FRUIT EVOLUTION AND DIVERSIFICATION IN CAMPANULID ANGIOSPERMS

Jeremy M. Beaulieu[1,2] and Michael J. Donoghue[1]

[1]*Department of Ecology and Evolutionary Biology, Yale University, P.O. Box 208106, New Haven, Connecticut 10620*

[2]*E-mail: jbeaulieu@nimbios.org*

With increases in both the size and scope of phylogenetic trees, we are afforded a renewed opportunity to address long-standing comparative questions, such as whether particular fruit characters account for much of the variation in diversity among flowering plant clades. Studies to date have reported conflicting results, largely as a consequence of taxonomic scale and a reliance on potentially conservative statistical measures. Here we examine a larger and older angiosperm clade, the Campanulidae, and infer the rates of character transitions among the major fruit types, emphasizing the evolution of the achene fruits that are most frequently observed within the group. Our analyses imply that campanulids likely originated bearing capsules, and that all subsequent fruit diversity was derived from various modifications of this dry fruit type. We also found that the preponderance of lineages bearing achenes is a consequence of not only being a fruit type that is somewhat irreversible once it evolves, but one that also seems to have a positive association with diversification rates. Although these results imply the achene fruit type is a significant correlate of diversity patterns observed across campanulids, we conclude that it remains difficult to confidently and directly view this character state as the actual cause of increased diversification rates.

KEY WORDS: Achene, Campanulidae, comparative methods, diversification, flowering plants, fruit type.

Fruits are among the most characteristic and diverse structural features of angiosperms and are critical for the dispersal and establishment of seeds. The ability to disperse longer distances can facilitate speciation through geographic isolation, which has long suggested a possible relationship between different fruit types and diversification patterns (e.g., Herrera 1989; Eriksson and Bremer 1991; Tiffany and Mazer 1995; Dodd et al. 1999; Smith 2001). For instance, fleshy fruits promote seed dispersal by birds and mammals, which can increase the chances of establishing isolated populations, and hence higher rates of speciation. In fact, the repeated evolution of fleshy fruits is considered to have been an important driver of angiosperm diversity during the Late Cretaceous and Early Cenozoic. By contrast, angiosperms with dry fruits tend to broadcast their seeds, mostly through mechanical means, over relatively short distances. However, the elaboration of various structures (e.g., specializations of the calyx) can promote more efficient dispersal of dry fruits by wind and water, thereby increasing the probability of isolation and speciation.

Despite a general interest in the impact of fruit type on angiosperm diversity, conflicting results are common among studies and no clear consensus has emerged. For example, fleshy fruits have been considered a major contributing factor to woody angiosperm diversity in the tropics only (e.g., Eriksson and Bremer 1991; Tiffany and Mazer 1995; Smith 2001), and it has also been noted that high diversity is often associated with dry fruited, herbaceous clades in temperate climates (e.g., Eriksson and Bremer 1991). Some studies have reported no association between species richness and dispersal syndrome (e.g., Ricklefs and Renner 1994; Dodd et al. 1999) and have implicated other factors, such as pollination or life history, as underlying drivers of angiosperm diversity.

Taxonomic scale may be an important factor leading to such conflicting results, as most studies have focused on comparing particular orders or families. Although such comparisons have merit, especially in cases where phylogenetic relationships at lower levels are uncertain, they can also be problematic. For

example, groups that are polymorphic for the fruit type of interest are either discarded from the analysis or are collapsed into clade pairs that represent contrasts between the predominate types. At best, this provides a coarse-grained analysis of the relationship between diversification and traits; at worst such a procedure not only compromises statistical power to detect a significant association even when one actually exists, but also introduces a bias by selectively and systematically excluding data.

Knowledge of relationships among flowering plants has improved greatly over the past decade (e.g., Jansen et al. 2007; Moore et al. 2007; Wang et al. 2009; Tank and Donoghue 2010; Soltis et al. 2011), as have methods for inferring very large phylogenies from sequences deposited in GenBank (e.g., Sanderson et al. 2008; Smith et al. 2009; Goloboff et al. 2009; Burleigh et al. 2011). Such methods make possible the rapid assembly of reasonably well-sampled trees for larger, older, and globally distributed clades. In the meantime, there has also been development of powerful new comparative methods that assess the dependence of speciation and extinction rates on particular character states (Maddison et al. 2007; FitzJohn et al. 2009). These methodological advancements allow us to take a fresh look at whether the evolution of particular fruit types has spurred radiations within angiosperms.

Here we explore fruit evolution within the Campanulidae (campanulids), a predominately herbaceous clade of angiosperms (Beaulieu et al. 2013a) containing some 35,000 species, including the familiar Asteraceae (composites, i.e., sunflowers and their relatives), Apiaceae (umbels, i.e., carrots and their relatives), and the Dipsacales (i.e., honeysuckles and their relatives). The campanulids exhibit a range of dry and fleshy fruits and here we focus on the transition rates among the major fruits types and their component attributes. Of special interest to us are the consequences of evolving an achene fruit—the dry, single-seeded, and indehiscent fruit that is the most common within the group. As we will show, the distribution of the diversity of species with achenes within campanulids is a consequence of both the near irreversibility of this fruit type and its positive association with diversification rate.

Methods and Materials
CAMPANULID PHYLOGENY AND CHARACTER MATRIX

The Campanulidae encompass four major lineages of angiosperms—the Aquifoliales, Asterales, Apiales, and the Dipsacales—as well as a number of smaller clades: Bruniales (Bruniaceae and Columelliaceae), Escalloniaceae *s.l.* (including *Eremosyne*, *Polyosma*, *Tribeles*; Escalloniales sensu Angiosperm Phylogeny Group, 2009), and Paracryphiaceae *s.l.* (including *Quintinia* and *Sphenostemon*; Paracryphiales sensu Angiosperm Phylogeny Group, 2009). We used the large DNA sequence-based phylogeny described in Beaulieu et al. (2013a), which

is based on a maximum likelihood (ML) analysis of a matrix of 12,094 nucleotide sites for 8911 species resulting from a concatenation of 12 genes (11 chloroplast genes and ITS, the nuclear ribosomal Internal Transcribed Spacer region). The sequence matrix was constructed using the procedures described in Smith et al. (2009) and implemented in the program PHLAWD. The inferred tree was converted to an ultrametric tree using the nonparametric dating method implemented in PATHd8 (Britton et al. 2007), with fixed age constraints taken from a more focused Bayesian divergence time analysis of 121 exemplar campanulid taxa (Beaulieu et al. 2013b). The relationships among the major campanulid lineages in this large sequence-based tree are identical to those of the more focused study of Tank and Donoghue (2010). Relationships among less inclusive clades also mirrored the cumulative results of hundreds of published studies focused on included clades (Beaulieu et al. 2012a). Overall, our tree provides a fairly complete synthesis of the knowledge of relationships within campanulids.

The fruits of campanulids, as with most groups, can be categorized as belonging to one of four broadly defined fruit types: drupe, berry, capsule, or achene (see Fig. 1). These categories encompass considerable variation, and the application of these terms can differ somewhat among clades. For example, although the fleshy fruits in the Aquifoliales are typically described as drupes, these are atypical drupes in that they contain more than one seed, each one of which is surrounded by endocarp derived from the inner wall of the ovary. Consequently, the drupes of Aquifoliales (also see *Sambucus* within the Dipsacales) are functionally more similar to the berry fruits in other groups, which also contain multiple seeds, although not surrounded by endocarp tissue. Within the campanulids, the capsule fruit type is also highly variable both in size and in the number of seeds that they contain (one to very many), and split open mechanically (dehisce), either along a septum in the ovary where two carpels meet (septicidal dehiscence) or along the carpel walls that delimit the seed containing locules within the ovary (loculicidal dehiscence). Some fruits that have been described as capsules do not dehisce at all (e.g., *Donatia* within the Stylidiaceae).

To circumvent these terminological issues, we scored the fruits of each of the 8911 species in our tree based on the three characteristics that underlie the definitions of the different fruit types: (A) indehiscent (0) versus dehiscent (1); (B) dry (0) versus fleshy (1); and (C) single-seeded (0) versus containing more than a single seed (1). From this standpoint, capsules are fruits that are dehiscent, dry, and multiseeded. Indehiscent, fleshy fruits are categorized as drupes if single seeded and as berries if multiple seeded (regardless of the presence or absence of endocarp tissue). Achenes are all fruits that, at maturity, are dry, single-seeded, and indehiscent. This includes the "cypselas" of the Asteraceae, the "samaras" of the Cardiopteridaceae, and the indehiscent,
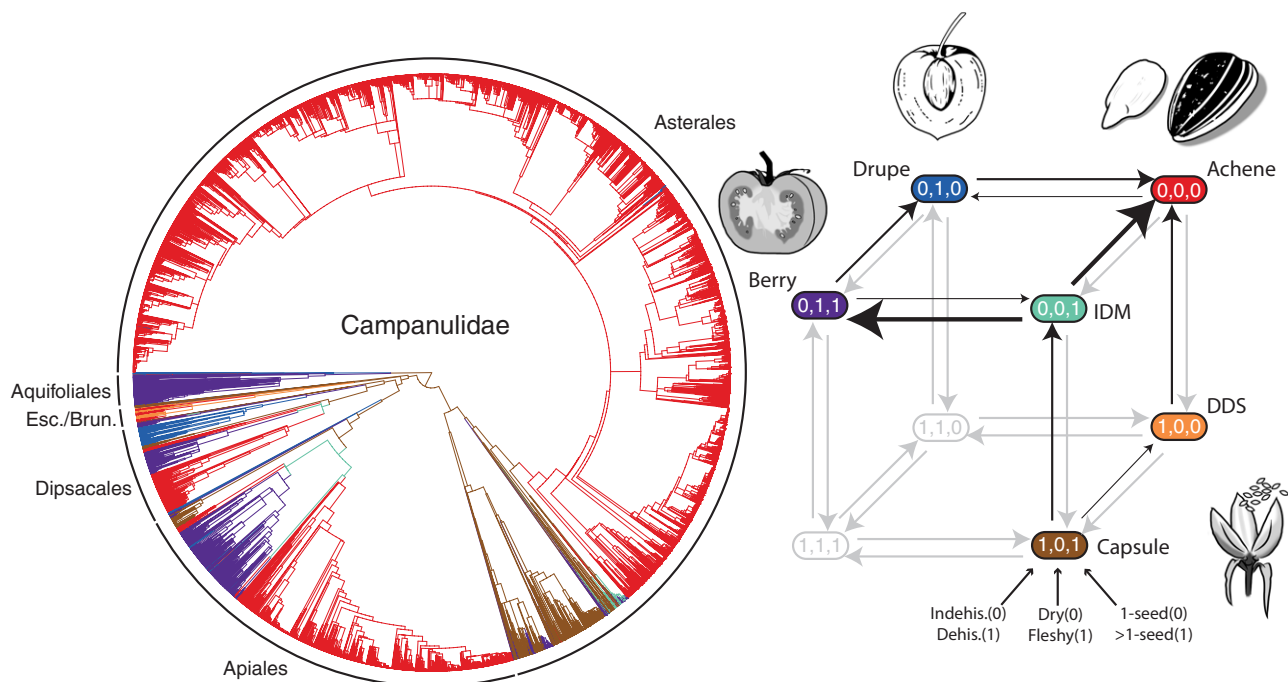
Figure 1. Time-calibrated phylogeny of 8911 species of Campanulidae (campanulid amgiosperms) taken from a maximum likelihood analysis based on a combined analysis of 11 chloroplast genes and one nuclear gene. The major clades of campanulids are labeled; Esc/Brun represents the Escalloniales and the Bruniales. Joint reconstructions of the likeliest fruit type are based on parameters estimated from the correlated paths model, which only allowed single step changes between eight possible combinations of the three binary characters that we used to constitute each fruit type (drawings of generic examples of the four main fruit types—achene [0,0,0], berry [0,1,1], capsule [1,0,1], and drupe [0,1,0]—were provided by Maxwell Rupp). Transitions between the possible combinations of character states are shown, with the thickness of the arrows corresponding to the rates (i.e., thicker arrows denoting higher rates). Transition pathways to and from the two unobserved fruit combinations within campanulids (i.e., [1,1,0] and [1,1,1]) were removed from the model, a priori.

single-seeded "mericarps" of the Apiaceae schizocarp. Beyond these four common forms, we recognized two other rare types of dry fruits that do not have common names. We use "IDM" to refer to fruits that are indehiscent, dry, and multiseeded, as observed within some Campanulaceae, Stylidiaceae, and Goodeniaceae, and we use "DDS" for dehiscent, dry, and single-seeded fruits, as seen in Bruniaceae. We note that the two other conceivable combinations of these three character states (dehiscent, fleshy, and single seeded; dehiscent, fleshy, and multiseeded) were not encountered among campanulids. The scoring of all characters was based on our observations in the field or on herbarium specimens, or on published accounts, including original taxonomic descriptions and floristic treatments.

MODELS OF FRUIT-TYPE EVOLUTION

Fruit evolution was modeled in two distinct ways to assess how differences in coding fruit characters might affect the interpretation. We first constructed a simple multistate likelihood-based model that assumed 30 transition rates between the six fruit types found in campanulids (i.e., drupe, berry, capsule, IDM, DDS, and achene). We refer to this model hereafter as the "multistate" model. The likelihood of this model is defined as being proportional to the probability of the data given a model of evolution, $\mathbf{Q}$, which defines a continuous-time Markov process and the transitions among the various character states. For the "multistate" model, $\mathbf{Q}$ is a 6×6 matrix describing the transition pathways between the different fruit types:

$$\mathbf{Q} = \begin{array}{c} Drupe \\ Berry \\ Capsule \\ IDM \\ DDS \\ Achene \end{array} \begin{bmatrix} - & q_{D \to B} & q_{D \to C} & q_{D \to IDM} & q_{D \to DDS} & q_{D \to A} \\ q_{B \to D} & - & q_{B \to C} & q_{B \to IDM} & q_{B \to DDS} & q_{B \to A} \\ q_{C \to D} & q_{C \to B} & - & q_{C \to IDM} & q_{C \to DDS} & q_{C \to A} \\ q_{IDM \to D} & q_{IDM \to B} & q_{IDM \to C} & - & q_{IDM \to DDS} & q_{IDM \to A} \\ q_{DDS \to D} & q_{DDS \to B} & q_{DDS \to C} & q_{DDS \to IDM} & - & q_{DDS \to A} \\ q_{A \to D} & q_{A \to B} & q_{A \to C} & q_{A \to IDM} & q_{A \to DDS} & - \end{bmatrix}. \quad (1)$$

With the multistate model, transitions between fruit types can (but need not always) occur in a single step. As a consequence, a transition from a berry to an achene ($q_{B \to A}$) can involve the same number of steps as a transition from a drupe to an achene ($q_{D \to A}$). Such an assumption may overly simplify the underlying changes necessary to transition among these fruit types. For example, the transition from a berry to an achene might involve two steps: the loss of fleshiness, followed by a reduction to a single seed, or vice versa. In contrast, a transition from a drupe to an achene would simply require the loss of fleshiness. To account for this, we constructed a second likelihood-based model that only allows single-step changes between the eight possible combinations of the three binary characters that we used to define the fruit types. Thus, for species exhibiting a capsule the observed character combination would be [1,0,1] for fruits that are dehiscent (1), dry (0), and multiple seeded (1), and so on for the other types. The likelihood of this model is the same as above and transitions among all character combinations are defined in the model, **Q**, as:

ever, found in angiosperms. The expression of genes for producing fleshiness can inhibit the expression of genes associated with dehiscence (Seymour et al. 2002), and the lack of fleshy dehiscent fruits may reflect genetic or developmental factors that have limited the possible paths of fruit evolution. Thus, it might be argued that such unobserved character combinations were never possible within campanulids for some genetic or developmental reasons. Of course, it could be that such fruit types once existed within campanulids but that they have not survived in any modern species. Perhaps such fruits are at a selective disadvantage, and there were very rapid transitions to other, more viable, character combinations. For this study, we removed the unobserved states from the correlated paths model completely, which we acknowledge has the obvious drawback of an a priori commitment to one interpretation over the other. However, we remain skeptical that inferring very rapid transitions to and from an unobserved character combination represents anything other than a methodological artifact of including them in the model.

$$
\mathbf{Q} =
\begin{array}{c}
000 \\ 100 \\ 010 \\ 001 \\ 110 \\ 101 \\ 011 \\ 111
\end{array}
\left[
\begin{array}{cccccccc}
- & q_{000\to100} & q_{000\to010} & q_{000\to001} & 0 & 0 & 0 & 0 \\
q_{100\to000} & - & 0 & 0 & q_{100\to110} & q_{100\to101} & 0 & 0 \\
q_{010\to000} & 0 & - & 0 & q_{010\to110} & 0 & q_{010\to011} & 0 \\
q_{001\to000} & 0 & 0 & - & 0 & q_{001\to101} & q_{010\to011} & 0 \\
0 & q_{110\to100} & q_{110\to010} & 0 & - & 0 & 0 & q_{110\to111} \\
0 & q_{101\to100} & 0 & q_{101\to001} & 0 & - & 0 & q_{101\to111} \\
0 & 0 & q_{011\to010} & q_{011\to001} & 0 & 0 & - & q_{110\to111} \\
0 & 0 & 0 & 0 & q_{111\to110} & q_{111\to101} & q_{111\to011} & -
\end{array}
\right]. \quad (2)
$$

Disallowing simultaneous changes in any two or three of the binary characters sets the paths among the different character combinations. For example, the transition rate going directly from a berry [0,1,1] to an achene [0,0,0] is set to 0 to ensure that these transitions must first either pass through one of two possible intermediate fruit types ([0,1,0] or [0,0,1]). This model is essentially a generalization of Pagel's model of binary character correlation (Pagel 1994), and we refer to it hereafter as the "correlated path" model.

We note that under a continuous-time Markov process the estimates of the transition rates among all possible character configurations are largely reflective of the observed state frequencies at the tips. Thus, when including transition pathways going to and from any unobserved character combination, the inferred rate estimates will always be extremely rapid—the only way a state will be unobserved is by being highly transitory. However, it will not always be clear whether there is any real biological meaning that can be attributed to these estimates. Here we are referring to the two fleshy/dehiscent combinations of character states (i.e., [1,1,0] and [1,1,1]) that are not seen in any extant campanulids. These fleshy/dehiscent combinations are rarely, if

We also note that both the multistate and correlated paths models implicitly assume that diversification rates are independent of the fruit type. As shown by Maddison (2006), methods can be misled if this assumption is not true, and the models of fruit type evolution described earlier are no exception. We initially attempted to use the multistate implementation (MuSSE; FitzJohn 2012) of the BiSSE model (Binary State Speciation and Extinction; Maddison et al. 2007) to jointly estimate transition rates and speciation and extinction, but we were unable to obtain meaningful results on a data set of this magnitude. Although we acknowledge potential issues when traits of interest are associated with differential diversification rates (see below), in our particular case we analyzed patterns of fruit evolution to generate specific hypotheses about fruit types that could then be tested explicitly (see "The macroevolutionary consequences of achene fruits").

For the multistate and correlated path models, Akaike's information criterion (AIC; Akaike 1974) was calculated from the log-likelihood to compare the fit of two models. The log-likelihood is equivalent to the probability of observing the states at the tips summed over the probability that the root is in each character

state. The first model assumed that transition rates among states are the same (i.e., equal rates model), whereas the second model assumed that all transition rates are different (i.e., all rates free model). For the correlated paths model, we also tested the fit of a model that assumed that the three traits evolved independently of one another.

The approximate branch and bound algorithm of Pupko et al. (2000, 2002) was used to assign the likeliest ancestral states to each internal node. These joint estimates are the set of ancestral states assigned to all nodes that jointly maximize the likelihood given the observed tip states and parameter estimates in **Q**. The algorithm works by first calculating the likelihood of each observed state given the transition probabilities in **Q**, then traversing the tree from the tips down to the root computing the likelihoods of all possible character states at each node, and only retaining states that produce the highest likelihood. The algorithm then traverses the tree from root to tip assigning each internal node the optimal character state. All analyses were carried out using the *corDISC* and *rayDISC* functions contained within the R package *corHMM* (Beaulieu et al. 2013a).

THE MACROEVOLUTIONARY CONSEQUENCES OF ACHENE FRUITS

Across campanulids, more than 80% of species exhibit the inde-hiscent, dry, and single-seeded character combination ([0,0,0]), or the achene fruit type. Several different processes could account for the predominance in this observed character frequency. The relative rarity of non-achene fruits within the campanulids could simply be the product of asymmetric transition rates: character transition toward an achene may be much more likely than the reverse, in which case the relative rarity of non-achenes is expected. However, the disparity in observed frequencies between achene and non-achene species could just as easily be explained by differences in the diversification process: clades composed of achenes may generally have higher net diversification rates as a consequence of increased speciation rates and/or decreased extinction rates. There is also the possibility that both asymmetric transition rates and differences in diversification work together to account for the overabundance of achene fruits. Traditional methods that estimate transition rates, such as the ones described earlier, implicitly assume that the diversification process is uniform among character states and, therefore, do not provide adequate means for disentangling the two processes (Maddison, 2006). We used the BiSSE model (Maddison et al. 2007) to jointly estimate state-dependent speciation and extinction rates and transition rates between non-achene (0) and achene (1) character states. We also assessed the generality of the results obtained from the full data set by fitting separate BiSSE models to the Asterales, Apiales, and the Dipsacales—the three major campanulid clades that comprise a majority of the achene-bearing species. In all analyses, we cor-

rected for sampling by incorporating the sampling frequency of non-achene (54.4%) and achene (33.1%) states contained within our tree (FitzJohn et al. 2009).

Our BiSSE model contained six parameters: two speciation rates (λ_0 and λ_1), two extinction rates (μ_0 and μ_1), and the two transition rates for going to ($q_{0\rightarrow1}$) and from ($q_{1\rightarrow0}$) the achene state. We conducted an ML search using the subplex optimization routine to obtain estimates of the parameters. As described elsewhere (Beaulieu et al. 2012b; Beaulieu et al. 2013a), the underlying second-order derivatives of this likelihood search, contained within the Hessian matrix, can be used as a means of calculating an approximate standard error of a parameter estimate. The variance–covariance matrix of the six parameters in our BiSSE model is computed by first inverting the Hessian matrix; the approximate standard errors are the square roots of the diagonals of this matrix.

It is possible for the standard errors of the speciation and extinction rates to be much wider if the parameters are correlated with one another. For example, a decrease in speciation rate (e.g., λ_0) has a similar effect as an increase in the extinction rate (e.g., μ_0): less extant species diversity. It is possible to have a ridge of nearly equal likelihood, where changing both the speciation rate and the extinction rate results in moving along the ridge, making estimates of speciation and extinction highly correlated and indistinguishable from one another; univariate standard errors would not capture this effect (Beaulieu et al. 2012b). We investigated the "distinguishability" of these parameters by also conducting a Bayesian analysis. Specifically, we assessed the correlation between the posterior distributions of the speciation and extinction parameters in our BiSSE model using Markov chain Monte Carlo (MCMC) methods. The prior followed an exponential distribution with a mean of 2*r*, where *r* is the net diversification rate that would produce our 8911 species tree (see FitzJohn et al. 2009). The Markov chain was run for 10,000 steps, with the first 2500 steps removed as burnin, resulting in 7500 samples comprising the marginal posterior distribution for each of the parameters. All analyses described earlier were carried out in the R package *diversitree* (FitzJohn et al. 2009; FitzJohn, 2012).

FRUIT-INDEPENDENT SHIFTS IN DIVERSIFICATION

We tested for shifts in diversification independent of whether they were associated with the achene fruit type by making use of the step-wise AIC framework proposed by Alfaro et al. (2009). This likelihood-based method measures the fit of shifts in net diversification rate, added in a stepwise manner, until the addition of new parameters exhausts the information contained within the tree. The method uses clade species diversity estimates to assess shifts in diversification while incorporating molecular divergence time information. However, because uncertainties surrounding the timing of events can confound estimates of diversification rates

(cf., Moore et al. 2004; but see Wertheim and Sanderson 2011), we performed these analyses using a distribution of 1000 randomly chosen trees obtained from a more focused Bayesian divergence time analysis (Beaulieu et al. 2013b). We note that the relationships among the major campanulid lineages from the more focused analysis are identical to those in our large sequence-based tree. We rely on the trees from the smaller analysis because they provide a sample of the uncertainty in the divergence times estimates, and because all inferred origins of the achene are resolved nodes. We attached the number of species represented by each campanulid lineage included as a "terminal" in our tree, with diversity estimates for the terminals obtained from Stevens (2012). The net diversification rate inferred for a node was represented as the average of the rates obtained across the 1000 random tree set. All analyses were carried out using MEDUSA in the R package GEIGER.

Table 1. The fit of alternative models of fruit-type evolution in campanulid angiosperms. The best model (shown in bold), based on Akaike weights, w_i, was the "correlated paths" model that assumed distinct rates for transitions ("all rates free") between each of the possible paths among the different character combinations.

Model	$-\ln L$	AICc	ΔAICc	w_i
Multistate, equal rates	-544.2	1090.4	240.4	<0.001
Multistate, all rates free	-397.2	854.3	4.31	0.103
Independent, equal rates	-672.5	1350.9	500.9	<0.001
Independent, all rates free	-584.8	1181.7	331.7	<0.001
Correlated paths, equal rates	-672.8	1347.6	497.6	<0.001
Correlated paths, all rates free	**-410.9**	**850.0**	**0.00**	**0.896**

Results

A MODEL OF FRUIT-TYPE EVOLUTION

The best-fit model overall, based the Akaike weights (w_i; Table 1), was the "correlated paths" model that assumed distinct rates for transitions between each of the possible paths among the different character combinations. The parameters estimated under the correlated paths model revealed a very general pattern of rates equal to 0 always being inferred for transitions involving the gain of multi-seededness from a single-seeded state. This included the gain of multi-seededness from the indehiscent, fleshy, single-seeded character combination ($q_{010\rightarrow011} = 0.0000$, SE < 0.0001); under the multistate model this is equivalent to a transition from a drupe to a berry. Also of note are the paths to and from dehiscent, dry, multiple-seeded fruits, or true capsules, where all paths leading to capsules were estimated to be 0. Interestingly, the most likely path from this state combination was estimated to involve the loss of dehiscence ($q_{101\rightarrow001} = 0.0082$, SE = 0.0013) rather than reduction to a single-seed ($q_{101\rightarrow100} = 0.0002$, SE < 0.0001). These indehiscent, dry, and multiseeded fruits were associated with some of the highest overall transition rates, and transitions from this state combination are far more likely to gain fleshiness ($q_{001\rightarrow011} = 0.1664$, SE = 0.0375) than reduce to a single seed ($q_{001\rightarrow000} = 0.0396$, SE = 0.0140). This character state combination, which is rarely encountered in extant species, is inferred to be an important intermediate step on the path from capsules to berries within campanulids.

The indehiscent, dry, and single-seeded fruit type (achenes) was the only one that had nonzero transition rates estimated for paths leading to it from all three "neighboring" character state combinations (Fig. 1). Although the most likely path was estimated to involve a reduction to a single seed from the indehiscent, dry, multiple-seeded combination, there was also a fairly high rate

estimated for the path that involved the loss of dehiscence from a dehiscent, dry, single-seeded fruit ($q_{100\rightarrow000} = 0.0102$, SE = 0.0029). Both of these paths had rate estimates that were nearly an order of magnitude higher than the rates estimated for the path leading through the loss of fleshiness ($q_{010\rightarrow000} = 0.0046$, SE = 0.0022). The only nonzero transition away from the indehiscent, dry, and single-seeded (achene) combination involved the gain of fleshiness, although this was estimated to have rarely occurred ($q_{101\rightarrow000} = 0.0001$, SE < 0.0001).

By comparison, the parameters estimated under the multi-state model largely agree with the direction and magnitude of transitions among the different fruit types (see Supporting Information). That is, all transitions leading to capsules were estimated to be zero, and transitions leading to single-seeded fruits, such as achenes, drupes, and DDS, and those containing multiple seeds, such as capsules, berries, and IDM, went from multi- to single-seeded fruits (Fig. S1). These mostly involved transitions toward the achene fruit type (e.g., $q_{B\rightarrow A} = 0.0001$; $q_{C\rightarrow A} = 0.0014$; $q_{D\rightarrow A} = 0.0037$; Fig. S1). The only exceptions to the multi- to single-seeded pattern were transitions going from an achene to a berry, which had a low, but nonzero rate ($q_{A\rightarrow B}$ <0.0001). We also note that nonzero transition rates were estimated for all transitions from a capsule to each of the different fruit types (Fig. S1). This included the IDM, where a transition from a capsule was the only nonzero rate for transitions toward this fruit type. Also, the only nonzero rate estimated for transitions going from the IDM was toward a berry ($q_{IDM\rightarrow B} = 0.1208$), which had a rate that was nearly two orders of magnitude higher than the rate of transitions going directly from a capsule to a berry ($q_{C\rightarrow B} = 0.0033$). Therefore, and similar to the estimates in the correlated paths model, a likely route by which capsules become fleshy within campanulids involved first transitioning to the IDM fruit type.
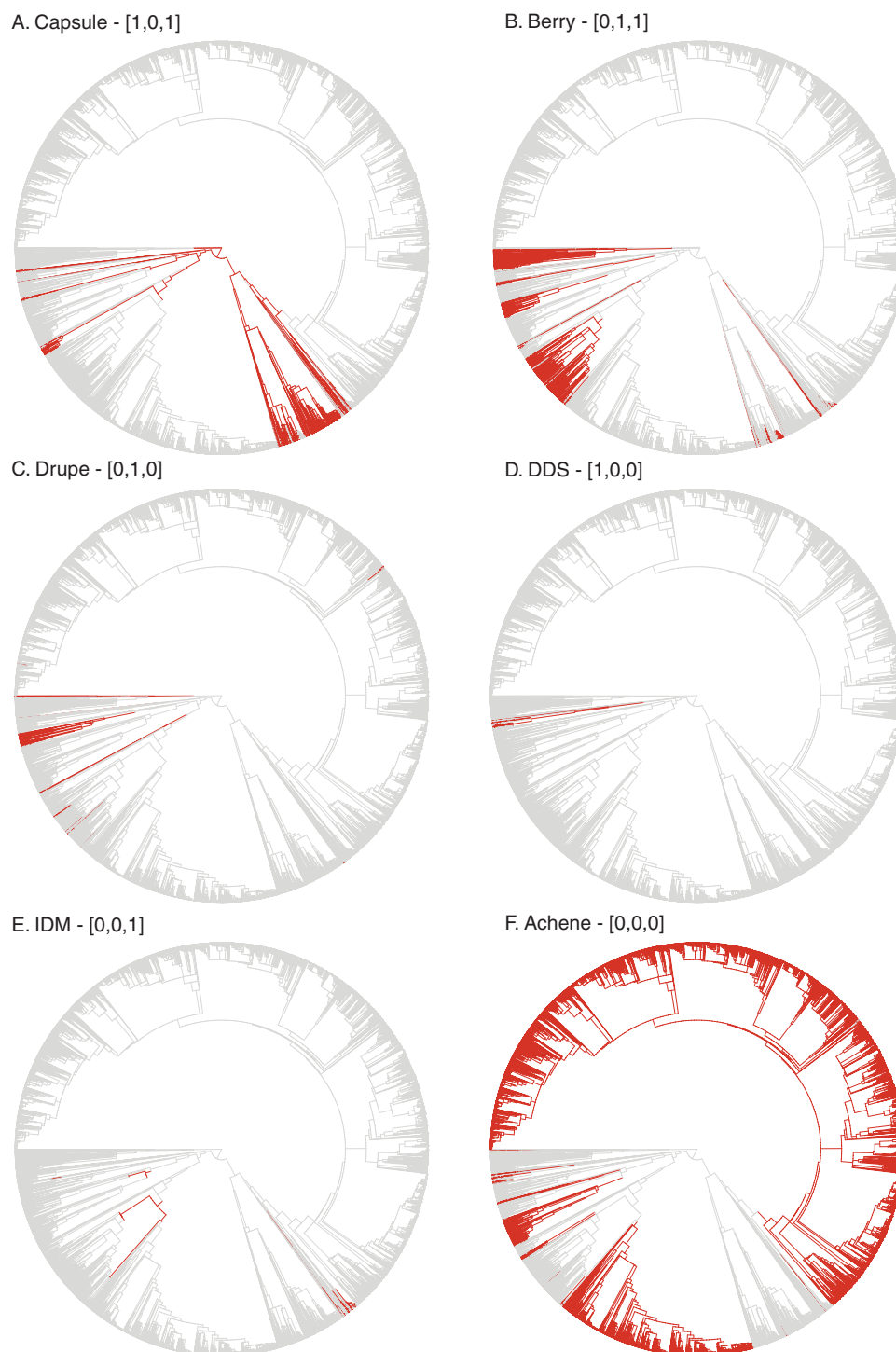
A. Capsule - [1,0,1]

B. Berry - [0,1,1]

C. Drupe - [0,1,0]

D. DDS - [1,0,0]

E. IDM - [0,0,1]

F. Achene - [0,0,0]

Figure 2. The distribution of branches inferred as being in each of the different fruit configurations in the correlated paths model. The general distribution of character states across campanulids indicate that the dry condition has been maintained throughout much of their history, and that the achene fruit type is by far the most predominant fruit type observed within the group.

RECONSTRUCTION OF CAMPANULID FRUIT EVOLUTION

Our correlated paths model inferred the relatively uncommon dehiscent, dry, multiple-seeded (capsule) fruit as the ancestral for Campanulidae. The capsule appears to have been retained through several early branching events and is inferred to be ancestral for Asterales, Apiales, Dipsacales, and several smaller lineages (Fig. 1 and 2). The Aquifoliales are a clear exception, shifting early to an indehiscent, fleshy, multiple-seeded (berry) fruit. Within Asterales, Apiales, and Dipsacales independent shifts
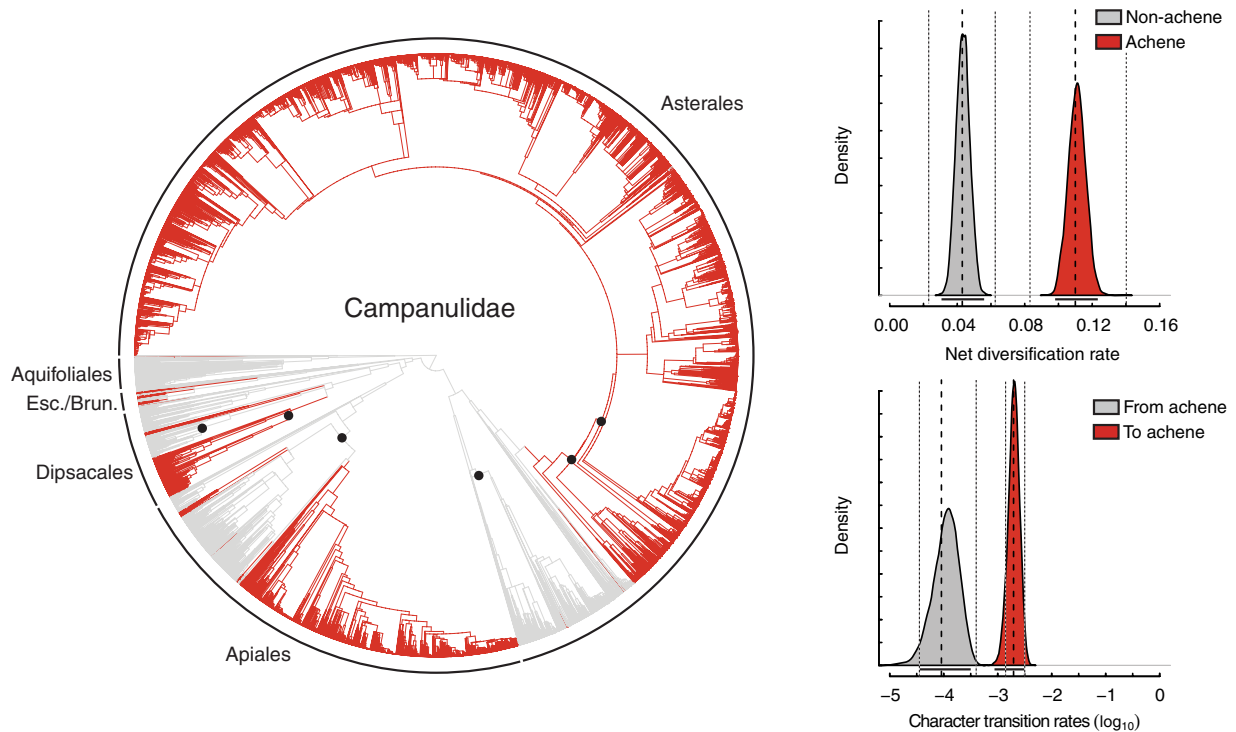
Figure 3. The distribution of branches in campanulids associated with the achene fruit type. The inference of whether a lineage is either a non-achene (gray) or an achene (red) state is based on the joint reconstruction using the parameters estimated from the BiSSE model and is identical to the reconstruction shown in Figure 2F. Posterior probability distributions from the BiSSE model indicated that the preponderance of achene or "achene-like" fruits within campanulids is largely a consequence of higher net diversification rates in achene lineages compounded by asymmetric transition rates that strongly favor transitions to the achene state rather than the reverse. A vertical dashed line indicates the parameter estimates from the maximum likelihood analysis, and the dotted lines indicate the associated approximate standard errors calculated directly from the likelihood search. The bar underneath each distribution represents the 95% credible interval obtained from the posterior distribution. We also identified six major shifts (represented by black dots) in diversification rate across the major lineages of campanulids. Three of these major shifts corresponded to nodes that are nested not far from several origins of achene fruits.

are inferred to an indehiscent, dry, single-seeded (achene) fruit (Figs. 1 and 2). A more detailed description of inferred fruit evolution within the major campanulid lineages is provided in the Supporting Information.

THE MACROEVOLUTIONARY CONSEQUENCES OF ACHENE FRUITS

According to the BiSSE model, the preponderance of indehiscent, dry, and single-seeded, or achene-like, fruits within campanulids results from the asymmetric transition rates to and from this character state combination, compounded by differences in the diversification process. Transitions going toward indehiscent, dry, and single-seeded fruits ($q_{0\to1} = 0.0020$) have a rate that is more than an order of magnitude greater than the reverse ($q_{1\to0} = 0.0001$), and lineages exhibiting this character combination are also estimated to have a net diversification rate ($r = \lambda - \mu$; $r_1 = 0.111$ lineages Myr^{-1}) that is nearly three times the rate of lineages that do not ($r_0 = 0.043$ lineages Myr^{-1}; Fig. 3; Table 2). The ML and

Bayesian analyses revealed very similar estimates, and the standard errors from the ML analysis and the credibility intervals from the Bayesian analysis did not overlap, implying that diversification rate differences between the achene and non-achene lineages are significant (Fig. 3).

Interestingly, both speciation and extinction were inferred to be higher in achene than in non-achene lineages. However, there was also a strong positive association between Bayesian posterior estimates of these parameters (achene: $r^2 = 98.9\%$; non-achene: $r^2 = 99.1\%$; Fig. S2), an indication of a "likelihood ridge" in the parameter space. Such a ridge represents multiple combinations of speciation and extinction parameters that are equally likely, rendering these combinations effectively indistinguishable from one another. This will naturally exert a strong influence on the relationship between the two parameters used to approximate the shape of a given tree: net diversification ($r = \lambda - \mu$) and the relative extinction rate ($\varepsilon = \mu/\lambda$). Indeed, there was a strong negative association between net diversification and the

Table 2. Comparisons of the full and constrained maximum likelihood BiSSE model fit to all campanulids and several of the major campanulid clades. The six BiSSE parameters are the speciation (λ_0 and λ_1) rates in non-achene (0) and achene (1) lineages, the extinction rates (μ_0 and μ_1), and the two transition rates for going to (q_{01}) and from (q_{10}) the achene state. However, because estimates of speciation and extinction are not distinguishable from one another (see text), we report the overall net diversification rate ($r = \lambda - \mu$). Nested models were compared with AIC, and significant differences between r_0 and r_1, and between q_{01} and q_{10}, are in bold and were based on an approximate standard error calculated directly from the likelihood search.

Model	−ln Lik	AIC	r_0	r_1	q_{01}	q_{10}
Campanulidae						
$\lambda_0=\lambda_1, \mu_0=\mu_1$	− 20893.1	41794.3	0.0632	0.0632	**0.0013**	**0.0001**
$\mu_0=\mu_1$	− 20668.3	41346.6	**0.0191**	**0.1451**	**0.0008**	**0.0002**
$q_{01}=q_{10}$	− 20542.7	41095.3	**0.0406**	**0.1108**	0.0005	0.0005
None	− 20528.7	41069.3	**0.0431**	**0.1108**	**0.0020**	**0.0001**
Asterales						
$\lambda_0=\lambda_1, \mu_0=\mu_1$	− 13689.0	27385.9	0.0903	0.0903	**0.0017**	**0.0002**
$\mu_0=\mu_1$	− 13605.7	27221.5	**0.0206**	**0.1352**	**0.0010**	**0.0002**
$q_{01}=q_{10}$	− 13585.1	27180.2	**0.0357**	**0.1248**	0.0004	0.0004
None	− 13580.7	27173.4	**0.0394**	**0.1231**	**0.0018**	**0.0001**
Apiales						
$\lambda_0=\lambda_1, \mu_0=\mu_1$	− 4532.6	9073.2	0.0725	0.0725	**0.0012**	**<0.0001**
$\mu_0=\mu_1$	− 4484.9	8979.8	**0.0316**	**0.1664**	0.0010	<0.0001
$q_{01}=q_{10}$	− 4423.8	8857.5	0.0852	0.0909	0.0003	0.0003
None	− 4422.2	8856.4	0.0869	0.0907	**0.0014**	**0.0002**
Dipsacales						
$\lambda_0=\lambda_1, \mu_0=\mu_1$	− 1394.9	2797.7	0.0749	0.0749	**0.0026**	**<0.0001**
$\mu_0=\mu_1$	− 1392.2	2794.5	0.0575	0.0968	**0.0021**	**<0.0001**
$q_{01}=q_{10}$	− 1394.5	2799.1	0.0599	0.0909	0.0009	0.0009
None	− 1392.1	2796.2	0.0604	0.0920	**0.0023**	**<0.0001**

relative extinction rate within achene and non-achene lineages (achene: $r^2 = 87.5\%$; non-achene: $r^2 = 85.0\%$), and for the relative extinction rates the Bayesian posterior estimates for the two states were clearly sampled from the same parameter space (Fig. S2). There was, however, very clear separation in the posterior sample of net diversification rates (Fig. S2), which showed very little overall variation despite the wide range of relative extinction rates. These results suggest that when interpreting the parameters of the BiSSE model for the evolution of the achene in campanulids, it is probably more appropriate to focus interpretation on the net diversification rate, as opposed to interpreting speciation and extinction separately.

We also note that the strength of the correlation between net diversification rate and the achene character state differed among the major lineages of campanulids (Fig. 4). The Asterales had diversification rates that were nearly identical to the rates inferred for the campanulids as a whole (Table 2). The achene state within the Asterales is almost entirely represented by the highly diverse Asteraceae, which not only makes up the majority of Asterales species diversity, but also the bulk of the diversity in campanulids as a whole. By contrast, despite some qualitative rate differences in the Dipsacales and Apiales that were consistent with the overall pattern, there was substantial overlap in both the

standard errors and the credibility intervals (Table 2; Fig. 4). We interpret these results as evidence for the general correlation between diversification and the achene state across campanulids as actually being inconclusive, and largely driven by high net diversification rates for achene lineages within the Asterales.

FRUIT-INDEPENDENT SHIFTS IN DIVERSIFICATION

We identified six major shifts in net diversification rate across the major lineages of campanulids when compared against the background rate ($r = 0.0609$ lineages Myr^{-1}). Three of these major shifts are noteworthy as they appear to be nested not far within clades that exhibit achene fruits (Fig. 3). Two of these shifts occur within the Asteraceae, with the largest shift in diversification overall ($r = 0.152$ lineages Myr^{-1}) being located along the branch leading to the "out of South America" clade (sensu Panero and Funk 2008) that contains the bulk of Asteraceae diversity (Fig. 3). This clade includes several major lineages, such as the Carduoideae (2780 species), Cichorioideae (3600 species), and the Asteroideae (16,360 species), and its origin appears to coincide with the radiation and worldwide spread of the clade (Beaulieu et al. 2013b). The remaining shift associated with achene clades is located at the crown of the Valerianceae-Dipsacaceae clade ($r = 0.088$ lineages Myr^{-1}). This shift appears to relate to

numerous adaptations for achene dispersal mediated by calyx evolution in Valerianaceae and "epicalyx" evolution in Dipsacaceae (Donoghue et al. 2003; Jacobs et al. 2010).

Additional major shifts in diversification elsewhere in campanulids were associated with the origin of the Campanulaceae *s.l.* ($r = 0.115$ lineages Myr^{-1}), the Araliaceae-Myodocarpaceae-Apiaceae clade ($r = 0.128$ Myr^{-1}), and the origin of *Lonicera* within the Dipsacales ($r = 0.110$ lineages Myr^{-1}). The shift in diversification associated with *Lonicera* occurs within the Caprifolieae clade, which is composed of species bearing berry-like fruits (i.e., indehiscent, fleshy, and multiseeded). In both Campanulaceae *s.l.* and Araliaceae-Myodocarpaceae-Apiaceae, the shift in diversification is more difficult to relate to any specific fruit type. Although most Campanulaceae *s.l.* have capsules, there are several nested clades with various fruit types, such as berries, achenes, and the IDM type. The Araliaceae-Myodocarpaceae-Apiaceae clade includes several subclades of achene-bearing species (i.e., Araliaceae).

Discussion

Our analysis of the fruit evolution in the Campanulidae strongly supports dry, dehiscent, multiseeded fruits, or capsules, as the ancestral condition within this major angiosperm clade, despite the fact that this type is relatively rare in the group. All of the earliest branches, including the earliest branches within the Aquifoliales, Asterales, Bruniales, Escalloniales, Apiales, and Dipsacales, were reconstructed as having capsular fruits, with subsequent fruit diversity derived from this ancestral fruit type. This includes the evolution of both the fleshy fruits characteristic of the Aquifoliales, Araliaceae, Adoxaceae, and Caprifolieae, and the dry, indehiscent, and single-seeded fruits that predominate in campanulids. The general distribution of character changes suggests that the dry condition has been maintained throughout much of the history of campanulids (Figs. 1 and 2). Dry fruits are perhaps related to being predominately herbaceous and/or occupying generally temperate or seasonally arid environments (Beaulieu et al. 2013b).

Our analyses strongly suggest that transitions away from the ancestral capsular condition—whether through the loss of dehiscence or through a reduction to a single-seed—were largely irreversible. That is, there were no inferred paths indicating that the capsule fruit could evolve again once any of its major component traits (i.e., being dehiscent, dry, or containing multiple seeds) were lost. Even when transitions among the different fruit types were not restricted, as with the multistate model, there were no nonzero rates estimated for transitions leading back to the capsule fruit. Thus, our data strongly support an explicit assumption made about the evolution of capsules in previous analyses of fruit type (Bremer and Eriksson 1992), namely, that because any change in the basic morphology of a capsule involves complicated structural
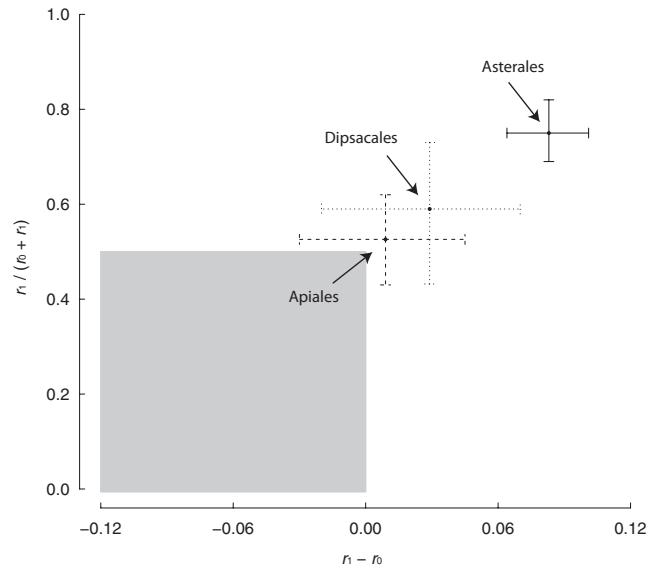

Figure 4. Posterior probability distributions of the difference between the net diversification rate in achenes (r_1) and non-achenes (r_0) (x-axis), and the proportion of net diversification rate of achenes (r_1) relative to the sum of both the achene (r_1) and nonachene rates (r_0) (y-axis), from individual BiSSE models fit to Dipsacales, Apiales, and Asterales separately. These clade-specific joint posterior probability distributions indicate that the high net diversification rate for achene lineages within campanulids as a whole is largely driven by the high rate associated with Asterales. The gray box denotes the region where the net diversification rate of non-achenes exceeds that of the achene lineages.

rearrangements, such modifications are unlikely to be reversible (cf. Stebbins 1974).

Furthermore, there appears to be a general trend toward a reduction in seed number, largely as a consequence of the multiple origins and subsequent success of the indehiscent, dry, and single-seeded, or achene-like, fruit type within several major campanulid clades. The achene fruit type evolved along several paths, the most frequent being the loss of dehiscence, followed by a reduction to a single seed (Fig. 1). Our analyses indicate that once the reduction to a single seed takes place, the resulting achene-like fruit becomes "locked in," as there were no inferred gains of multiseededness or dehiscence after the loss of these characters. In fact, the only transition out of the achene fruit type involves the gain of fleshiness, which occurred only a few times and only in particular species of Asteraceae (Barker et al. 2009). These "fleshy achenes" reflect shifts in dispersal syndrome, where birds, as opposed to abiotic factors are the primary dispersal agents.

Although we are confident that strong asymmetries in transition rates have played an important role in achene fruits being the predominant fruit type within campanulids, it is somewhat less clear how higher diversification rates have contributed. The test we used to estimate diversification rate in relation to the achene

character state (Maddison et al. 2007; FitzJohn et al. 2009) did indicate that the achene fruit type might be a significant correlate of diversity patterns observed across campanulids. The BiSSE method is an improvement over the simple sister-clade comparisons that have commonly been employed in studying fruit evolution and diversification (e.g., Eriksson and Bremer 1991; Ricklefs and Renner 1994; Tiffany and Mazer 1995; Dodd et al. 1999; Smith 2001). Using BiSSE, all origins of the achene are incorporated in a single model, which greatly improves statistical power, and the explicit inference of differential speciation and extinction has the potential for a more fine-grained analysis of diversification. However, several aspects of the model are problematical. For instance, estimates of speciation and extinction seem strongly compensatory, rendering the two parameters difficult to distinguish, although we suspect that the lack of independence between speciation and extinction is due largely to the inability of the model to accurately estimate relative extinction (Rabosky 2010; Fig. S2). In any case, all we can conclude with confidence is that the achene state has been positively associated with overall net diversification.

More importantly, these methods assume a homogenous birth–death process through time and across taxa, which we find less defensible, particularly when examining trait-dependent diversification patterns across larger, older, and globally distributed clades such as the campanulids. Perhaps especially at this phylogenetic scale, a characters' influence on diversification is likely contingent on other factors, such as the assembly of particular combinations of characters and/or movement into new environmental conditions of geographic regions (de Querioz 2002; Vamosi and Vamosi 2004; Donoghue 2005; Moore and Donoghue 2007; Poulin 2009). Thus, the evolution of a trait might be associated with higher rates of diversification in some clades but not in others. When we use BiSSE to consider all campanulid clades in which achenes have evolved, we see a strong positive correlation with diversification. However, as we have emphasized, there are major clade-specific differences in the strength of this correlation, and the strong correlation is largely the effect of the one major clade that includes the Asteraceae (Fig. 4).

It is noteworthy that we located several major shifts in diversification rate that were nested not far from the several origins of the achene fruit type. This is consistent with the view that the origin of a trait may not, in itself, be sufficient to increase diversification rate, but rather, requires the right combination of traits (cf. Donoghue 2005; Smith et al. 2011). For instance, the achenes of the Asteraceae-Calyceraceae clade might have evolved initially only as a mechanism to fit many small fruits into a compressed head inflorescence (capitulum) composed of many small flowers. It is possible that the upward shifts in diversification nested not far within Asteraceae was a consequence of the elaboration of structures that often accompany the achene, such as the pappus (specialized calyx), that promote more efficient dispersal, and possibly increase the probability of allopatric speciation. However, such reasoning certainly does not rule out other factors, and it is interesting to note that the location of this shift in diversification within Asteraceae coincides with the beginning of a worldwide biogeographic expansion (Panero and Funk 2008), as well as with an increase in the rate of growth form evolution (Beaulieu et al. 2013a).

It is important to bear in mind, of course, that the features that have driven these diversification patterns may not relate to anything having to do with fruit type. Instead, these shifts could reflect instances where the proximate drivers of diversification are traits that are nested within these achene clades. It is difficult to determine all of the factors that have contributed to these major shifts in diversification, especially as gene and genome duplications may also be involved (e.g., Carlson et al. 2011). Moving forward, we advocate the further development of methods that simultaneously estimate shifts in diversification rate in connection with the different origins of a character (cf. Moore and Donoghue 2009). Such an approach might be similar to one recently derived for identifying different rates of binary character evolution among different lineages (Beaulieu et al. 2013a). Such a method would provide a powerful extension to existing methodology and allow for a more refined understanding of how particular character states affect the diversification process.

With respect to the evolution of fruit type, one general implication of our correlated paths model concerns the way in which our allowance or disallowance of particular evolutionary pathways can influence our inferences. In particular, we are struck by the several instances of IDM fruits being inferred as the likeliest ancestral state for nodes whose descendents do not today exhibit this configuration. Transitioning through such rare states could be interpreted as a pathological behavior of the model, especially in those few cases where the condition is inferred to have extended through a considerable time period (e.g., toward the base of Apiales). However, it is also possible that character combinations that are rarely found in modern species were once more common, and that there were transitions (possibly rapidly) through these states on the path to the more common combinations that may be functionally advantageous. Ultimately, information from the fossil record will prove important in evaluating such possibilities, even though, at the moment, it remains too sparse to analyze the frequency of campanulid fruit types through time.

The correlated paths-type model illustrates the immediate benefit of using very large phylogenies for the study of fruit evolution. Studies of these questions have until now been based on a sparse sampling of species, with generalities about fruit evolution based on scoring standard fruit types with standard names (capsules, drupes, etc.). As a consequence, unique and possibly "transitional" forms, intermediate between the broadly

defined fruit types (e.g., the IDM and DDS fruit types), may end up lumped within the standard botanical categories. It is important to recognize that these particular combinations of states may provide us with unique insights into how transitions among fruit types took place. Now, with far larger, more comprehensive phylogenies in hand, coupled with complex and parameter-rich models, we can better represent the variation observed and may be able to discern the dynamics underlying the evident evolutionary patterns.

LITERATURE CITED

Akaike, H. 1974. A new look at the statistical model identification. IEEE Trans. Autom. Contr. 19:716–723.

Alfaro, M. E., F. Santini, C. Brock, H. Alamillo, A. Dornburg, D. L. Rabosky, G. Carnevale, and L. J. Harmon. 2009. Nine exceptional radiations plus high turnover explain species diversity in jawed vertebrates. Proc. Natl. Acad. Sci. USA 106:13410–13414.

Angiosperm Phylogeny Group. 2009. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG III. Bot. J. Linnean Soc. 161:105–121.

Barker, N. P., S. Howis, B. Nordenstam, M. Källersjö, P. Eldenäs, C. Griffion, and H. P. Linder. 2009. Nuclear and chloroplast DNA-based phylogenies of *Chrysanthemoides* Tourn. ex Medik. (Calenduleae; Asteraceae) reveal extensive incongruence and generic paraphyly, but support the recognition of infraspecific taxa in *C. monilifera*. South Afr. J. Bot. 75:560–572.

Beaulieu, J. M., R. H. Ree, J. Cavender-Bares, G. D. Weiblen, and M. J. Donoghue. 2012a. Synthesizing phylogenetic knowledge for ecological research. Ecology 93:S4–S13.

Beaulieu, J. M., D.-C. Jhwueng, C. Boettiger, B. C. O'Meara. 2012b. Modeling stabilizing selection: expanding the Ornstein-Uhlenbeck model of adaptive evolution. Evolution 66:2369–2383.

Beaulieu, J. M., B. C. O'Meara, and M. J. Donoghue. 2013a. Identifying hidden rate changes in the evolution of a binary morphological character: the evolution of plant habit in campanulid angiosperms. Syst. Biol. doi:10.1093/sysbio/syt034.

Beaulieu, J. M., D. C. Tank, and M. J. Donoghue. 2013b. A Southern Hemisphere origin for campanulid angiosperms, with traces of the break-up of Gondwana. BMC Evol. Biol. 13:80

Bremer, B., and O. Eriksson. 1992. Evolution of fruit characters and dispersal modes in the tropical family Rubiaceae. Biological Journal of the Linnean Society 47:79–95.

Britton, T., C. J. Anderson, D. Jacquet, S. Lundqvist, and K. Bremer. 2007. Estimating divergence times in large phylogenetic trees. Syst. Biol. 56:741–752.

Burleigh, J. G., M. S. Bansal, O. Eulenstein, S. Hartmann, A. Wehe, and T. J. Vision. 2011. Genome-scale phylogenetics: Inferring the plant Tree of Life from 18,896 gene trees. Syst. Biol. 60:117–125.

Carlson, S. E., D. G. Howarth, and M. J. Donoghue. 2011. Diversification of CYCLOIDEA-like genes in Dipsacaceae (Dipsacales): implications for the evolution of capitulum inflorescences. BMC Evol. Biol. 6:315.

de Queiroz, A. 2002. Contingent predictability in evolution: Key traits and diversification. Syst. Biol. 51:917–929.

Dodd, M. E., J. Silvertown, and M. W. Chase. 1999. Phylogenetic analysis of trait evolution and species diversity variation among angiosperm families. Evolution 53:732–744.

Donoghue, M. J. 2005. Key innovations, convergence, and success: macroevolutionary lessons from plant phylogeny. Paleobiology 31:77–93.

Donoghue, M. J., C. D. Bell, and R. C. Winkworth. 2003. The evolution of reproductive characters in Dipsacales. Intl. J. Plant Sci. 164:S453–S464.

Eriksson, O., and B. Bremer. 1991. Fruit characteristics, life forms, and species richness in the plant family Rubiaceae. Am. Nat. 138:751–761.

FitzJohn, R. G. 2012. Diversitree: comparative phylogenetic analyses of diversification in R. Methods Ecol. Evol. 3:1084–1092.

FitzJohn, R. G., W. P. Maddison, and S. P. Otto. 2009. Estimating trait-dependent speciation and extinction rates from incompletely resolved phylogenies. Syst. Biol. 58:595–611.

Goloboff, P. A., S. A. Catalano, J. M. Mirande, C. A. Szumik, J. S. Arias, M. Källersjö, and J. S. Farris. 2009. Phylogenetic analysis of 73,060 taxa corroborates major eukaryotic groups. Cladistics 25:211–230.

Herrera, C. 1989. Seed dispersal by animals: a role in angiosperm diversification? Am. Nat. 133:309–322.

Jacobs, B., C. Bell, and E. Smets. 2010. Fruits and seeds of the *Valeriana* clade (Dipsacales): diversity and evolution. Intl. J. Plant Sci. 171:421–434.

Jansen, R. K., Z. Cai, L. A. Raubeson, H. Daniell, C. W. dePamphilis, J. Leebens-Mack, K. F. Müller, M. Guisinger-Bellian, R. C. Haberle, A. K. Hansen, et al. 2007. Analysis of 81 genes from 64 plastid genomes resolves relationships in angiosperms and identifies genome-scale evolutionary patterns. Proc. Natl. Acad. Sci. USA 104:19369–19374.

Maddison, W. P. 2006. Confounding asymmetries in evolutionary diversification and character change. Evolution 60:1743–1746.

Maddison, W. P., P. E. Midford, and S. P. Otto. 2007. Estimating a binary character's effect on speciation and extinction. Syst. Biol. 56:701–710.

Moore, B. R., and M. J. Donoghue. 2007. Correlates of diversification in the plant clade Dipsacales: geographic movement and evolutionary innovations. Am. Nat. 170:S28–S55.

———. 2009. A Bayesian approach for evaluating the impact of historical events on rates of diversification. Proc. Natl. Acad. Sci. USA 106:4307–4312.

Moore, B. R., K. M. A. Chan, and M. J. Donoghue. 2004. Detecting diversification rate variation in supertrees. Pp. 487–533 *in* O. R. P. Bininda-Emonds, Phylogenetic supertrees: combining information to reveal the tree of life. Kluwer Academic, Dordrecht, The Netherlands.

Moore, M. J., C. D. Bell, P. S. Soltis, and D. E. Soltis. 2007. Using plastid genome-scale data to resolve enigmatic relationships among basal angiosperms. Proc. Natl. Acad. Sci. USA 104:19363–19368.

Pagel, M. 1994. Detecting correlated evolution on phylogenies: a general method for the comparative analysis of discrete characters. Proc. R. Soc. B 255:37–45.

Panero, J. L., and V. A. Funk. 2008. The value of sampling anomalous taxa in phylogenetic studies: major clade of the Asteraceae revealed. Mol. Phylogenet. Evol. 47:757–782.

Poulin, R. 2009. Character combinations, convergence and diversification in ectoparasitic arthropods. Intl. J. Parasitol. 39:1165–1171.

Pupko, T., I. Pe'er, R. Shamir, and D. Graur. 2000. A fast algorithm for joint reconstruction of ancestral amino-acid sequences. Mol. Biol. Evol. 17:890–896.

Pupko, T., I. Pe'er, D. Graur, M. Hasegawa, and N Friedman. 2002. A branch-and-bound algorithm for the inference of ancestral amino-acid sequences

when the replacement rate varies among sites: application to the evolution of five gene families. Bioinformatics 18:1116–1123.

Rabosky, D. L. 2010. Extinction rates should not be estimated from molecular phylogenies. Evolution 64:1816–1824.

Ricklefs, R. E., and S. S. Renner.1994. Species richness within families of flowering plants. Evolution 48:1619–1636.

Sanderson, M. J., D. Boss, D. Chen, K. A. Cranston, and A. Wehe. 2008. The PhyLoTA browser: processing GenBank for molecular phylogenetics research. Syst. Biol. 57:335–346.

Seymour, G. B., K. Manning, E. M. Eriksson, A. H. Popovich, and G. J. King. 2002. Genetic identification and genomic organization of factors affecting fruit texture. J. Exp. Bot. 53:2065–2071.

Smith, J. F. 2001. High species diversity in fleshy-fruited tropical understory plants. Am. Nat. 157:646–653.

Smith, S. A., J. M. Beaulieu, and M. J. Donoghue. 2009. Mega-phylogeny approach for comparative biology: an alternative to supertree and super-matrix approaches. BMC Evol. Biol. 9:37.

Smith, S. A., J. M. Beaulieu, A. Stamatakis, and M. J. Donoghue. 2011. Understanding angiosperm diversification using small and large phylogenetic trees. Am. J. Bot. 98:404–414.

Soltis, D. E., S. A. Smith, N. Cellinese, K. J. Wurdack, D. C. Tank, S. F. Brockington, N. F. Refulio-Rodriguez, J. B. Walker, M. J. Moore, B. S. Carlsward, et al. 2011. Angiosperm phylogeny: 17 genes, 640 taxa. Am. J. Bot. 98:704–730.

Stebbins, G. L. 1974. Flowering plants: evolution above the species level. Harvard University, Cambridge, MA.

Stevens, P. F. 2012. Angiosperm phylogeny website. Version 12, July 2012. Available via http://www.mobot.org/MOBOT/research/APweb/.

Tank, D. C., and M. J. Donoghue. 2010. Phylogeny and phylogenetic nomenclature of the Campanulidae based on an expanded sample of genes and taxa. Syst. Bot. 35:425–441.

Tiffney, B. H., and S. J. Mazer. 1995. Angiosperm growth habit, dispersal and diversification reconsidered. Evol. Ecol. 9:93–117.

Vamosi, J. C., and S. M. Vamosi. 2004. The role of diversification in causing the correlates of dioecy. Evolution 58:723–731.

Wang H., M. J. Moore, P. S. Soltis, C. D. Bell, S. F. Brockington, R. Alexandre R., C. C. Davis, M. Latvis, S. R. Manchester, and D. E. Soltis. 2009. Rosid radiation and the rapid rise of angiosperm-dominated forests. Proc. Natl. Acad. USA 10:3853–3858.

Wertheim, J. O., and M. J. Sanderson. 2011. Estimating diversification rates: how useful are divergence times? Evolution 65:309–320.

Associate Editor: L. Jesson

Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Figure S1. Time-calibrated phylogeny of 8911 species of Campanulidae (campanulid amgiosperms) taken from a maximum likelihood analysis based on a combined analysis of 11 chloroplast genes and one nuclear gene.

Figure S2. The joint posterior probability distributions of the speciation and extinction parameters from the BiSSE model.