. This number typifies Vitaceae, the closest outgroup, as well as the woody clade, and is maintained in major subclades such as Saxifragaceae and Grossulariaceae. Several prominent increases in carpel number occur, and these often characterize major clades and can serve as potential synapomorphies in some cases. For example, there is an increase to a variable number of carpels in Paeoniaceae. There is a change to four carpels in Haloragaceae s.l. (Haloragaceae s.s., plus Aphanopetalaceae, Tetracarpaeaceae, Penthoraceae)—all of these taxa are typified by four carpels with a further increase to five carpels in Penthoraceae. There is also an increase to four carpels in Peridiscaceae. An increase to five carpels occurred in the ancestor of Crassulaceae. Although five carpels

characterize nearly all members of this family, there is a decrease to four carpels in the large genus *Kalanchoe*—this carpel number serves as a synapomorphy for that genus.

An increase in carpel number above five is also seen in some Crassulaceae; in fact, the transition from five carpels to 6–10 carpels is the most frequent change in carpel number across the tree, without reversal (see Appendix S7). Furthermore, this increase in carpel number is seen only in taxa that also exhibit an increase in stamen number (above the typical five). The ancestral character state for a subclade of Crassulaceae that is largely endemic to Macaronesia (i.e., the GAMA clade) is 8–12 carpels. However, within the Macaronesian clade, the *Aeonium* subclade shows a further increase, with most taxa having flowers with 12–32 carpels. There are other examples of individual taxa or smaller clades that exhibit an increase in carpels in Crassulaceae, including *Pseudosedum longidentatum*, *Sedum sarcalosum*, *S. pubescens*, *S. palmeri*, *S. sedoides*, and *S. sediforme*. Within Crassulaceae, a small clade of species of *Sempervivum* and *Jovibarba* also shows an increase to 8–16 carpels. Hence, there have been multiple increases in carpel number across the Crassulaceae phylogeny, and reconstructing the ancestral state for the clade is difficult, as was found for petal number. This correlation in petal and carpel number is discussed next.

Petal number—The ancestral number of five petals has been retained in most Saxifragales, but there have been a few major shifts that characterize clades. Three transitions in petal number are roughly equally common in Saxifragales (all other stamen transition types rare or absent): 5 to 0; 5 to 4; 5 to 6–10 (see Appendix S7). An increase in petal number above the typical five is seen in Paeoniaceae and also in multiple cases in Crassulaceae. In fact, the same Crassulaceae showing an increase in stamen number (above) exhibit an increase in petal number (compare d and e in Appendices S2, S4, and S5). The GAMA clade is characterized by 6–10 petals (a putative synapomorphy); within the GAMA clade, a subclade of *Greenovia* and *Aeonium* species shows a further increase to more than 10 petals, which again represents a potential synapomorphy. Other Crassulaceae also exhibit independent increases to 6–10 or more petals, including: *Pseudosedum longidentatum*, *Sedum sarcalosum*, *S. pubescens*, *S. palmeri*, *S. sedoides*, and *S. sediforme*; a small clade of species of *Sempervivum* and *Jovibarba*.

A transition from five to four petals has occurred multiple times, with major shifts to four exemplified by Haloragaceae s.s. and the large genus *Kalanchoe* (Crassulaceae); these shifts are also accompanied by a change in carpel number to four.

The complete loss of petals also defines some clades in Saxifragales. *Cercidiphyllum* + *Daphniphyllum*, *Chrysosplenium*, and *Altingia* are examples (although the support for a sister group of *Cercidiphyllum* + *Daphniphyllum* is not high in most studies). In addition, two distinct subclades within Hamamelidaceae have lost petals: (1) *Fothergilla*, *Distylium*, *Sycopsis*, *Distyliopsis*, *Shaniodendron*, *Parrotia*, and *Parratiopsis*; (2) *Molinodendron* and *Sinowilsonia*.

Stamen to petal ratio—A 1× stamen to petal ratio is reconstructed as ancestral for Saxifragales. Changes from this condition are apparent synapomorphies for several clades. For example, a ratio of >2× characterizes one large clade: the woody clade plus Paeoniaceae. Within Hamamelidaceae, there is a major shift to a ratio of 1×, but multiple shifts to 2× (labile). A ratio

of 2× is found in most Crassulaceae, but *Crassula* has a ratio of 1×—an apparent synapomorphy for that subclade.

There has been an overall reduction in the stamen to petal ratio through the evolution of Saxifragales, although several more dramatic reductions have occurred independently multiple times in the Crassulaceae and other families such as Haloragaceae, Grossulariaceae and Saxifragaceae. This trend can readily be seen in examination of the ancestral state reconstruction (Appendix S2, f and Appendix S5, f). Peridiscaceae, most members of the woody clades, and Paeoniaceae have high stamen to petal ratios (>2), whereas the ratio is lower in most other families. In every case, this decrease seems to be independent of a decrease in petal number; therefore it is the number of stamens that has decreased in Saxifragales.

Character correlations—The correlation between some characters was apparent from visual inspection of the reconstructions. Crassulaceae provide the best example with multiple independent examples of a correlated increase in petal and carpel number. For example, an increase in petal number above the typical five (seen in most Saxifragales) to 10 or more is seen in the GAMA clade; members of this group also exhibit an increase to 8–12 carpels. Within GAMA, the *Aeonium* + *Greenovia* subclade shows further increases with most taxa having flowers with more than 10 petals and 12–32 carpels. Other examples of individual taxa or smaller clades that exhibit concomitant increases in carpel and petal numbers include *Pseudosedum longidentatum*, *Sedum* spp.; *Sempervivum* spp. and *Jovibarba* spp.

However, not all increases in petal number or carpel number are correlated. *Lithophragma* (Saxifragaceae) is characterized by an increase in carpel number from two to three, but the petal number does not have a corresponding increase (it remains as five).

Habit evolution and diversification—Whether a lineage consists of woody or herbaceous or annual or perennial taxa seems to have a strong effect on its diversification patterns. According to the results obtained with diversitree, both woodiness and perenniality are associated with lower net diversification rates, although through different mechanisms. Woody lineages appear to have lower speciation rates than herbaceous taxa, which results in lower net diversification in spite of also having lower extinction rates compared to herbaceous taxa (Fig. 2). Conversely, perenniality results in higher speciation rates, but this habit is also associated with higher rates of extinction. Annual species appear to have a significantly lower extinction rate, which leads to higher net diversification. This result was verified by combining both habit traits in a single multistate character. The analysis of the resulting multistate trait confirmed that annual lineages of the Saxifragales are less speciose than perennial ones (Fig. 2), whether herbaceous or woody. However, extinction rates of perennial lineages are remarkably high, which results in significantly higher diversification rates for annual lineages in comparison.

The association between annuality and higher diversification rates has been previously described (e.g., Smith and Donoghue, 2008). However, to our knowledge, this is the first time that this higher net diversification has been shown to be due to the lower extinction rates of short-lived taxa. Again, we acknowledge the known issues of estimating extinction rates from molecular phylogenetics based upon extant taxa in the absence of a complete fossil record (Rabosky, 2009; 2010; reviewed in

Quental and Marshall, 2010), and these findings warrant future investigation.

It is unclear at this point why extinction rates appear to be lower in annual taxa, and it will be interesting to determine whether this is the case in other groups as well. However, it is possible that this phenomenon is caused by the intrinsic seasonal tuning of annual plants. In annuals, a significant part of the life cycle is spent in a dormant state as seeds in the soil bank. During this time, seeds can cycle in and out of dormancy, and germination will only occur under very specific sets of conditions (Baskin and Baskin, 1998). This provides a bet-hedging mechanism to buffer environmental variation and maximize geometric fitness (Childs et al., 2010) and ultimately minimize extinction, as has been proposed for other organisms with a dormancy behavior (Liow et al., 2009).

Our diversification analyses also suggested that the transition between annuality and perenniality, when not controlling for other traits, appears to be largely unidirectional in Saxifragales: annuality is much more easily lost than gained. Our analysis actually favored (albeit not significantly) the model in which the transition from perennial to annual is negligible (perennial \rightarrow annual = 0). Figure 3 splits annual and perennial into two states each: annual + woody, annual + herbaceous, perennial + woody, and perennial + herbaceous, at a cost of no longer controlling for diversification. This shows that the time spent in annual + woody once entering that state (node size) is short. According to the inferred model, if a plant evolves that combination, it quickly evolves into a perennial + woody state, and perennial + woody plants are unlikely to evolve into annual + woody. In contrast, perennial + herbaceous and annual + herbaceous evolve into each other at about equal rates (see Appendix S7). Thus, for woody plants, there is a much higher rate of gaining annuality than losing it, but the rates are more similar for herbaceous plants. When ignoring the woody/herbaceous distinction, as in the BiSSE analyses, this results in the observed rate difference.

Evolutionary analyses of groups such as *Castilleja* (Orobanchaceae) appear to indicate that perennial taxa are often derived from annuals (Tank and Olmstead, 2008). Our findings confirm this trend, and although the opposite shift cannot be ruled out, the transition from annual to perennial is the most probable in nearly any lineage. Our results also largely confirm the long-held hypothesis that annual angiosperms are derived from perennial ancestors (e.g., Stebbins, 1965), perhaps because perennial lineages tend to be lost due to their higher extinction rates, thus increasing the relative frequency of annual lineages that derive from perennial ancestors.

A recent study in *Lupinus* (Leguminosae) revealed increased rates of diversification for perennial taxa and the colonization of montane habitats and that both traits were strongly correlated (Drummond et al., 2012). These findings support the idea that annual species are favored in warmer, drier climates, while perennial species are more successful in cooler, wetter climates (e.g., Smith and Beaulieu, 2009). While we did not correlate habitat to life history in this study, it remains a possibility that the differences between perennial and annual lineages found in our analyses may be attributable to other factors.

When combining the characters in a multiple trait analysis, we found that the low likelihood of the perennial to annual transition may be caused by the association between annuality and herbaceousness. All annual taxa are herbaceous. Our model formally forbids simultaneous change in growth form and life history. However, we note that while the model allows transitions

in both directions between woody perennial and herbaceous perennial, and between herbaceous perennial and herbaceous annual, and allows plants to stay in any of those three states for significant amounts of time, it has a low rate of entering woody annual and a very fast rate of evolving a woody perennial life history from that state (Fig. 3). Thus, while transitions between annual and perennial habits may occur for herbaceous plants, entering a woody annual state almost immediately results in a woody perennial state, leading to nearly simultaneous evolution of these two states if an herbaceous annual plant evolves woodiness and thus a strong bias in annual to perennial transitions.

Our analyses for Saxifragales agree with other recent studies in highlighting the value of very large phylogenetic trees in the analysis of diverse evolutionary questions. The large phylogenies used here provide novel insights into diversification and character evolution that are not obvious with smaller trees. Diversification analyses indicate that perennial or woody lineages have higher rates of speciation than do annual or herbaceous lineages. However, higher probabilities of extinction offset these differences. Importantly, to our knowledge, this is the first time that this higher diversification has been shown to be due to the lower extinction rates of short-lived taxa. Moreover, our results have revealed a pattern that reconciles two seemingly opposing hypotheses that would probably not be detectable with smaller-scale phylogenies (e.g., at the genus or family level): that the emergence of perennial lineages from annual ancestors is relatively frequent and that most annual plants derive from perennial ancestors. Although the transition from annual to perennial might occur at a high rate in individual groups, perennial lineages are lost due to extinction. It will be important to determine whether this result characterizes other lineages of plants, as well. Two major shifts in the order are revealed from a woody ancestor to the herbaceous habit, but with multiple secondary changes from herbaceous to woody. Some changes are correlated, which was also not obvious with smaller trees. A major increase in petal number is correlated with a large increase in carpel number—these have co-occurred multiple times in Crassulaceae. Concomitantly, this study also reveals new possible synapomorphies for some clades (e.g., loss of petals; three carpels; annual habit; high petal number (6–10 and >10).

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