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# Evolutionary history of floral key innovations in angiosperms

Elisabeth Reyes

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NNT : 2016SACLS489

THESE DE DOCTORAT  
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Sciences du Végétal : du Gène à l'Ecosystème

Spécialité de Doctorat : Biologie

Par

**Mme Elisabeth Reyes**

Evolutionary history of floral key innovations in angiosperms

**Thèse présentée et soutenue à Orsay, le 13 décembre 2016 :**

**Composition du Jury :**

M. Ronse de Craene, Louis	Directeur de recherche aux Jardins Botaniques Royaux d'Édimbourg	Rapporteur
M. Forest, Félix	Directeur de recherche aux Jardins Botaniques Royaux de Kew	Rapporteur
Mme. Damerval, Catherine M. Lowry, Porter	Directrice de recherche au Moulon Curateur en chef aux Jardins Botaniques du Missouri	Président du jury Examineur
M. Haevermans, Thomas	Maître de conférences au MNHN	Examineur
Mme. Nadot, Sophie	Professeur à l'Université Paris-Sud	Directeur de thèse
M. Sauquet, Hervé	Maître de conférences à l'Université Paris-Sud	Invité (co-directeur de thèse)



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# GENERAL INTRODUCTION

## 1. THE MANY FACES OF THE FLOWER

The flower is a short axis bearing, from the center out, female reproductive organs consisting of ovules protected by carpels, male reproductive organs called stamens and a perianth (Bateman et al. 2006). The flower is one of the most distinctive features of angiosperms, the clade of land plants with the highest diversity. “Diversity” is here used in the sense of having a large number of species, and is accompanied by a variety of flower forms that is exemplified in Figure I.1. The image that the word “flower” evokes to the layman probably greatly resembles the upper left picture of Figure I.1 and consists of reproductive organs of both sexes surrounded by a specialized two-whorl perianth in which the inner whorl is colorful and showy but delicate, while the outer whorl is more inconspicuous and stiff as it protects the developing bud; it will also probably have five petals and five sepals. That mental picture includes the organs being separate and those of a given category being the same size and shape. Adaptation to pollinators, self-pollination, abiotic pollination or simply evolutionary history has led to the development among flowers of aspects that are radically different from that “layman’s mental image”. For instance, the “sepal and petal” perianth is not the only form of perianth that exists and is actually a derived state (see Figure I.3 and Chapter 3). Species from the early-diverging clades of angiosperms can have only one type of perianth part, or two types that are different in shape but of similar color, petal-like or sepal-like. Flowers from these early-diverging clades can also have more than two perianth whorls or a spiral perianth, usually associated with a large number of perianth parts. While most species in such cases are found in early-diverging angiosperms, the above situations occasionally appear within clades in which the “sepal and petal” two-whorled perianth is otherwise the norm. The whorled flowers of magnoliids and monocots are mostly trimerous (three perianth parts per whorl) rather than pentamerous (five parts per whorl). Some flowers show variation in perianth part size and shape within the same whorl, which may result in a perianth with bilateral symmetry, disymmetry (two perpendicular planes of symmetry) or no symmetry at all, instead of the more common radial symmetry. Certain types of inflorescences (flower aggregations) are made of a large number of extremely small or simplified flowers. Simplified flowers can lose their perianth and consist only of reproductive organs, which themselves can be reduced to a very small number; there are flowers that effectively consist of a single carpel and/or a single stamen, such as

members of *Cryptocoryne* (Araceae) and *Sarcandra* (Chloranthaceae). While we will be focusing on the perianth in this thesis, stamens and carpels can also be subject to variation in number, shape, differentiation, symmetry, and presence/absence. Some species of angiosperms have the two sexes separated in different flowers on the same individual (monoecy) or different individuals (dioecy). In such situations, the organs corresponding to the absent sex are either reduced or absent.



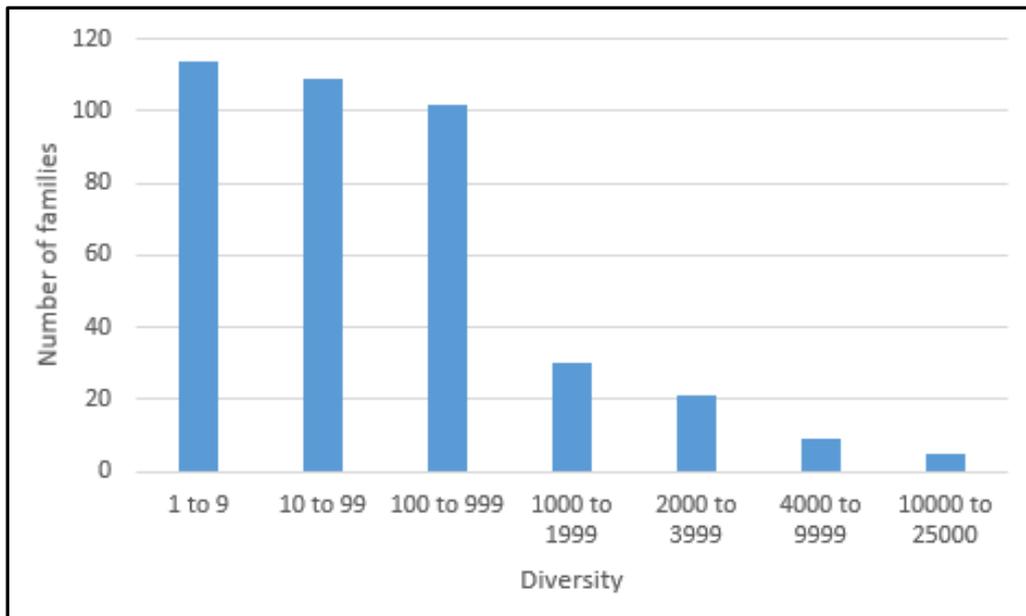
**Figure I.1:** Examples of the variation in flower and inflorescence form. From upper left to lower right: Row 1, *Anagallis arvensis* (Primulaceae), *Eucryphia cordifolia* (Cunoniaceae), *Alisma plantago-aquatica* (Alismataceae), *Stellaria holostea* (Caryophyllaceae), *Mimulus luteus* (Phrymaceae), *Dactylorhiza maculata* (Orchidaceae) *Lotus corniculatus* (Fabaceae), Row 2, *Beta vulgaris* (Amaranthaceae), *Akebia quinata* (Lardizabalaceae), *Butomus umbellatus* (Butomaceae), *Brugmansia sanguinea* (Solanaceae), *Hyacinthoides non-scripta* (Asparagaceae) *Arbutus unedo* (Ericaceae), *Cypripedium calceolus* (Orchidaceae), Row 3, *Leucanthemum vulgare* (Asteraceae), *Plantago lanceolata* (Plantaginaceae), *Anthoxanthum odoratum* (Poaceae), *Piper excelsum* (Piperaceae), *Isopogon anemonifolius* (Proteaceae), *Ficaria verna* (Ranunculaceae), *Nymphaea* sp. (Nymphaeaceae). Photos by Hervé Sauquet.

All this variation in flower form has appeared via various changes in flower development across the evolutionary history of angiosperms. In the days before widespread gene sequencing, shared morphological features, including those of flowers when they were present, were used to classify species into groups. Molecular data broke up some of the families from the time, but confirmed that the members of others were indeed related. Clades in which most or all species share a given morphological attribute can be safely assumed to have inherited that attribute from a common ancestor. Some morphological attributes are encountered in more species than others, and are sometimes a characteristic shared by entire, very diverse (in terms of species number) clades. Because of this, it has been speculated that such attributes could be a cause of the high diversity of these clades.

## 2. A SERIES OF ABOMINABLE MYSTERIES

Charles Darwin strongly believed in gradual evolution. He found that the fossil record of several groups did not confirm this belief, with the rapid appearance of many different angiosperms being the most extreme example. Such groups were part of the “abominable mystery” mentioned in the letter written to Joseph Hooker in 1879. However, concerning angiosperms specifically, he suggested that fast appearance of multiple forms may have a biological explanation in the form of co-evolution with pollinators (Friedman 2009). A century and a half after these words were written, we now know that the diversity of the approximately 300,000 living species of angiosperms is unevenly distributed across the whole clade, that the five largest families, which account for 30% of that diversity, are not closely related to each other and that pollinator interaction is one of the probable causes of the large number of flower forms (Armbruster 2014). When we look at angiosperm families separately, we indeed find species diversity ranging from one (this is the known diversity of about 30 families, including Amborellaceae, sister to all other angiosperms) to more than 25,000 species in the Asteraceae. However, rather than solving the mystery, this fact is closer to giving us several abominable mysteries to resolve instead of only one. Out of 424 families recognized today (APG IV, 2016), only five boast five-digit species diversity (Asteraceae, Orchidaceae, Fabaceae, Poaceae and Rubiaceae). Families with four-digit diversity, while much more frequent in comparison, can still be exceptionally diverse compared to their most closely related families (e.g. the 3460-species strong Gesneriaceae are sister to the 260-species Calceolariaceae). A distribution of family diversities is shown in Figure I.2.

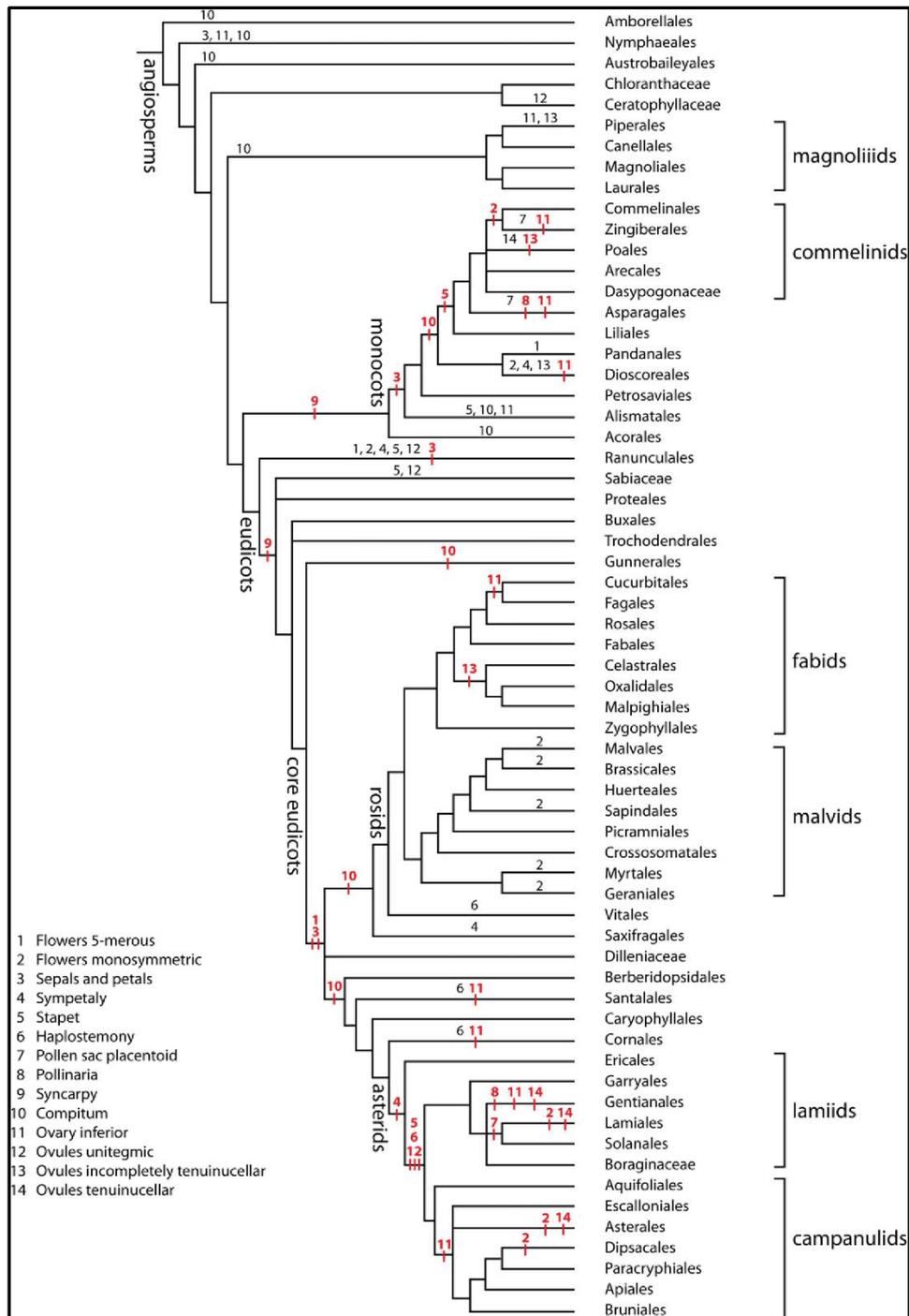
One of the proposed causal factors of this imbalance is the origin of key innovations, which are character states whose appearance in a clade results in higher diversification (Hunter 1998). Several character states, which include the aforementioned bilateral symmetry and specialization of the perianth into sepals and petals, have been proposed as candidate key innovations (Sargent 2004; Vamosi and Vamosi 2010; Endress 2011; Armbruster 2014; De Vos et al. 2014; O’Meara et al. 2016). Following this initial definition, the flower can itself be considered a key innovation within land plants, which led to angiosperm diversification.



**Figure I.2:** Histogram of species diversity per family across angiosperms.

However, since there have in reality probably been multiple bursts of diversification within angiosperms (Magallón et al. 2015; Tank et al. 2015), the causes must be multiple: a different cause for each outburst, the same cause appearing several times, or a mix of both. In fact, only cases in which a candidate morphological or environmental character state has appeared several times independently and is correlated with higher diversification rates can be potentially hypothesized to be key innovations. This extra requirement is due to a caveat in tests deducing key innovation properties in traits found only in a single subsection of the clade in which they are being tested (Maddison and FitzJohn 2015). Indeed, if a character state is present only in one clade that is extremely diverse, it is easy to assume that this character state is the cause of the higher diversity, the flower's presence in land plants being an example of this. In reality, the character state may be a neutral one that appeared in the clade before, after or at the same time as the actual cause of diversification and was never eliminated due to a lack of negative effects on the clade's fitness. For example, we could be attributing the higher diversification of angiosperms (compared to their closest living relatives, the gymnosperms, or to any other clade among land plants) to the presence of flowers when in reality, something else that is exclusive to angiosperms could have played a much bigger role in the higher diversity. In such a case, the character state would be simply correlated to the higher diversification and hitchhiking on one or several of the character states that are the true factors responsible the higher diversification (Vamosi and Vamosi 2011). Because of this, only character states that appear repeatedly and in several parts of the angiosperm tree will be considered as potential

key innovations in this thesis. Figure I.3 shows a summary of the hypothesized origins of character states that could be considered as potential key innovations across the angiosperms: several of them have appeared several times, even on the scale of the order-level tree.

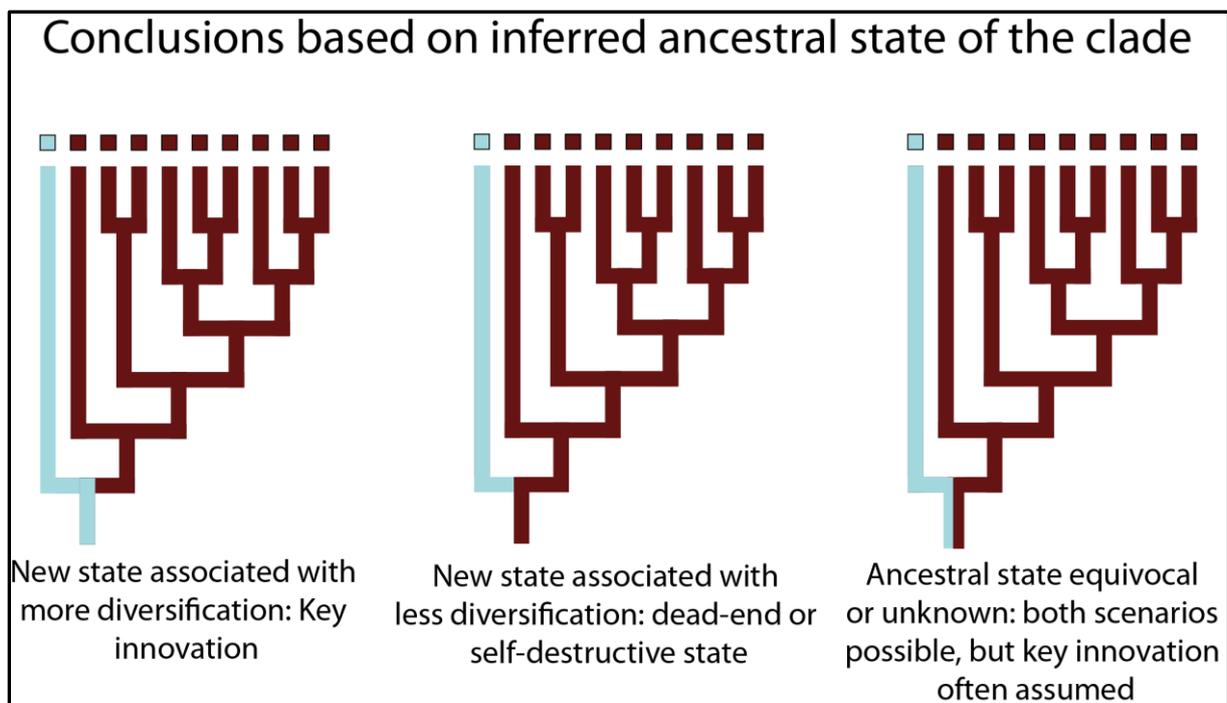


**Figure I.3:** Phylogenetic tree of angiosperms showing relationships among orders (Figure 1 from: **Endress PK. 2011.** Evolutionary diversification of the flowers in angiosperms. *American Journal of Botany* **98(3):** 370–396). Numbers are red when the corresponding trait is a potential key innovation in the clade, black when it appears without having the consequences expected of a key innovation. Reproduced with permission.

### 3. THE NEGLECTED FLIP SIDE OF THE ABOMINABLE MYSTERY

Character states that have the opposite effect of key innovations have been called “self-destructive” (Bromham et al. 2016) and lineages that have very few species have been called “depauperons” (Donoghue and Sanderson 2015). Such character states have been encountered in various studies (Gyllenberg and Parvinen 2001; Agnarsson et al. 2006; Rankin et al. 2007; Helanterä et al. 2009; Schwander and Crespi 2009; Beck et al. 2011; Kokko and Heubel 2011; Wright et al. 2013; Igc and Busch 2013; Pruitt 2013; Burin et al. 2016). Many self-destructive traits have been called evolutionary dead-ends, a term that has also been used to cover derived states that cannot revert to their ancestral state (without necessarily being detrimental to the lineage) and lineages with no present-day descendants (Bromham et al. 2016). They have received less focus than key innovations, perhaps due to having a negative connotation (Donoghue and Sanderson 2015). Being aware that self-destructive character states exist alongside key innovations is important. If one state of a binary character is found to be associated with higher species diversification than the other state, labelling it a “key innovation” implies that it is, indeed, an innovation and that the other state, associated with lower diversification, is the ancestral one. If the ancestral state of the clade is unknown or ambiguous, suggesting that the state associated with the most diverse subclade must be the derived one may at first seem valid; natural selection suggests that innovations that are advantageous are kept, while those that are harmful are “discarded” and thus unlikely to be observed. This rationale seems to have been followed for floral phyllotaxy. The notion that the spiral perianth is a “primitive” state seems to be at least as old as 1907, as an article from that year mentions it (Arber and Parkin 1907). The spiral state is much less frequent than the whorled one, which is present in all hyperdiverse (extremely high diversity) clades. The ancestral character state was assessed as equivocal between whorled and spiral in the most recent common ancestor of all angiosperms in all recent optimizations using parsimony. While studies dating from the end of the 20<sup>th</sup> century have found the ancestral state to be spiral (Endress 1990; Soltis et al. 2000), later studies have consistently found the state to be equivocal (Ronse De Craene et al. 2003; Zanis et al. 2003; Soltis et al. 2005; Endress and Doyle 2007, 2009). These results can legitimately be interpreted either as the angiosperm perianth being ancestrally spiral or ancestrally whorled, especially considering that all spiral lineages that are not in the orders Amborellales or Austrobaileyales tend to be reconstructed as being part of a larger clade that is either ancestrally whorled or equivocal. However, out of the five studies cited above, three make mention of transitions from the spiral to whorled state while discussing the results, and

two are non-committal concerning the direction of the transition; this indicates that the idea of spiral flowers being ancestral is still quite strong, but that the possibility of whorled flowers being ancestral is starting to emerge. In the latter scenario, the whorled state would have been conserved by Nymphaeales and the common ancestor of Magnoliidae, while Amborellales and Austrobaileyales would have a derived spiral state. Figure I.4 illustrates the potential pitfall that the existence of self-destructive character states creates in the “most diverse clade equals key innovation” reasoning. If the most diverse clade has the derived state, this state is possibly a key innovation. However, if the most diverse clade exhibits the ancestral state, the less diverse one may have developed a self-destructive state. However, when the ancestral state is uncertain, such as the root of the largest clade in which it is possible to diagnose the character state at all being equivocal (e.g. floral phyllotaxy above), both situations are possible.



**Figure I.4:** Three scenarios of ancestral state reconstruction and how they tend to be interpreted. The ancestral state is blue in the left-hand scenario, brown in the middle scenario, blue or brown in the right-hand scenario.

#### 4. THE CRITICAL ROLE OF ANCESTRAL STATE RECONSTRUCTION IN THE STUDY OF CHARACTER EVOLUTION

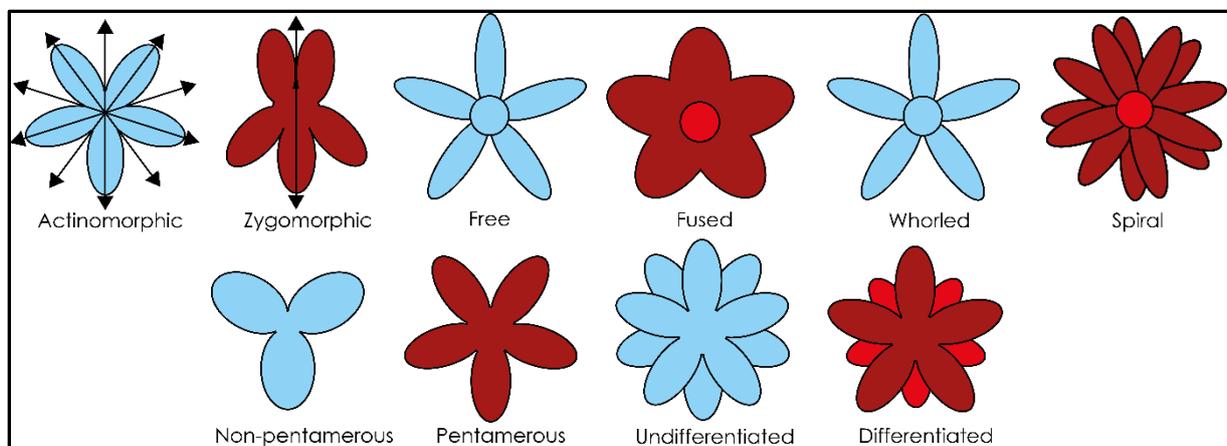
Many studies of character evolution base their conclusions on ancestral state reconstructions that assume that the ancestral state reconstructed is reliable. However, there are several models available for ancestral state reconstruction, several methods for sampling

species and several ways to take rate heterogeneity within the clade into account (or not). A change in any of these factors may have an impact on the ancestral state that will be reconstructed for a given node. In this thesis, I am mostly interested in ancestral state reconstruction as a step towards determining whether a given character state may be a key innovation or not. Because of this, what I look for in ancestral state reconstructions is confirmation that the character state I consider a potential key innovation is derived and has repeated origins. Demonstrating repeated origins of the potential key innovation states was one of the purposes of the trees presented in the supplementary material of Chapter 1. I also did a reconstruction for the same purpose for a study of Proteaceae symmetry, of which I am co-author (Citerne et al 2016, Figure 1). However, ancestral state reconstruction has other applications, such as (i) establishing the evolutionary pattern of a character that had not been previously optimized on a phylogenetic tree, (ii) determining whether a given character state can be considered a synapomorphy of a given clade, and (iii) explaining the choice of study species in an evolution and development (evo-devo) study. Another application of ancestral state reconstruction is implied by Käfer and Mousset (2014), who suggest that clades in a derived state had less time to diversify than their sister clades in the ancestral state. Using their proposed correction for this factor involves testing if a clade actually does present a derived state.

## **5. THESIS OBJECTIVES**

This thesis focuses on floral characters in angiosperms and their impact on species diversification. It is part of the eFLOWER project, whose aims include reconstructing the morphology of flowers in the deep nodes of the angiosperm tree and finding out which floral innovations are linked with major increases in diversification rates. One of the means used for these objectives is the collaborative database of floral morphological traits PROTEUS (Sauquet 2016). Chapter 1 presents an initial test of the key innovation nature of two character states on a small clade, the family Proteaceae. The next step was to do a similar test on all angiosperms with five characters (symmetry, fusion, phyllotaxy, pentamery, differentiation). One of the essential elements for doing this was ancestral state reconstruction for all five characters, as I needed to know in which clades the derived state of each of these characters had appeared, and eventually reverted to the ancestral state. The ancestral state reconstruction done on my test characters provided interesting results in itself. In addition, the methods I had used on Proteaceae proved to be inapplicable at the scale of all angiosperms and were severely criticized

in 2015 (Maddison and FitzJohn 2015; Rabosky and Goldberg 2015). This caused ancestral state reconstruction to become the primary focus of the following chapters, in which I experimented with a taxon sampling method that focused on ensuring the inclusion of as many putative character state transitions as I could find across angiosperms. In Chapter 2, the sampling method was used on one character, perianth symmetry. The ancestral states were reconstructed via parsimony, because the sampling method produced unusable results with maximum likelihood. In Chapter 3, we used the same sampling method, this time on five characters simultaneously (Figure I.5) instead of only one, which altered the species sampling sufficiently to be able to use a maximum likelihood approach. This enabled me to test several modifications of the maximum likelihood model and their impact on the states that were reconstructed on a selection of nodes. Aside from the objectives from the third chapter, the three studies have made me work on two taxonomic scales, use two different ancestral state reconstruction methods and test three different species samplings, which let me gain an overview of elements that affect ancestral state reconstruction. The discussion will explore the properties of the sampling method used in Chapters 2 and 3, discuss what is missing to better understand the diversification of angiosperms, and review other studies that are approaching the latter question.



**Figure I.5:** Characters optimized in Chapter 3 and the states in which they were divided. The state colored in blue is the hypothesized ancestral state while the derived one is colored in red.

# **CHAPTER 1: Presence in Mediterranean hotspots and floral symmetry affect speciation and extinction rates in Proteaceae**

*Written by Elisabeth Reyes, Hélène Morlon and Hervé Sauquet. Published in New Phytologist online in December 2014, in Volume 207 of the paper edition in July 2015.*

Many members of the flowering plant family Proteaceae are found in Mediterranean hotspots, which are known to have a very high species diversity. A previous article has found a correlation between presence in hotspots and high diversification rates in the family (Sauquet et al. 2009). Proteaceae also have many species with a perianth displaying bilateral symmetry (Figure I.6), a character trait that has been found to be correlated with higher diversification in angiosperms (Sargent 2004). Two methods for analyzing species diversification were tested on the characters of surrounding climate and perianth symmetry to verify if presence in hotspots and bilateral perianth symmetry may have had an impact on species diversification in the family. The family was chosen for this test because the diversity of each genus and the relationships among the genera are well-known. The floral morphology of the species is also well known; flowers have a well-conserved groundplan (four tepals, four opposite stamens, a monocarpellous ovary; Figures I.6, V.1 in general discussion) despite the size of the family (1750 species).



**Figure I.6:** Species of Proteaceae with zygomorphic flowers: *Adenanthos sericeus*, *Triunia montana* and *Lomatia ferruginea*. Photos by Hervé Sauquet.

Bilateral symmetry, also known as monosymmetry or zygomorphy, is a character state that is characteristic of several large families of angiosperms, such as Fabaceae and Orchidaceae. Zygomorphy has been shown to be a key innovation across angiosperms (Sargent 2004). The first method I used to analyze species diversification in the Proteaceae phylogenetic tree is MEDUSA (Alfaro et al. 2009), which detects shifts in diversification rate within a phylogenetic tree. The second is BiSSE (Maddison et al. 2007; FitzJohn et al. 2009), which is used to test if a given character state is correlated with higher diversification rates than the other. The ideal situation to produce a phylogeny that most accurately reflects evolutionary history is a fully-resolved and completely sampled species level phylogeny. Getting to such a situation is rare and becomes more difficult as the study clade gets larger. Both BiSSE (used to search for correlations between character states and species diversification) and MEDUSA (used to find diversification rate shifts) can accommodate non-resolved higher clades (genera in my application) on several conditions. One is that the tree must be fully resolved outside of these unresolved clades. Another is that the tree needs to be dated. For each unresolved clade, BiSSE has the additional requirement that the proportion of species in each character state must be known. The tree published by Sauquet et al. (2009) was a completely resolved genus-level tree with presumably monophyletic genera of known diversity, which enabled me to fill the requirements common to both methods. Hotspot presence data collected from Sauquet et al. (2009), and availability of perianth symmetry data for a majority of Proteaceae species enabled me to meet the extra requirement from BiSSE.

The results of the MEDUSA analysis showed five locations in which a significant rate shift had occurred: four in which the diversification rate increased and one in which the rate

decreased. These results, while giving an idea of which clades could have benefitted from a key innovation, are independent of the character state of the species and only show that shifts are present. The BiSSE results showed that species found in Mediterranean hotspots had a diversification rate significantly higher than that of species found only outside of these hotspots. They also demonstrated that the transition rate from hotspot to non-hotspot is higher than the transition rate from non-hotspot to hotspot. These results confirmed, with more appropriate methods, what had been found in Sauquet et al. (2009) using the methods available at that time. In addition to this, I observed that the diversification rate of non-hotspot clades was negative ( $r=-0.002$ ) and the net transition rate from hotspot (1) to non-hotspot (0) climate had a value high enough to compensate for it ( $q_{10}-q_{01}= 0.0027$ ). In other words, my results suggest that presence in a non-hotspot climate is globally a macroevolutionary sink for Proteaceae i.e., a character state that is correlated with extinction among the species that have it, but is acquired by new species frequently enough to compensate for the loss of those that go extinct (Burin et al. 2016). The results for perianth symmetry showed that perianth zygomorphy has no discernible impact on diversification, but that there was a higher frequency of state change from actinomorphic to zygomorphic than from zygomorphic to actinomorphic. My conclusion was that Proteaceae are an exception to the rule and that some of the other morphological characteristics of the family could be of greater importance to account for species diversification than the zygomorphy of individual flowers.

This article was published around the same time as two articles discrediting BiSSE and other methods derived from it. The first, Maddison and FitzJohn (2015), was accepted but not yet published at the time at which our manuscript was in review. From the perspective of my study, it was merely a warning against a potential caveat of the BiSSE method. That caveat was concluding that a character state has an impact on diversification even when it appears in only one clade that happens to have a high diversification rate. To give support to my results, I demonstrated via maximum likelihood ancestral state reconstruction that both species living in Mediterranean hotspots and species with perianth zygomorphy were found across several clades that were distributed in the entire family tree (Appendices II.S3, S7). I also used split-BiSSE (FitzJohn 2012), a form of BiSSE that allowed the rates for both traits to change in a chosen subclade. Here, I conducted five tests. In each one, the rates were allowed to change in a different subclade defined by one of the nodes at which MEDUSA had found a rate shift. In all of these tests, the portion allowed to have a different rate from the rest was too small to have enough statistical power; however, the remaining part of the tree consistently had results similar

to those of the full tree (Table II.S5, S6). This showed that neither of the potential caveats mentioned by Maddison and FitzJohn (2015) applied to my data. In a later article, Rabosky and Goldberg (2015) demonstrated that BiSSE and its variants had a tendency to conclude that neutral character states have an impact on diversification. In my case, however, perianth symmetry (which had no effect on diversification) was found to be neutral in Proteaceae and presence in a non-hotspot climate was found to be a macroevolutionary sink rather than simply causing species to not diversify as fast as hotspot species. These two results are hence likely to reflect a level of biological reality.

A derivative of this work, a species-level Proteaceae chronogram with sampling proportional to genus diversity on which the evolution of perianth symmetry was optimized (Figure V.1 in general discussion), later became part of Citerne et al. (2016), an article in which I am a co-author, as are my supervisors, Sophie Nadot and Hervé Sauquet. I built the species-level dataset with the help of Peter Weston and I was in charge of phylogenetic reconstruction and dating. The parsimony reconstruction found a minimum 10 origins and 12 reversals. The maximum likelihood reconstruction found 16 origins of zygomorphy and six reversals to actinomorphy.

# **Presence in Mediterranean hotspots and floral symmetry affect speciation and extinction rates in Proteaceae**

Elisabeth Reyes<sup>1,2</sup>, H  l  ne Morlon<sup>2,3</sup>, Herv   Sauquet<sup>1</sup>

<sup>1</sup>Universit   Paris-Sud, Laboratoire   cologie, Syst  matique,   volution, CNRS UMR 8079,  
91405 Orsay, France

<sup>2</sup>CMAP,   cole polytechnique, CNRS UMR 7641, Route de Saclay, 91128 Palaiseau, France

<sup>3</sup>Institut de Biologie de l'  cole Normale Sup  rieure, CNRS UMR 8197, 46 rue d'Ulm, 75005  
Paris, France

Reyes E, Morlon H, Sauquet H. 2015. *New Phytologist* 207(2): 401-410

## ABSTRACT

- Proteaceae are a large angiosperm family displaying the common pattern of uneven distribution of species among genera. Previous studies have shown that this disparity is a result of variation in diversification rates across lineages, but the reasons for this variation are still unclear. Here, we tested the impact of floral symmetry and occurrence in Mediterranean climate regions on speciation and extinction rates in Proteaceae.
- A rate shift analysis was conducted on dated genus-level phylogenetic trees of Proteaceae. Character-dependent analyses were used to test for differences in diversification rates between actinomorphic and zygomorphic lineages and between lineages located within or outside Mediterranean climate regions.
- The rate shift analysis identified five to ten major diversification rate shifts in the Proteaceae tree. The character-dependent analyses show that speciation rates, extinction rates, and net diversification rates of Proteaceae have been significantly higher for lineages occurring in Mediterranean hotspots. Higher speciation and extinction rates were also detected for zygomorphic species, but net diversification rates appeared to be similar in actinomorphic and zygomorphic Proteaceae.
- Presence in Mediterranean hotspots favors Proteaceae diversification. In contrast to observations at the scale of angiosperms, floral symmetry is not a trait that strongly influences their evolutionary success.

**Key words:** Mediterranean hotspots, floral symmetry, Proteaceae, diversification rates, speciation rates, extinction rates.

## INTRODUCTION

Species richness is notoriously unevenly distributed across taxonomic groups. This uneven distribution is a general feature of life that has been observed in insects, amphibians, birds, mammals, and plants (Harmon 2012). In phylogenetic trees, the difference in numbers of species across groups manifests itself by what is often referred to as tree imbalance – the extent to which sister clades across a phylogeny have different numbers of species (Colless 1982; Mooers and Heard 1997). While tree imbalance has been documented across the Tree of Life, the processes driving this pattern are less clear. One of the most intuitive explanations for tree imbalance is that diversification rates depend on a heritable evolving trait (Heard 1996). While this explanation is widely accepted (although see Pigot et al., 2010 for possible ‘neutral’ explanations), the heritable trait(s) responsible for diversification rate variation are rarely known.

Proteaceae are a flowering plant family with more than 1700 species widely distributed throughout the Southern Hemisphere that provides a good illustration of uneven species distribution across genera: 25 out of 81 genera are monospecific and 12 have only two species, while the largest genus, *Grevillea*, contains 362 species and the second largest, *Banksia*, has 169 species. Although Proteaceae have been widely studied by botanists, ecologists and evolutionary biologists, both the evolutionary success of the family and the reason for the uneven distribution of species within genera remain poorly understood.

Proteaceae are found in a variety of habitats including tropical rainforests, sclerophyllous forests, and open shrublands. Yet, they are mostly concentrated in the Cape Floristic Region (CFR) of South Africa and Southwest Australia (SWA), both of which are Mediterranean-type regions characterized by dry summers, mild winters, nutrient-poor soils and frequent fires (Cowling et al. 1996). These environments may seem inhospitable for plants, but Proteaceae have developed a number of adaptive traits such as serotiny and dense cluster roots (Lambers et al. 2006; He et al. 2011). Hence, the species richness of Proteaceae in general, and of CFR and SWA lineages in particular, could conceivably be linked to their adaptation to the conditions of Mediterranean climates.

Various intrinsic features have played a role in the radiation of clades of angiosperms (flowering plants) and may have contributed to the high diversity of Proteaceae. For instance, self-incompatibility has been shown to be associated with higher long-term diversification (Igic et al. 2008; Goldberg et al. 2010; Vamosi and Vamosi 2011) and fused carpels (syncarpy) have been proposed to be a key innovation in angiosperms (Endress 2011). Flowers may have radial symmetry (actinomorphy), which is the ancestral state for angiosperms as a whole, or bilateral symmetry (zygomorphy), which evolved numerous times independently (Citerne et al. 2010). Zygomorphy characterizes many species-rich plant families (e.g., Orchidaceae, Fabaceae) and zygomorphic clades tend to be more diverse than actinomorphic ones (Sargent 2004), suggesting that bilateral symmetry may favor diversification. Potential advantages of zygomorphy include an attractive visual signal for pollinators and a single access angle that maximizes the efficiency of pollen deposition (Sargent 2004). In addition, zygomorphy could facilitate adjustment of the shape of the flower to that of the pollinating animal and thus enhance plant-pollinator specialization. In Proteaceae, floral symmetry is remarkably variable, despite a well-preserved floral groundplan of four tepals with opposite stamens and a single carpel (Weston 2006). Zygomorphy is expressed in various ways in the family, from differential curvature of a tubular perianth to pronounced shape differentiation among tepals (e.g., with one tepal longer or broader than the others). Therefore, we might expect that zygomorphy could have a positive effect on diversification rates in Proteaceae.

The objective of the present study was to identify shifts in diversification rates throughout Proteaceae and to test the impact of Mediterranean hotspots and/or zygomorphy on speciation and extinction rates in the family. Sauquet et al. (2009) assembled a fossil-calibrated, dated phylogenetic tree of Proteaceae including all genera. Analyses of this phylogeny suggested that some clades within the family diversified significantly faster than the family-wide background rate, and that clades present in Mediterranean-type hotspots diversified faster (Sauquet et al. 2009). However, the methodological tools available at the time of the study did not allow the authors to locate putative diversification rate shifts, nor to disentangle the separate effect on speciation and extinction rates of the occurrence in Mediterranean-type regions.

There has recently been fast development of phylogenetic methods for studying diversification (Pennell and Harmon 2013; Stadler 2013; Pyron and Burbrink 2013; Morlon 2014). These methods mostly stem from the original work of Nee et al. (1994), who showed that although molecular phylogenies contain information of only extant taxa, patterns in their branching times contain information about both speciation and extinction rates. Recently developed methods in particular allow us to study when shifts in diversification rates occurred during clade evolution (Rabosky et al. 2007; Alfaro et al. 2009; Silvestro et al. 2011; Morlon et al. 2011; Rabosky 2014), and how specific traits -- such as the occurrence in Mediterranean-type hotspots or characters reflecting floral symmetry -- affected the speciation and extinction rates of clades (FitzJohn et al. 2009; FitzJohn 2012).

A complete species-level tree for Proteaceae would be desirable to answer our questions, but it does not exist yet for the entire family. Recent studies have addressed similar questions using well-sampled species-level phylogenies of two large genera of Proteaceae (Valente et al. 2010; Schnitzler et al. 2011; Cardillo and Pratt 2013). Interestingly, these studies did not find support for increased diversification rates in Mediterranean-climate hotspots. However, it remains unknown whether their results can be generalized across the entire family. Thus, we chose here to use the genus-level tree of Sauquet et al. (2009) with methods that have been developed to specifically work on such backbones with tips representing large terminally unresolved clades (Alfaro et al. 2009; FitzJohn et al. 2009). These methods analyze rates of speciation and extinction based on a combination of this backbone tree and standing diversity (total number of species) assigned to each terminal taxon. The same methods have recently been used on similar data to study the diversity dynamics of very large clades such as the vertebrates (Alfaro et al. 2009) and the Malpighiales (Xi et al. 2012).

Using the phylogeny of Sauquet et al. (2009) and these recently developed phylogenetic tools (Maddison et al. 2007; Alfaro et al. 2009; FitzJohn et al. 2009), we identify shifts in diversification rates in the history of Proteaceae, and analyze if and how the presence in Mediterranean-type regions and floral symmetry have influenced diversification in the family.

## MATERIALS AND METHODS

### Phylogeny

We used the dated phylogeny (chronogram) obtained by Sauquet et al. (2009), in which all of the Proteaceae genera recognized at that time (79) were represented. Two recently described genera, *Lasjia* (5 spp.) and *Nothorites* (1 sp.) (Mast et al. 2008) are not sampled in this tree. This phylogeny was constructed from eight molecular markers (the nuclear ITS and seven plastid markers: *atpB*, *atpB-rbcL*, *matK*, *rbcL*, *rpl16* intron, *trnL* intron, *trnL-trnF*) using BEAST (Drummond and Rambaut 2007) and 25 phylogenetically analyzed fossil calibration points; the tree was in general very well-supported. Specifically, we used the maximum clade credibility tree with mean ages from the posterior sample of 10 combined runs of BEAST (4 million generations each) as the main chronogram for our core analyses. In addition, we repeated our analyses with 4000 chronograms randomly sampled from this posterior (see below). We pruned all outgroup taxa. Two genera, *Protea* and *Banksia*, were represented by three and seven tips in the original tree, respectively. This corresponded to very low sampling fractions given the standing diversity of these two genera. Therefore, we preferred to treat both of them as terminally unresolved clades as we did for the rest of the genera in the tree. Thus, we also pruned two and six tips, respectively, from these two genera so that the final tree used in our analyses had exactly one tip per genus of Proteaceae.

### Diversification rate shift analyses

In order to identify major diversification rate shifts, we used the MEDUSA approach of Alfaro *et al.* (2009). MEDUSA is a likelihood-based approach that identifies shifts in diversification and relative extinction rates using a stepwise approach based upon the Akaike Information Criterion (AIC). More specifically, we used the multiMEDUSA function, which applies the MEDUSA algorithm to each tree in a collection of chronograms and draws statistics from these results, such as the number of trees supporting a rate shift at a specific node. Other recently developed methods to detect diversification rate shifts such as the Bayesian approaches implemented in BayesRates and BAMM (Silvestro et al. 2011; Rabosky 2014) would be attractive alternatives to analyze our data but also have limitations. BayesRates does not yet handle higher-level trees where tips represent large clades with known diversity but unknown

phylogeny (terminally unresolved clades), such as the genus-level phylogeny used here. On the other hand, BAMM does not yet handle multiple trees and therefore cannot take phylogenetic and dating uncertainty into account. Therefore, MEDUSA was the most suitable approach for our data. MEDUSA now provides the choice between three different models: Yule (pure birth), birth-death, or a mixed model, that is, a model with a combination of both Yule and birth-death processes within the same tree. We chose the birth-death model for our analyses because it is the most biologically appropriate for Proteaceae, but we also generated results with the other two models for comparison. Total numbers of species in each genus are provided in Table II.S1 and were taken from Sauquet et al. (2009). All analyses were performed in R using the multiMEDUSA function from package MEDUSA v0.93 4.33 (<https://github.com/josephwb/turboMEDUSA>).

### Character-dependent analyses

In order to test the effect of Mediterranean-type regions and floral symmetry on diversification, we used the BiSSE (Binary-State Speciation and Extinction) approach of Maddison et al. (2007), extended by FitzJohn et al. (2009) to handle terminally unresolved trees, which corresponds to our situation of a tree resolved at the genus rather than at the species level. For polymorphic genera, the proportion of species in each state was specified (see below). All BiSSE analyses were conducted using the diversitree package in R.

Should the possible states of a given character be named 0 and 1, the rates calculated by BiSSE are  $\lambda_0$  and  $\lambda_1$  (speciation rate of a lineage in state 0 and 1, respectively),  $\mu_0$  and  $\mu_1$  (extinction rate of a lineage in state 0 and 1, respectively), and  $q_{01}$  and  $q_{10}$  (transition rate from state 0 to state 1 and from state 1 to state 0, respectively). We fitted by maximum likelihood (ML) eight models of increasing complexity, ranging from a 3-parameter model in which speciation, extinction and transition rates did not depend on the character state ( $\lambda_0 = \lambda_1$ ,  $\mu_0 = \mu_1$ ,  $q_{01} = q_{10}$ ), to a 6-parameter model in which speciation, extinction and transition rates depended on the character state ( $\lambda_0 \neq \lambda_1$ ,  $\mu_0 \neq \mu_1$ ,  $q_{01} \neq q_{10}$ ). We found that, for this data set, different initial parameter values led to different ML estimates of model parameters and, occasionally, a different best model based on AIC scores, suggesting multiple peaks in the likelihood surface. Hence, we ran the full procedure (i.e., the ML fit of the 8 BiSSE models) multiple times for each analysis and present the results for which the highest maximum likelihood score was obtained. We then selected the best model based on the AIC criterion and ran an MCMC

(Markov Chain Monte Carlo) algorithm to generate credibility intervals for the parameter estimates.

By using the BiSSE model, we are restricted to analyzing the effect of a binary trait on diversification, and on character changes happening along the branches of the trees (and not at cladogenetic events). Hence, a character state corresponding to species spanning both Mediterranean hotspots and non-Mediterranean regions cannot be accounted for, nor allopatric speciation, whereby a species spanning both regions would split into two species, one in each region. Other models, such as GeoSSE (Goldberg et al. 2011) or BiSSE-ness (Magnuson-Ford and Otto 2012) would allow taking such processes into account. However, GeoSSE is not yet implemented for unresolved clades and BiSSE-ness only allows binary traits without overlapping states. In addition, extant Proteaceae species spanning both Mediterranean hotspots and non-Mediterranean regions were rare (less than 10%), and for these species, most of the distribution was either clearly Mediterranean or not. Last, the recently developed Bayesian implementation of BiSSE (Silvestro *et al.* 2014) could be potentially useful to take into account phylogenetic uncertainty, but this approach cannot yet be applied to phylogenies with terminally unresolved clades such as ours (D. Silvestro, pers. comm.). Hence, the BiSSE model was the most appropriate to deal with our data.

As currently implemented, BiSSE does not accommodate unresolved clades of more than 200 species (FitzJohn et al. 2009), which has proven to be a problem in other studies (e.g., Wiens 2011; McDaniel et al. 2012). This was problematic here for the genus *Grevillea*, which includes 359 species and for which there is currently no comprehensive phylogeny. Therefore, we used the morphological taxonomic classification of the genus to break this clade into four pseudo-clades. Makinson (2000) recognized thirty-three subgroups, some of which were assumed to be more or less closely related to each other based on their morphology. Using these putative relationships, we assembled these subgroups into four clusters: “Grevillea1” (one species), “Grevillea2” (112 species), “Grevillea3” (90 species), and “Grevillea4” (156 species). To approximate a polytomy giving birth to all four clusters, they were arranged in a way so that the clusters diverged from each other early within a short time span (0.5 Ma): (Finschia:11.1947, ((Grevillea1:10.5, Grevillea4:10.5):0.5, (Grevillea2:10.5, Grevillea3:10.5):0.5): 0.1947). Divergence times within *Grevillea* are actually unknown. However, the genus is the largest in the family, the majority of its species are in Mediterranean hotspots, and most of its species are zygomorphic (Table II.S1). We therefore chose the short

divergence times above specifically to remain conservative with respect to the questions asked in our study. We acknowledge that this strategy might introduce an artificial short burst of diversification, but longer divergence times and therefore younger subclades would result in an even higher artificial increase of diversification rates in *Grevillea*. We then randomly resolved the relationships among these four clades and tested the robustness of our results to three alternative resolutions differing only in topology (same branch lengths): tree 1 = (Grevillea1, Grevillea4), (Grevillea2, Grevillea3); tree 2 = (Grevillea1, Grevillea2), (Grevillea3, Grevillea4); tree 3 = (Grevillea1, Grevillea3), (Grevillea2, Grevillea4). We also tested a different set of branch lengths in which divergences were more evenly distributed: (Finschia:11.1947, ((Grevillea1:3.7316, Grevillea4:3.7316): 3.7315, (Grevillea2:3.7316, Grevillea3:3.7316):3.7315):3.7316).

To run the version of BiSSE that handles unresolved terminal clades, we needed the number of species in each state for each tip (here equivalent to a genus). The number of species in each genus that occur within or outside Mediterranean hotspots were taken from Sauquet et al. (2009) and revised for *Grevillea* based on a thorough review of the data in the Flora of Australia (Makinson 2000). Numbers of hotspot and non-hotspot species in each cluster were compiled using the maps from Flora of Australia and FloraBASE (Western Australia). Species with part of their distribution in SWA and the rest outside of SWA were treated as hotspot species. This gave us a total of 735 non-hotspot species and 1021 hotspot species. These numbers are provided in Table II.S1.

Floral symmetry data were found in the Flora of Australia and other sources for non-Australian species (Rebelo 2001; Weston 2006). These data are also provided in Table II.S1. Precise data on floral symmetry in the genus *Banksia* were not available. Weston (2006) stated that both *Banksia* and the formerly segregate genus *Dryandra* (now a subgroup of *Banksia*) were polymorphic for floral symmetry (i.e., with actinomorphic species and zygomorphic species). As a consequence, *Banksia* was considered unknown in the general results, but we also tested the impact of assuming different ratios of actinomorphy vs. zygomorphy (100/0, 75/25, 50/50, 25/75, 0/100). This gave us a total of 643 actinomorphic species and 944 zygomorphic species when *Banksia* was considered unknown (812:944 when *Banksia* was

assumed to be entirely actinomorphic and 1113:643 when *Banksia* was assumed to be entirely zygomorphic).

There is a possibility that our BiSSE analyses could spuriously identify an effect of the traits of interest on diversification rates when there is none. This could happen if there were heterogeneity in diversification rates across the tree linked to other factors, and if one (or potentially few) large clades coincidentally happened to contain many species with the trait in a given character state. This scenario is most likely to occur in cases with few transitions between character states, which results in a lack of (or pseudo) replication) (Maddison and FitzJohn 2015). To assess whether our analyses are likely subject to this potential pitfall, we mapped ancestral character states on the phylogeny (see Methods S1 for details). In addition, we carried out “split BiSSE” analyses, which allow different rates on different parts on the tree. For each of the two studied traits, we conducted five split analyses, each with a “split” at one of the five nodes identified by MEDUSA as supporting a diversification rate shift. This procedure provides the model with an alternative to attributing the rate shifts to the trait of interest.

## RESULTS

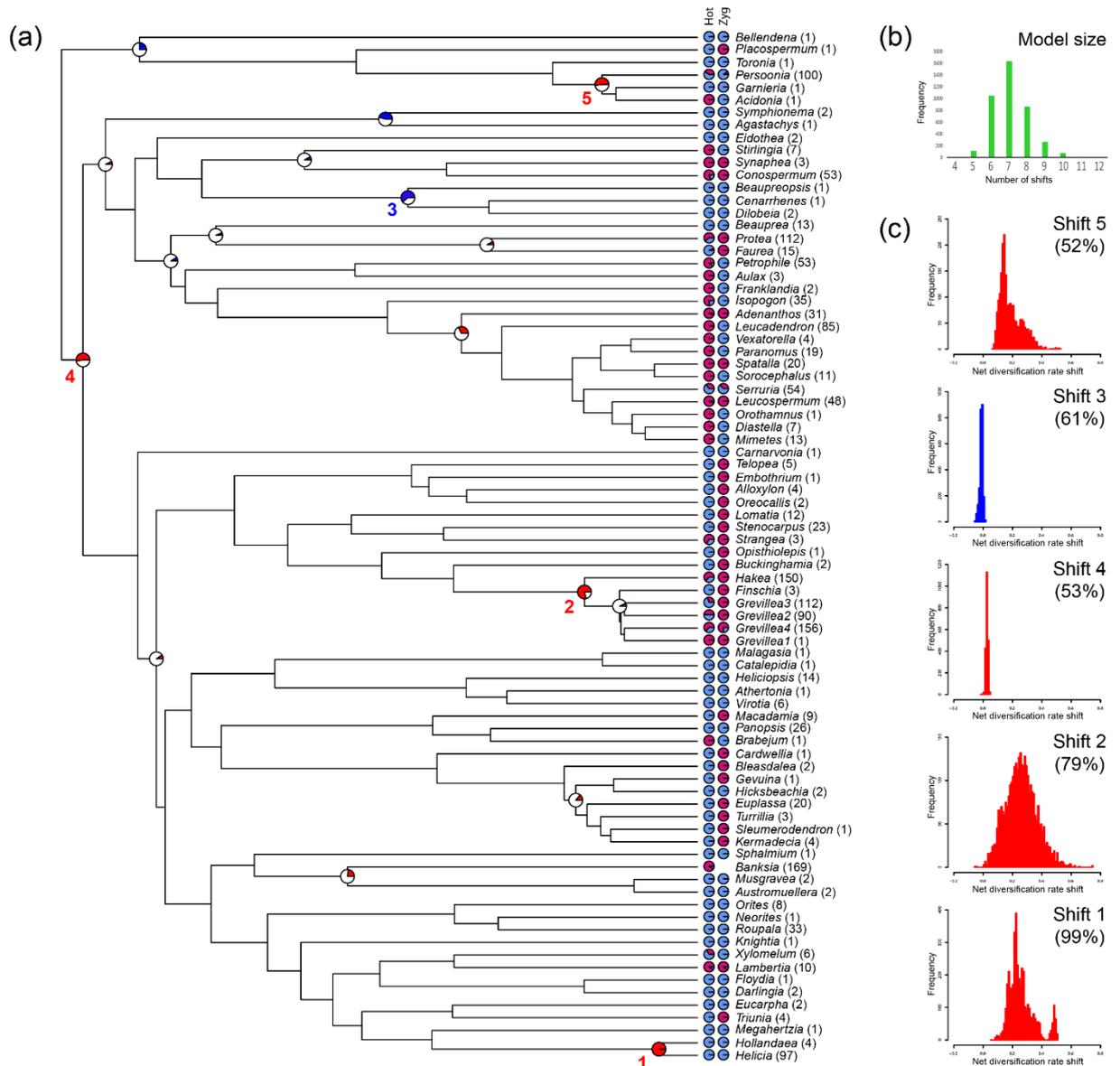
Our MEDUSA analyses of 4000 timetrees of Proteaceae with the birth-death model identified five to eleven rate shifts per tree, with a peak frequency of seven rate shifts (Fig. II.1). Five diversification rate shifts were found in more than 50% of trees. Four of these shifts corresponded to an increase in net diversification rate, and one corresponded to a decrease in net diversification rate. MEDUSA analyses with a mixed model (e.g., the possibility of having both Yule and birth-death processes on the same tree) applied to the same set of trees found a number of rate shifts per tree ranging from seven to sixteen, with a peak frequency of 10 rate shifts, and seven shifts supported in more than 50% of trees. These seven shifts included the five most recurring shifts from the birth-death model. MEDUSA analyses with the Yule model found a number of rate shifts per tree ranging from seven to 17, with a peak frequency of 11 rate shifts, and eight shifts supported in more than 50% of trees. Out of these eight shifts, only

three are shared with the birth-death model top five shifts and four with the mixed model top seven shifts.

Applied to occurrence in Mediterranean-type hotspots, character-dependent analyses identified the model with all parameters different as the best supported model (i.e., lowest AIC; Table II.S2), suggesting that speciation and extinction differ between hotspots and non-hotspot regions, and that transition rates from/to hotspots and non-hotspot regions are significantly different (Table II.1; Fig. II.2a,c; Fig. II.S1). Specifically, we found both speciation and extinction rates to be higher in Mediterranean hotspots than outside them (Table II.1; Fig. II.S1). Interestingly, the difference between speciation and extinction rates (i.e., the net diversification rate) was found to be positive and significantly higher in Mediterranean hotspots than in other regions, where it was found to be negative (Fig. II.2a). In addition, the transition rate from non-hotspot to hotspot was estimated to be much lower than the hotspot to non-hotspot rate (Table II.1; Fig. II.2c). These results were robust to various subclade arrangements within *Grevillea* (results not shown) as well as when the splits within *Grevillea* were more evenly distributed (Table II. S3; Fig. II.S2). The mapping of ancestral states shows that the ‘hotspot’ and ‘non-hotspot’ states are not located in one single or a few clades, suggesting that our results are likely not a spurious outcome of pseudoreplication (Fig. II.S3). The results of the split BiSSE analyses corresponding to the main part of the tree were consistently similar to those of the main analyses, further supporting our results. The only exception was the analyses with a split at shift 2, which yielded a positive diversification rate instead of a negative one outside hotspots, but it was still less than a tenth of the diversification rate inside hotspots. We choose to not report results for the portions of the tree that were split off, as some results indicate that they may be too small to have decent statistical power.

Applied to floral symmetry, character-dependent analyses also identified the model with all parameters different as the best-supported model, suggesting that speciation and extinction rates differ between actinomorphic and zygomorphic species and that transition rates from/to actinomorphy and zygomorphy are significantly different (Table II.2; Table II.S2; Fig. II.2d; Fig. S1b,d). Specifically, we found speciation and extinction rates to be higher for zygomorphic than actinomorphic species, and transitions to be more frequent from actinomorphy to zygomorphy than from zygomorphy to actinomorphy (Table II.2; Fig. II.S1). However, net

diversification rates have completely overlapping credibility intervals and therefore do not appear to differ significantly between actinomorphic and zygomorphic species (Fig. II.2b). Because data on floral symmetry were missing or ambiguous for the genus *Banksia*, we initially treated it as unknown for this trait. The results were robust when we tested different ratios of actinomorphic vs. zygomorphic flowers in the genus (Figs. II.S4, S5, S6). Only when treating the entire genus as zygomorphic did we find a (non-significant) tendency for zygomorphic species to have higher net diversification rates than actinomorphic species (Fig. II.S4f). These results were robust to various subclade arrangements within *Grevillea* (results not shown) as well as when the splits within *Grevillea* were more evenly distributed (Table II.S6; Fig. II.S2). Similarly to the ‘hotspot’ analyses, the mapping of ancestral symmetry showed that zygomorphy appeared several times in Proteaceae, yielding confidence that the BiSSE results are not spurious (Fig. II.S7). The results of the split BiSSE analyses corresponding to the main part of the tree consistently show the zygomorphic species to have a diversification rate slightly higher than that of actinomorphic species. However, the difference between the two the diversification rates is small and similar to the ML results from the core BiSSE analysis, suggesting that they are not significantly different. We choose to not report results for the portions of the tree that were split off, as some results indicate that they may be too small to have decent statistical power.



**Figure II. 1.** (a) Genus level time-calibrated phylogenetic tree of Proteaceae from Sauquet *et al.* (2009), with significant shifts in diversification rates obtained from the multiMEDUSA analysis of 4000 trees randomly sampled from the BEAST posterior. The numbers of extant species in each genus are indicated in parentheses. Node pie charts indicate the proportion of trees in which a significant shift was detected at the node, for the 17 nodes where a shift was detected in at least 5% of the trees. Terminal pie charts represent the proportion of species (magenta) in each state for the two traits of interest considered in the character-dependent analyses (Hot = proportion of species in Mediterranean-climate regions; Zyg = proportion of zygomorphic species). (b) Distribution of the number of rate shifts detected across the sample of trees. (c) Distributions of the magnitude (difference in net diversification rate) of the rate shifts for each of the five most recurrent shifts (identified with the same node numbers as on the tree). Colors indicate whether the shift corresponds to an increase (red) or a decrease (blue) in net diversification rate. Percentages specify the proportion of trees concerned.

**Table II.1.** Parameter estimates obtained from the BiSSE analysis testing the impact of occurring in Mediterranean hotspots on diversification rates in Proteaceae (mean estimates followed with 95% confidence intervals).  $\lambda$  = speciation rate;  $\mu$  = extinction rate;  $r$  = net diversification rate ( $\lambda - \mu$ );  $\varepsilon$  = relative extinction rate ( $\mu / \lambda$ );  $q$  = transition rate.

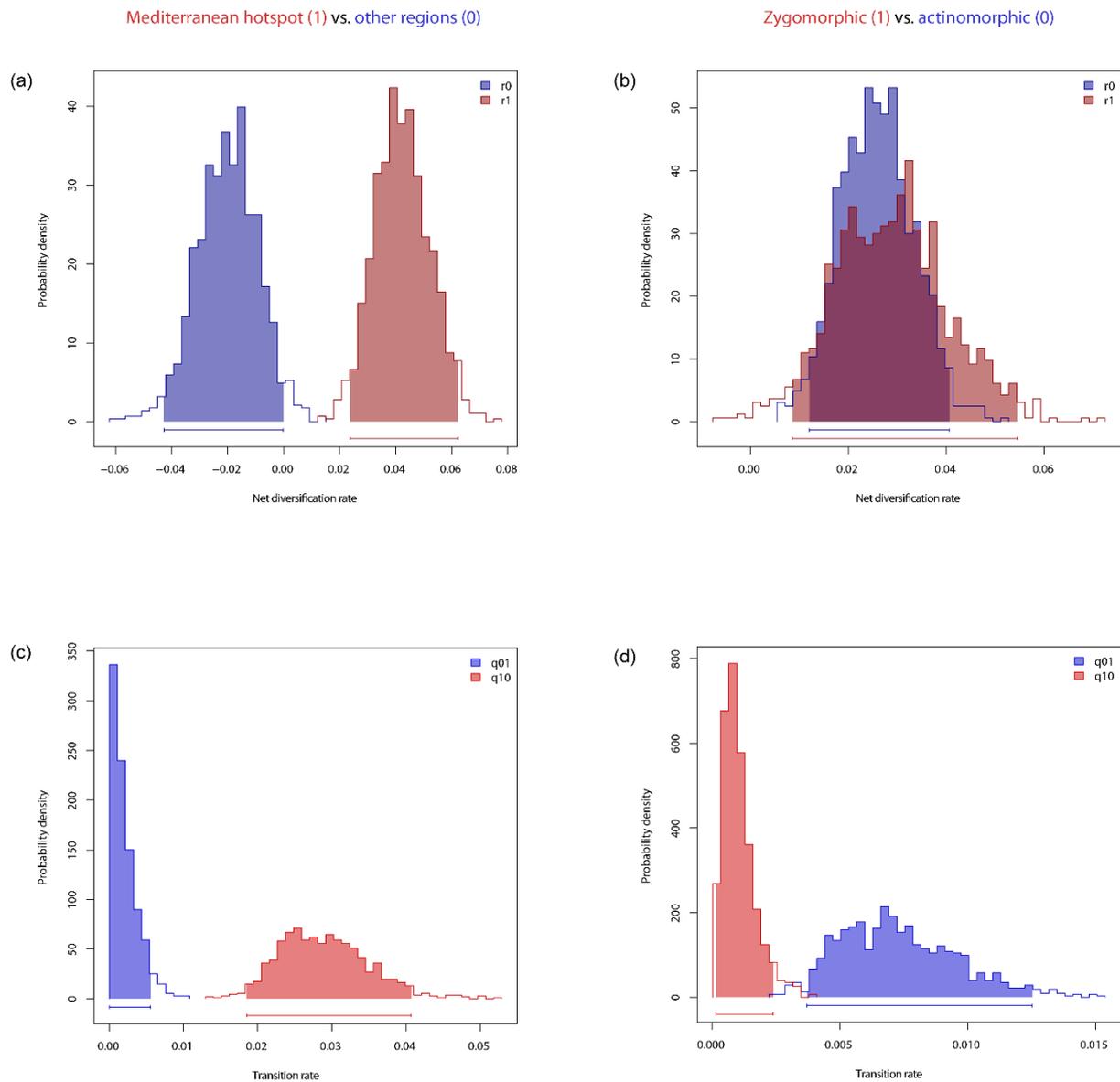
	$\lambda$	$\mu$	$r$	$\varepsilon$	$q_{01}$	$q_{10}$
non-hotspot (0)	1.06 (0.84 – 1.25)	1.08 (0.89 – 1.27)	-0.02 (-0.04 – 0.01)	1.02 (1.00 – 1.04)	0.002 (0.000 – 0.006)	0.029 (0.019 – 0.041)
hotspot (1)	1.62 (1.39 – 1.81)	1.58 (1.34 – 1.78)	0.04 (0.02 – 0.06)	0.97 (0.96 – 0.99)		

## DISCUSSION

### Proteaceae diversification rates

The estimates of the background net diversification rate for Proteaceae are slightly lower, but similar to those previously reported for the family as a whole (Sauquet et al. 2009). Diversification rates, however, are not homogeneous across the Proteaceae family (Fig. II.1). Our analyses of rate shifts confirm results by Sauquet et al. (2009), which showed that some clades within Proteaceae have diversified much faster than others. Previous studies with the MEDUSA model have identified a similar number of significant shifts for clades much larger than Proteaceae. Among angiosperms, 13 diversification rate shifts have been found by Baker & Couvreur (2013) for palms (~2500 species) and six among the Malpighiales (~16,000 species) by Xi et al. (2012). Among Metazoa, Moreau and Bell (2013) found ten shifts among 12,500 ant species, and the original Alfaro et al. (2009) study on the approximately 60,000 gnathostome species found nine diversification rate shifts. Many more empirical studies will be required to assess whether there is a scale effect. Indeed, one shift was identified within mammals by Alfaro et al. (2009) and 11 within the same clade by Rabosky et al. (2012), but the two studies differed in the number of terminal unresolved clades used to represent the 5,279

mammals (4 and 149, respectively; see also Yu et al. 2012). While these previous studies also show that finding several rate shifts is not exceptional, we note that the other known groups in which a similar number of shifts has been found so far have much higher diversity than Proteaceae.



**Figure II.2.** Parameter estimates obtained for the best BiSSE models selected for each trait of interest. (a,c) Presence in Mediterranean climate regions (red) vs. other regions (blue). (b,d) Floral zygomorphy (red) vs. actinomorphy (blue), assuming *Banksia* is unknown for this trait. (a,b) Net diversification rates. (c,d) Transition rates. Additional parameter estimates for these analyses are illustrated in Supporting Information (Figs. II.S1, S4).

### *Diversification within versus outside Mediterranean hotspots*

Our results confirm that diversification of Proteaceae in the two Mediterranean biodiversity hotspots, the Cape Floristic Region and Southwest Australia, has been higher than in any other area of their wide distribution, as suggested in a previous study (Sauquet et al. 2009). This contradicts the idea that the great species diversity of SWA and the CFR could be the result of long-term accumulation of species rather than increased diversification rates, at least for certain clades (Linder 2008; Schnitzler et al. 2011). The character-dependent models we used here (Maddison et al. 2007; FitzJohn et al. 2009) allowed a finer analysis than in Sauquet et al. (2009) in which we could disentangle the effect of Mediterranean-climate regions on both speciation and extinction rates. These analyses suggest that higher net diversification rates in Mediterranean hotspots result from significantly increased speciation rates, but not from decreased extinction rates, which instead appear to have also increased. Interestingly, they also suggest that other regions act as a sink of Proteaceae biodiversity, whereby extinction events exceed speciation events but species also migrate from the hotspots (see below). This is compatible with the observation of many species-poor genera, some of them very old, in tropical regions of the Proteaceae distribution, in particular the Australian Wet Tropics. This would be consistent with a “museum” interpretation of tropical rainforests for Proteaceae, a view also matched by the fossil record (Dettmann and Jarzen 1998). Specifically, negative net diversification rates would explain here why tropical Proteaceae are species-poor, while comparatively lower extinction rates would explain why old lineages of Proteaceae are more common in the Tropics than in Mediterranean hotspots. Last, all of our analyses estimated high relative extinction rates (close to 1), suggesting a high turnover of species during the evolutionary history of Proteaceae, a result at least compatible with the abundant fossil record known for the family. However, these results are to be taken with caution, especially due to the difficulty in accurately estimating extinction rates from molecular phylogenies (Rabosky 2010; Quental and Marshall 2010). These results are also conditional on the assumption of constant rates in each region, which might be incorrect if diversification experienced slowdowns, either due to adaptive radiations or other processes, in these new environments (Moen and Morlon 2014).

**Table II.2.** Parameter estimates obtained in the BiSSE analysis of the impact of floral symmetry on diversification rates in Proteaceae (mean estimates followed with 95% confidence intervals).  $r$  = net diversification rate ( $\lambda - \mu$ );  $\varepsilon$  = relative extinction rate ( $\mu / \lambda$ );  $q$  = transition rate.

	$\lambda$	$\mu$	$r$	$\varepsilon$	$q_{01}$	$q_{10}$
actinomorphic (0)	0.65 (0.46 – 0.82)	0.62 (0.42 – 0.80)	0.03 (0.01 – 0.04)	0.96 (0.92 – 0.98)	0.007 (0.004 – 0.013)	0.001 (0.000 – 0.004)
zygomorphic (1)	1.38 (1.23 – 1.54)	1.35 (1.20 – 1.52)	0.03 (0.01 – 0.05)	0.98 (0.96 – 1.00)		

Several factors have been proposed to explain higher net diversification rates of Proteaceae and other plant clades in Mediterranean hotspots, including edaphic specialization, microhabitats, adaptation to fire, and nutrient-poor soils (Cowling et al. 1996; Linder 2003; Barraclough 2006; Lambers et al. 2006; Verdú et al. 2007). However, establishing a causal link between these factors and diversification rates requires the timing of the onset of specific conditions of Mediterranean-climate regions to be consistent with the diversification of taxa in these regions. This question remains open for Proteaceae in general because too few species-level phylogenies are available to address it properly, therefore we prefer not to elaborate further on these factors here.

Our results at first glance seem to contradict those of similar analyses performed at a lower taxonomic scale in the South African genus *Protea* and the Australian genus *Banksia* (Valente et al. 2010; Cardillo and Pratt 2013). Indeed, BiSSE analyses of well-sampled species-level phylogenies of both genera revealed no significant differences in diversification rates for Mediterranean-climate lineages compared to other lineages. Two main reasons might explain this apparent contradiction. First, it is possible that the sample size of these two species-level studies is insufficient for detecting an impact on diversification rates with the BiSSE approach even if it existed (Davis et al. 2013). In particular, both genera are inferred to have originated in the Mediterranean-climate regions and only one (*Protea*) and two (*Banksia*) subclades migrated to other regions, representing 17 (20%) out of 87 and 15 (9%) out of 158 total sampled

species, respectively. For comparison, there are 735 (42%) non-hotspot species in Proteaceae and these are more widespread across the family tree than in these two genera (Fig. II.1). It remains uncertain whether the BiSSE approach has the power of detecting the effect of a trait on diversification rates with so few transitions and such small fractions of species in the non-hotspot state. Second, these two genera might represent exceptions to the general pattern seen in Proteaceae. Similar analyses carried out on a densely sampled species-level phylogeny of Proteaceae, when available, will help understand further this apparent contradiction (Cardillo and Pratt 2013).

An interesting result found in our character-dependent analyses is that the mean rate estimated for the transition to a Mediterranean hotspot ( $q_{01} = 0.002$ ; CI = 0–0.007) is about ten times lower than the mean rate for the transition to a non-Mediterranean region ( $q_{10} = 0.029$  ; CI = 0.019–0.041; Table II.1; Fig. II.2c). This result may be due to the nature of prerequisites for moving from an area to the other: it may be easier for a species that is optimized for absorbing nutrients to thrive on a richer soil than it is for a species that usually grows on richer soils to move to poor soils (e.g., Lambers et al. 2012). This tendency was confirmed in a recent study focusing on the genus *Banksia* (Cardillo and Pratt 2013), in which the authors suspect the more diverse portion of SWA to be a “biodiversity pump” for both its less diverse portion and the non-hotspot parts of Australia. In other words, the situation may closely resemble the biodiversity counterpart to a source-sink dynamic (Pulliam 1988). Most new species would be emerging in the hotspot, but only a fraction of them would be able to last a significant amount of time in the region due to high competition. The non-hotspot regions, on the other hand, would be places where extinction is much more frequent than speciation and in which diversity would be maintained by a flux of species coming from the hotspots. It may seem strange for the non-hotspot regions to be a sink for Proteaceae when it appears that they should thrive in richer soils and less demanding environments. Our results show that extinction is lower outside hotspots, but that the phenomenon is even stronger for speciation, thus resulting in negative diversification. This could be due to Proteaceae having to compete with species that have had a longer time to adapt to non-hotspot environments.

### Floral symmetry and diversification

Our results suggest that floral symmetry had no significant impact on Proteaceae net diversification rates, but that transitions from actinomorphy to zygomorphy are more likely to occur (i.e., have a higher rate) than their reversals in the family. Current developmental evidence on the genetic basis of floral symmetry would suggest instead that zygomorphy can be lost more easily than it is gained (e.g., by a single mutation in a *CYCLOIDEA*-like gene involved in dorsoventral asymmetry; Citerne et al., 2010). Thus, one interpretation of our result is that loss of zygomorphy is sufficiently disadvantageous when it occurs to be counter-selected at the microevolutionary level, perhaps because specialized pollinators would ignore or be less effective on actinomorphic mutant flowers. Nevertheless, our results do support both higher speciation and higher extinction rates for zygomorphic species in the family. Although we note that we should remain careful about interpretation of such analyses in terms of speciation and extinction rather than net diversification (e.g., Leslie et al., 2013), these results are interesting from a biological point of view. Indeed, increased efficiency in pollen transfer allowed by zygomorphic flowers and increased pollinator specialization may increase speciation rates, but at the same time increased specialization may also make zygomorphic species more vulnerable to extinction.

However, the lack of impact of zygomorphy on net diversification in Proteaceae is inconsistent with the global pattern found by Sargent (2004) in angiosperms. It is possible that the balance between speciation and extinction in zygomorphic Proteaceae is different from the one at the scale of angiosperms as a whole. Many Proteaceae species (e.g., *Protea* and *Leucospermum*) have zygomorphic flowers densely clustered in actinomorphic inflorescences, which are the primary visual signal to pollinators, making the possession of zygomorphic flowers less relevant than in other zygomorphic angiosperm clades with solitary flowers or loose inflorescences (e.g., Orchidaceae, Fabaceae, Lamiales). One of the characteristics of SWA Proteaceae, which are more diverse than the CFR Proteaceae, is that they are more likely to be pollinated by vertebrates (e.g., birds and mammals; Hopper & Gioia, 2004), while the visual and pollen deposition advantages for zygomorphy have only been confirmed with insect pollinators (Citerne et al. 2010).

### Methodological limitations

Maddison et al. (2007) and Maddison and FitzJohn (2014) have noted that the BiSSE method can suffer from pseudoreplication. A character may seem to have an influence on speciation and extinction because it occurs in a clade that underwent a significant increase in diversification rate for reasons unrelated to the given character. This is unlikely to be the case for the two characters studied here, because hotspot as well as zygomorphic species occur in multiple replicates across the tree (Figs. II.S3, S7). In addition, our analyses isolating major shifts have confirmed that none of the clades that have undergone an increase in diversification are singlehandedly responsible for the effects of the two studied characters on diversification (Tables II.S5, II.S6). Finally, the fact that floral symmetry has no effect on net diversification demonstrates that the tree does not have a shape that would make BiSSE conclude that *any* tested character has an influence on net diversification.

### Conclusions and perspectives

We used here a genus-level phylogeny of Proteaceae because a species-level phylogeny of the family is not yet available. Analyses at the species level would show more precise, and potentially different results. For example, diversification shifts found at the root of some genera might instead be identified within these genera; new diversification rate shifts may also be detected (e.g., Yu et al., 2012). Thus, similar analyses carried on a detailed species-level phylogeny of Proteaceae, and with other candidate traits potentially influencing diversification, will eventually allow a refined understanding of the factors that have influenced the success of Proteaceae and of particular clades within the family. Still, our study strongly suggests that presence of species in Mediterranean hotspots is associated with higher diversification rates in Proteaceae. There is mounting evidence that diversification rates play a major role in explaining species richness in tropical regions, the predominant hotspots on Earth (Pyron and Wiens 2013; Rolland et al. 2014). If our results expand to other taxonomic groups, this would suggest that diversification rates also play a major role in explaining species richness in the second most predominant hotspots, Mediterranean regions.

## **ACKNOWLEDGEMENTS**

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# CHAPTER 2: Perianth symmetry changed at least 199 times in angiosperm evolution

*Written by Elisabeth Reyes, Hervé Sauquet and Sophie Nadot. Published in the October 2016 issue of Taxon.*

This part of my thesis work was initially intended to test whether zygomorphy was indeed a key innovation in angiosperms. All of the methods available required knowing which portions of the tree included species (or more generally taxa) with a zygomorphic perianth. Existing optimizations of floral symmetry on phylogenetic trees did not provide the needed information, so I decided to produce my own optimization, which became more time consuming than initially planned and transformed into the second chapter of this thesis. The first step hence became to find where zygomorphy had appeared in the angiosperm tree, as well as where it had returned to the ancestral state of actinomorphy (i.e., radial symmetry; Figure I.5). Two of the requirements for this phylogeny were for it to reflect the actual taxonomic diversity of angiosperms and to include as many putative changes in perianth symmetry as possible. Families in which symmetry was monomorphic could be represented by one species, while families in which perianth symmetry changed between actinomorphic and zygomorphic at least once were represented by the number of species necessary to display all the changes undergone by the family. To keep the amount of work reasonable, polymorphic genera were never represented by more than one species in each state. The starting point of my sampling was the species selection used by Magallón et al. (2015), the most recent dated phylogeny including most of the angiosperm families recognized today and for which most of the morphological data were already in the PROTEUS database due to being part of the eFLOWER project. In the process of building my own species sample, several species were added or deleted from the Magallón tree for various purposes. One of the purposes was to add species from families that were not in the tree, another was to add representatives of the other character state in families that were polymorphic for perianth symmetry and had species representing only one state in the Magallón tree. In other cases, we could not find symmetry data for the species from the Magallón tree, but could find such information for another member of the same family. In such cases, the member for which we had the information on symmetry was preferred, regardless of the presence or absence of molecular sequences in GenBank. Several species from my final

sample did not have molecular sequences from closely related species that could be used as surrogates and were the only representatives I could find for some instances of perianth symmetry shifts. In addition, generating new sequences was not part of the objectives of the work for this chapter. Because of this combination of factors, I did not use molecular sequence data to make a chronogram and instead used other methods to build the phylogenetic tree.

To build the tree, we first used the PROTEUS database (Sauquet 2016) to output a tree that reflected the relationships among orders, families and sometimes within families, based on the relationships that had been summarized in the Angiosperm Phylogeny Website (Stevens 2016) in 2011. Resolution was manually added to some families using recently published studies. The family groupings were from APG III (APG 2009); they are similar enough to those of APG IV (APG 2016) to bring no change in the inferences for this study. The function used for dating the tree requires the user to enter ages for as many nodes as possible, and calculates an estimate of the age of the remaining nodes by evenly distributing them between the pre-dated ones. The user-entered dates used came from the Magallón et al. (2015) tree and were entered for 398 family-level and deeper nodes when nodes were shared between my tree and that of Magallón et al. (2015). Unfortunately, the transition rates reconstructed by maximum likelihood on the tree with branch lengths (dated tree) were extremely high, which resulted in all the nodes having identical state probabilities. We were only able to reconstruct ancestral states with parsimony, a non model-based method insensitive to branch-length. Due to parsimony having the same results regardless of branch lengths, we displayed the results on a non-dated tree. The parsimony results showed a number of changes far superior to those reported in the latest article to have counted them, Citerne et al. (2010). Where the latter had estimated about 70 origins of zygomorphy across angiosperms, I found close to 200 changes, among which at least 130 were origins. The true number of changes might be even higher, as the sampling method used limits the number of changes per genus to one, and some of the larger genera may have more. In addition, methods that take branch lengths into account allow more changes to happen on longer branches, which makes the number of changes found by parsimony likely to be a low estimate (see also the difference between parsimony and maximum likelihood reported in the derivative of the study presented in Chapter 1). I considered this to be an interesting enough result on its own to make an article out of it.

I decided to accompany the tree with a review of various aspects of perianth symmetry, which were to be occasionally linked to the changes I observed. My review of ontogeny showed that perianth zygomorphy could be expressed at various stages in flower development, ranging

from sepal initiation to pre-anthesis stages. This stage can vary between closely related clades. In addition, some species that are actinomorphic at anthesis can have early zygomorphy (during development) while not being closely related to zygomorphic species. These two facts show that the developmental stage at which perianth zygomorphy becomes detectable is a labile trait. My review of the genetic control behind zygomorphy revealed that all zygomorphic clades in which it has been studied have recruited either *CYCLOIDEA* or a closely related gene for this control (Hileman 2014; Horn et al. 2014). Through the review of the selective advantages and disadvantages of zygomorphy, I saw that zygomorphy could be a promoter of species diversification or the cause of too extreme specialization that can lead to extinction depending on the group. I also considered the possibility that perianth zygomorphy could be a hitchhiking character state (e.g. a state that tends to appear alongside another trait that is the actual cause of higher diversification) in some clades where it is seemingly correlated with higher diversity.

# **Perianth symmetry changed at least 199 times in angiosperm evolution**

Elisabeth Reyes, Hervé Sauquet, Sophie Nadot

Écologie Systématique Evolution, Univ. Paris-Sud, CNRS, AgroParisTech, Université Paris-Saclay, 91400, Orsay, France

Reyes E, Sauquet H, Nadot S. 2016. *Taxon*. 65(5): 945-964

## ABSTRACT

Perianth bilateral symmetry (zygomorphy) has evolved repeatedly from radial symmetry (actinomorphy) throughout angiosperms. Zygomorphy has previously been linked with plant-pollinator specialization and higher species diversification. However, the exact number of transitions to and from zygomorphy has remained so far unknown. We recorded perianth symmetry from 761 species, selected to represent all 61 orders and 426 currently accepted families of angiosperms and to include all presumed origins of perianth zygomorphy. We then reconstructed the evolution of perianth symmetry on a consensus backbone tree, using parsimony. We found perianth zygomorphy in 32 orders and 110 families. There was a minimum of 130 origins, almost the double of what was previously estimated, and 69 reversals to actinomorphy. Among the origins, two were in magnoliids, 29 in monocots, 17 in basal eudicots, 35 in superrosids and 47 in superasterids. Among the reversals, eight were in monocots, four in basal eudicots, 18 in superrosids and 39 in superasterids. This study shows that there has been many more origins of perianth zygomorphy and reversals to actinomorphy than previously shown. We then use this new framework to review the developmental evidence of changes in floral symmetry, showing convergence in the early stages of zygomorphy across angiosperms at the developmental level. We also review the evidence on the genetic control of floral symmetry, suggesting that a restricted number of genes has been recruited multiple times independently to achieve zygomorphy. In contrast to its relative homogeneity at the early developmental and molecular level, zygomorphy appears to be highly variable in its morphological expression at anthesis, involving various processes such as perianth part displacement and differentiation. We then review recent hypotheses on the relationship between floral symmetry, floral orientation, and pollination mode in terms of selective advantages and constraints. Our comprehensive angiosperm-wide reconstruction of floral symmetry evolution provides a new context for future studies on the developmental, functional, and macroevolutionary aspects of floral symmetry.

Key words: actinomorphy, ancestral state reconstruction, angiosperms, floral evolution, parsimony, perianth symmetry, zygomorphy.

## INTRODUCTION

Flowers, the reproductive structures that set angiosperms apart from other land plants, have a relatively conserved groundplan consisting of a short axis bearing, from the center out, ovules protected by carpels, stamens and a perianth (Bateman et al. 2006). Each of these elements can vary greatly in size, shape, color and number, leading to the diversity seen in extant flowers. Some derived floral traits may be found in many clades among the angiosperms. One of such recurring traits is perianth zygomorphy, or bilateral symmetry, which may affect the androecium and gynoecium as well. Actinomorphy, or radial symmetry, on the other hand, is considered to be ancestral in angiosperms (Endress 2011). Zygomorphic flowers restrict the angle by which a pollinator can approach and move within them, hence making pollen placement on the pollinator more precise and inducing reproductive isolation between flowers that place pollen on different parts of the pollinator's body (Sargent 2004).

Some of the most species-rich families, such as Orchidaceae and Fabaceae, include a majority of zygomorphic species. The recurrence of speciose clades in which the majority of species have zygomorphic flowers has led to the widespread idea that zygomorphy in flowers is a key innovation, a trait that has a significant, positive impact on species diversification (Hunter 1998). However, this conclusion has been reached mostly by comparing sister clades with contrasted floral symmetries (Sargent 2004), while ignoring other related clades. When considering all angiosperms, it appears that many entirely actinomorphic families are quite speciose while there are zygomorphic families with very few species. Families that include a majority of zygomorphic species, on the other hand, may in fact contain several origins of zygomorphy.

Patterns in the evolution of floral symmetry, in terms of exact number of transitions, still remain incompletely known. The most recent angiosperm-wide survey found a minimum of 70 origins of zygomorphy (Citerne et al. 2010). However, understanding of phylogenetic relationships within angiosperms is constantly being improved (Moore et al. 2010; Soltis et al. 2011; Wickett et al. 2014; Zeng et al. 2014), enabling patterns of transitions in floral characters to become more and more open for detailed scrutiny.

The main objective of this study was to identify the majority of shifts in floral symmetry throughout angiosperm evolution. To do so, we took an exemplar approach, sampling 761 species chosen to represent all of these transitions as well as all currently recognized angiosperm families. We show with parsimony reconstructions on the latest phylogeny of angiosperms that the total number of transitions to and from zygomorphy is much higher than previously estimated. Using this new landmark reconstruction, we then review current evidence on the developmental control of floral symmetry at the molecular level, the diversity of zygomorphy expression at the morphological level, and the selective advantages and constraints of zygomorphy.

## **MATERIALS AND METHODS**

We used an exemplar approach to record perianth symmetry in a large sample of angiosperm species. Since our purpose was to reconstruct all changes in perianth symmetry across angiosperms, our choice of exemplar species was guided by this particular trait, as explained below. Our focus on the perianth makes this character inapplicable to entirely perianthless families (i.e., those in which all known species lack any kind of perianth part, such as Piperaceae and Ceratophyllaceae), regardless of the symmetry of reproductive organs. However, we decided to include representatives of such families (scored with missing data) so that all currently recognized families would be sampled. All data were scored in the PROTEUS database (Sauquet 2016). APG III (APG 2009) was followed for order and family delimitations, except where new families have been described and widely accepted since (e.g., the former paraphyletic Olacaceae s.l. that are now seven monophyletic families in Santalales (Nickrent et al. 2010)). For names of all clades above the order level, we followed Cantino et al. (2007) and Soltis et al. (2011).

### *Character coding and taxon sampling*

It was first necessary to determine which families included zygomorphic species. Both visible differentiation in the shape or size of perianth parts and differential curvature of the perianth were considered forms of zygomorphy, which is defined here as the presence at anthesis of a single plane of perianth symmetry (Endress 1999). Following this definition, we included several genera subject to “non-structural” zygomorphy, such as *Alstroemeria* L. and *Thismia* Griff. (Rudall and Bateman 2004). It might seem more appropriate to score with

different character states the many forms of zygomorphy observed across angiosperms. However, at present, insufficient developmental and structural data are available to objectively attempt such characterization without assuming that all species of a systematic group follow the same process as those few species that have been investigated more thoroughly. In this study, our goal is instead to highlight the recurrent evolution of the attribute of perianth bilateral symmetry that most botanists easily recognize, without phylogenetically guided assumptions on the genetic and developmental processes that lead to this attribute. By mapping this attribute onto the phylogeny, we can then infer a number of independent origins, which might help guide more research in understanding the processes leading to this functionally important, recurring attribute. This is similar to studies investigating wood vs. herbaceous habit evolution in seed plants, where this trait has been scored as a binary character (e.g. Ricklefs and Renner 1994; Yang et al. 2008; Soltis et al. 2013).

As a starting point, we used family-level descriptions from *The Families of Flowering Plants* (Watson and Dallwitz 2016), and the online *Flora of North America*, *Flora of China* and *Flora of Pakistan*. These sources were only used to score species explicitly cited in them. Additional sources including morphological studies, taxonomic descriptions and revisions, and other online databases (in total 282) were investigated for families or species not covered by these sources (Appendix III.1). When descriptions did not explicitly mention perianth symmetry, drawings attached to taxonomic descriptions and trusted photos from *Encyclopedia of Life* (ww.eol.org) were used. For all families presenting no variation in perianth symmetry, we selected and scored a single representative species, chosen from the list of species explicitly listed in the flora. Polymorphic families were sampled so as to include at least one actinomorphic and one zygomorphic species. Available phylogenies for polymorphic families were taken into account so as to represent all transitions to and from zygomorphy in such families (e.g. Knapp 2010; Reyes et al. 2015). When no phylogeny was available for a polymorphic family, at least two species in each state were sampled as a reminder that multiple changes were possible. Species presenting both actinomorphy and zygomorphy due to sexual dimorphism (e.g. *Cissampelos pareira* L.) or intra-inflorescence variation (e.g. ray and disc florets of many species of Asteraceae) were scored as polymorphic in PROTEUS, but then rescored as zygomorphic for the present study, as our primary purpose here was to record origins of zygomorphy.

There are two other, rarer types of symmetry in angiosperms: disymmetry (two perpendicular symmetry planes) and asymmetry (no recognizable symmetry). We treated them as missing data in the analyses presented here. In families in which they coexist with other types of symmetry (e.g. Papaveraceae, Begoniaceae, Marcgraviaceae), they were usually ignored. Marantaceae and Cannaceae (Zingiberales) are entirely asymmetric. Gunneraceae, Halophytaceae and Didiereaceae are disymmetric. Urticaceae and Begoniaceae are each represented by a species in which one sex has a disymmetric perianth, while the other is either actinomorphic (*Urtica* L., some *Begonia* L.) or zygomorphic (*Begonia*); these species are represented by the data of their non-disymmetric sex. Although some authors have described non-zygomorphic Brassicaceae as actinomorphic (Hileman and Cubas 2009; Busch et al. 2012), careful morphological studies show that they are instead disymmetric (Endress 1992; Patchell et al. 2011). We indicate the location of disymmetric Brassicaceae species on our angiosperm tree with missing data to illustrate variation within the family, which is quite frequent. In total, perianth symmetry was recorded for 761 species representing all 61 orders and 426 families of flowering plants.

### Phylogenetic tree

The backbone tree used here is the consensus tree from the Angiosperm Phylogeny Website (Stevens 2016). It was not possible to build a new molecular phylogenetic tree including all the species from our sample, given available sequence data in GenBank; due to excessive missing data, the resulting tree would have been incomplete and unreliable. An alternative approach would have been to design a study based on available angiosperm-wide dated molecular trees (Sauquet et al. 2015). However, without considerable additional DNA sequencing effort, this approach does not allow us to sample all transitions in a given trait, which was the primary focus of the study presented here. We thus chose to build on the Angiosperm Phylogeny Website tree topology, and to use recently published phylogenetic studies to resolve within-family relationships where possible (Table. III.S1).

### Ancestral state reconstruction

The character state matrix for perianth symmetry was exported from PROTEUS as a NEXUS matrix. Ancestral state reconstruction was performed using parsimony in Mesquite 3.04 (Maddison and Maddison 2015) (Appendix III.2). Polytomies were treated as hard polytomies, in which all branches are treated as having diverged from the same common

ancestor. We counted the number of origins of zygomorphy in two ways. The minimum number of origins treats the branches of the tree with equivocal reconstruction as zygomorphic. The maximum number of origins for the tree was found by treating all branches with equivocal reconstruction as actinomorphic.

## RESULTS

We found perianth zygomorphy in 30 orders (Fig. III.2) and 110 families of angiosperms (Table III.S2). Of these, two orders and 41 families are entirely zygomorphic to the best of our knowledge. In total, we found 199 transitions in perianth symmetry (Fig. III.2). The minimum number of origins is 130, in which case there are 69 reversals. The maximum number of origins is 164, in which case there are 35 reversals. Hereafter, the main result will be given for the minimum origins scenario, followed by the number of the maximum origins scenario (in parentheses) when relevant.

Actinomorphy is ancestral in angiosperms and zygomorphy evolved in magnoliids, eudicots, and monocots (Fig. III.1). The number of origins was two in magnoliids, 29(--33) in monocots, 17(--18) in basal eudicots, 35(--44) in superrosids and 47(--67) in superasterids.

### Magnoliids

Two independent origins were found in magnoliids, which consist of 19 families and four orders. They concern *Aristolochia* L. (Aristolochiaceae) and *Glossocalyx* Benth. (Siparunaceae) (Fig. III.2A).

### Monocots

Twenty-six of the 80 families of monocots include zygomorphic species; they are distributed in eight out of the 11 orders (Fig. III.2A and III.2B). Despite the presence of zygomorphy in the majority of orders, more than 40% of the origins are found in Asparagales. Within the order, five of the 10 origins are in the Amaryllidaceae. There are two unambiguous reversals in Commelinales and two more in Asparagales (both in Orchidaceae). Zygomorphy is inferred to be ancestral in Acorales and Zingiberales.

### Basal eudicots

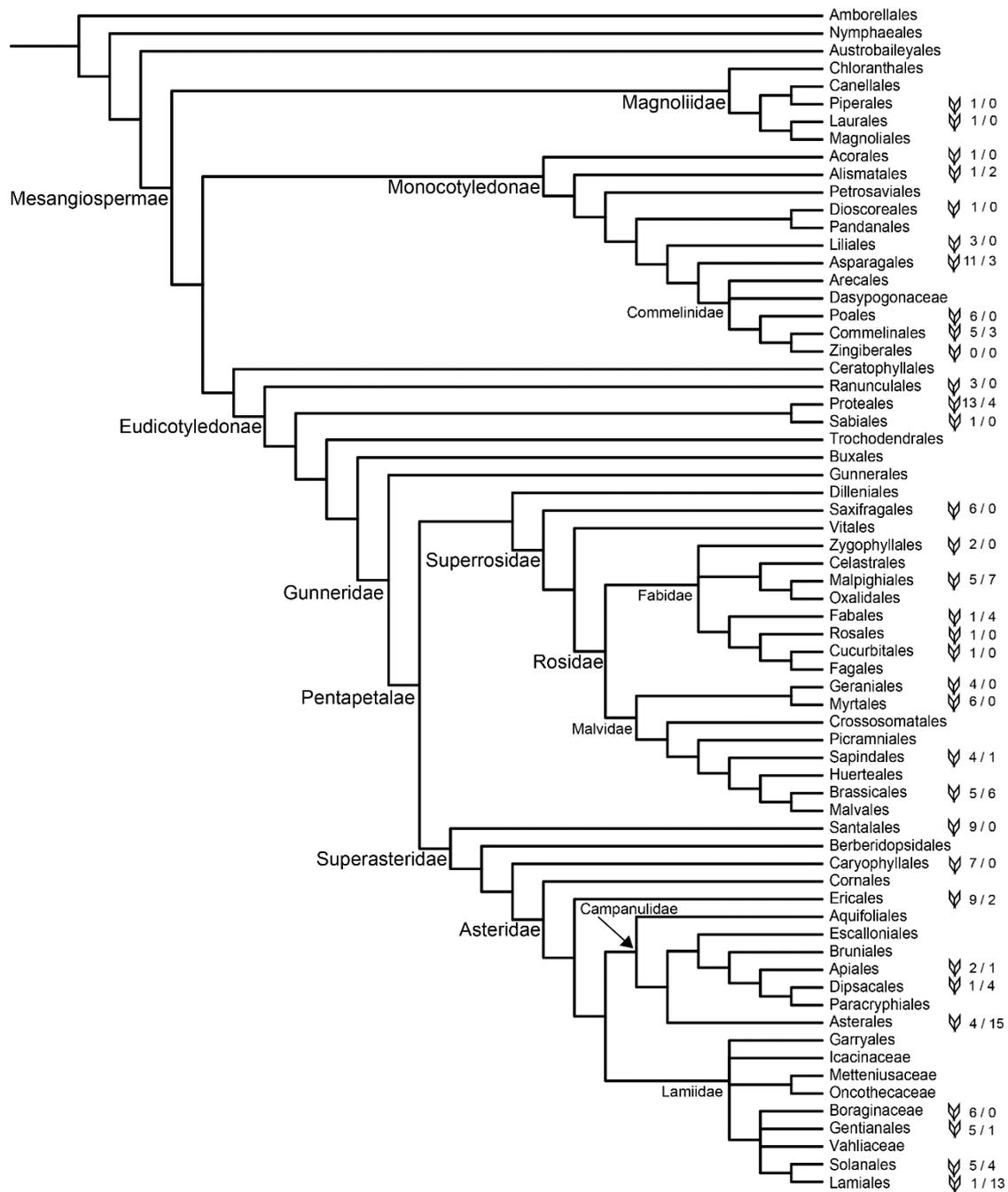
Five families and three orders of basal eudicots, which have 13 families and five orders, have zygomorphic species (Fig. III.2B). Out of the 17 origins of zygomorphy, 13 are found in Proteaceae (Proteales), three in Ranunculales, and one in Sabiaceae. All reversals are in Proteaceae.

### Superrosids

Thirty-one of the 153 superrosid families, and 10 out of 19 orders have zygomorphic species (Fig. III.2C and III.2D). Most origins are independent from each other in both minimum and maximum scenarios. One of the exceptions is the clade formed by Capparaceae, Cleomaceae and Brassicaceae. The two clades with multiple reversals are Fabales and Malpighiaceae.

### Superasterids

Forty-four of the 149 families of superasterids have zygomorphic species (Fig. III.2E, III.2F and III.2G). They are distributed in nine out of 16 orders. Unambiguous reversals are inferred in Asteraceae, Lamiales, Solanaceae, and Caprifoliaceae. In the minimum origins scenario, zygomorphy is ancestral, and a synapomorphy of the entire order Lamiales.



**Fig. II.1.** Order-level phylogeny of angiosperms based on the Angiosperm Phylogeny Website (Stevens, 2001–) highlighting all orders in which perianth zygomorphy evolved. For each order with zygomorphy, we indicate the number of origins followed by the number of reversals in the minimum origins scenario. Ordinal and supra-ordinal names follow APG III (2009) and Cantino et al. (2007), respectively.

Figure III.2A

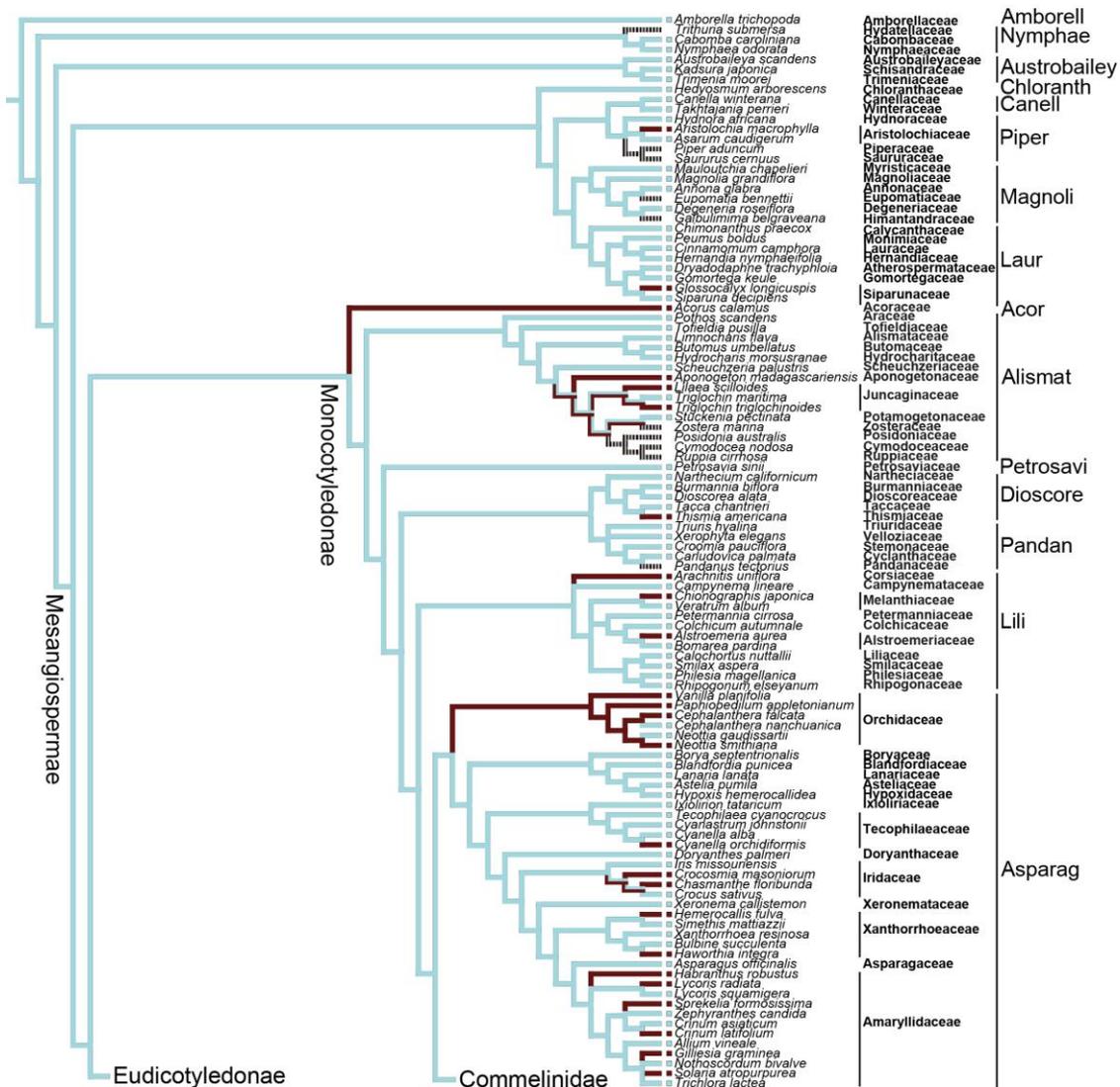
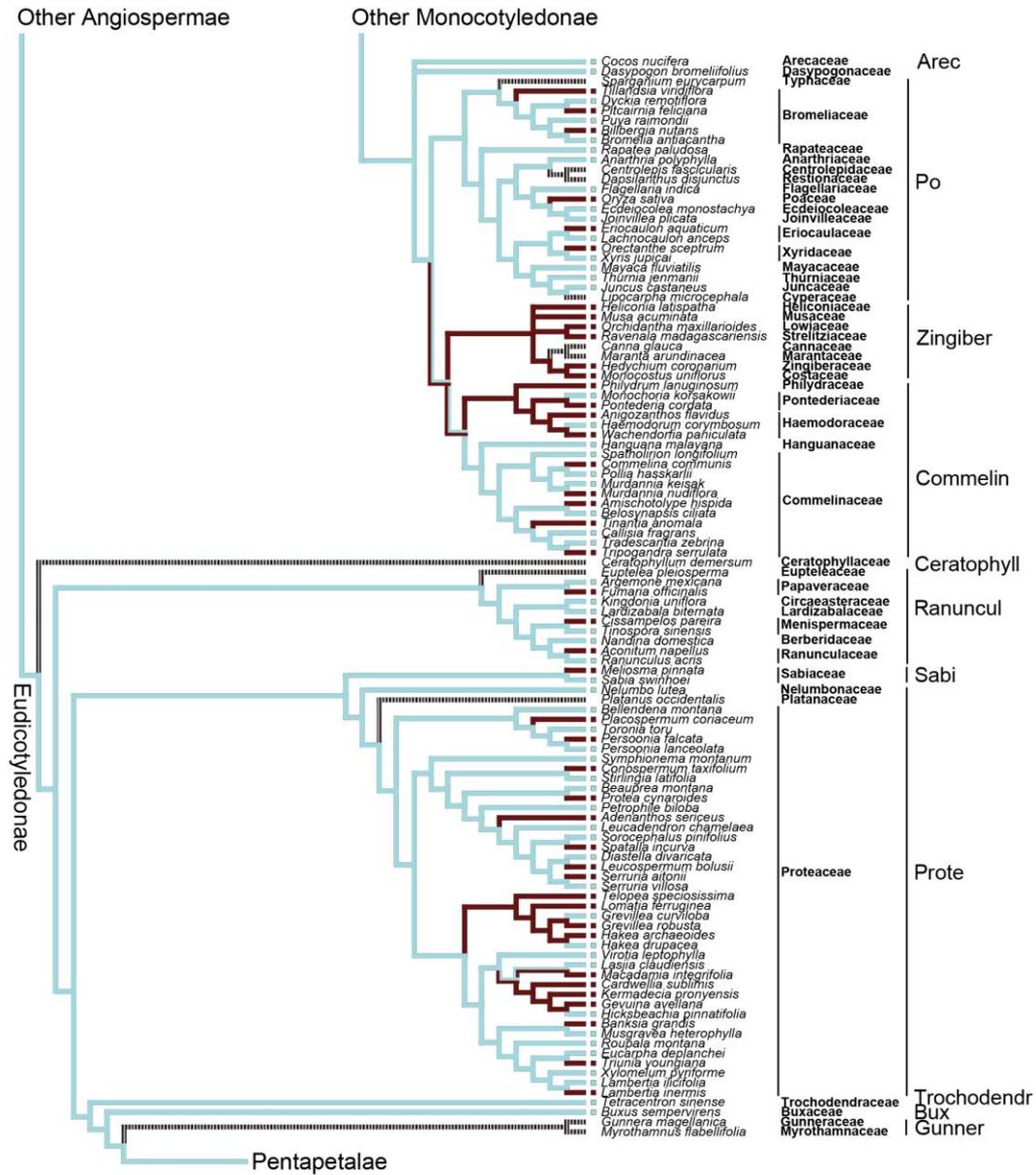


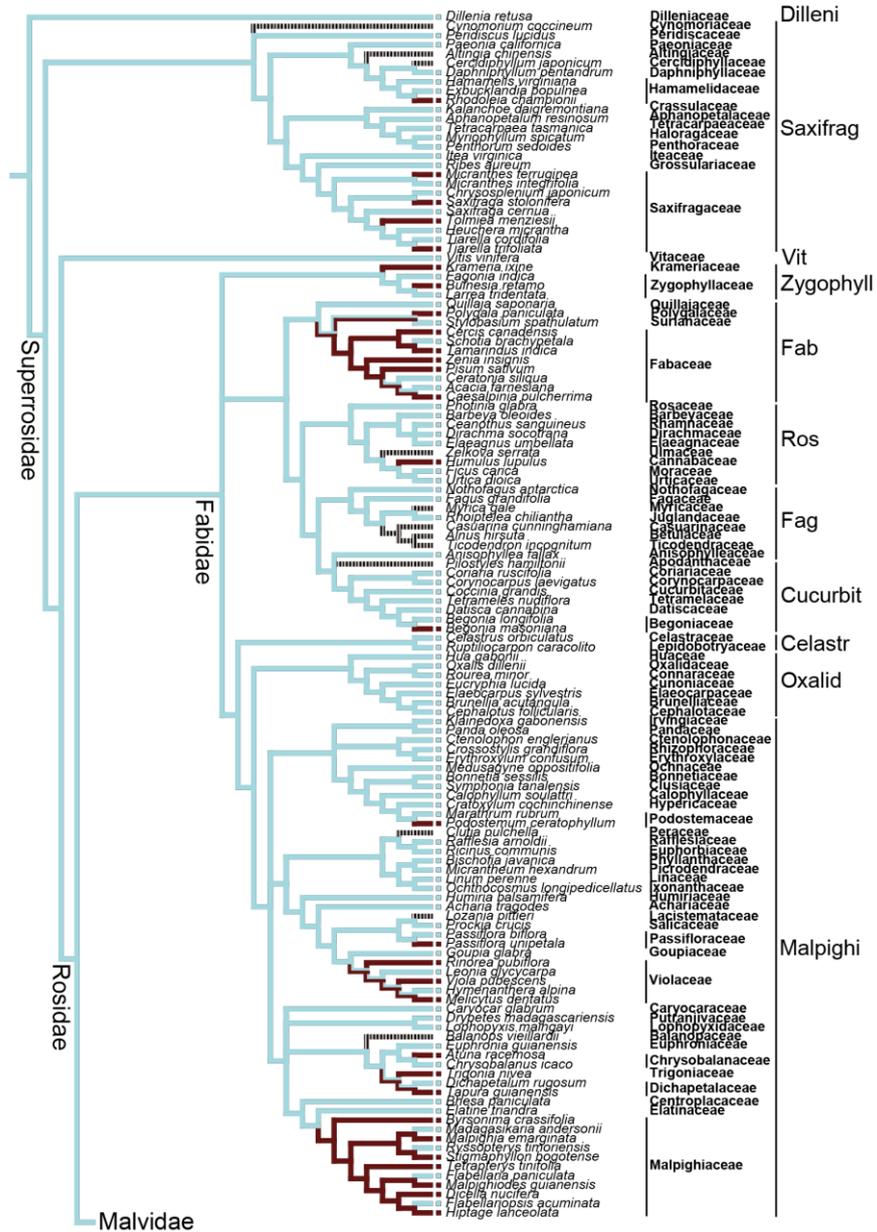
Fig. II.2. A, Basal angiosperms (including magnoliids), monocots excluding Commelinidae; B, Commelinidae, basal eudicots; C, basal superrosids; D, Malvidae; E, Basal superasterids; F, Campanulidae; G, Lamiidae. The order names are transcribed without the “ales” suffix.

Figure III.2B



- actinomorphic
- zygomorphic
- equivocal
- data missing or not applicable

Figure III.2C



■ actinomorphic  
■ zygomorphic  
■ equivocal  
 data missing or not applicable

Figure III.2D

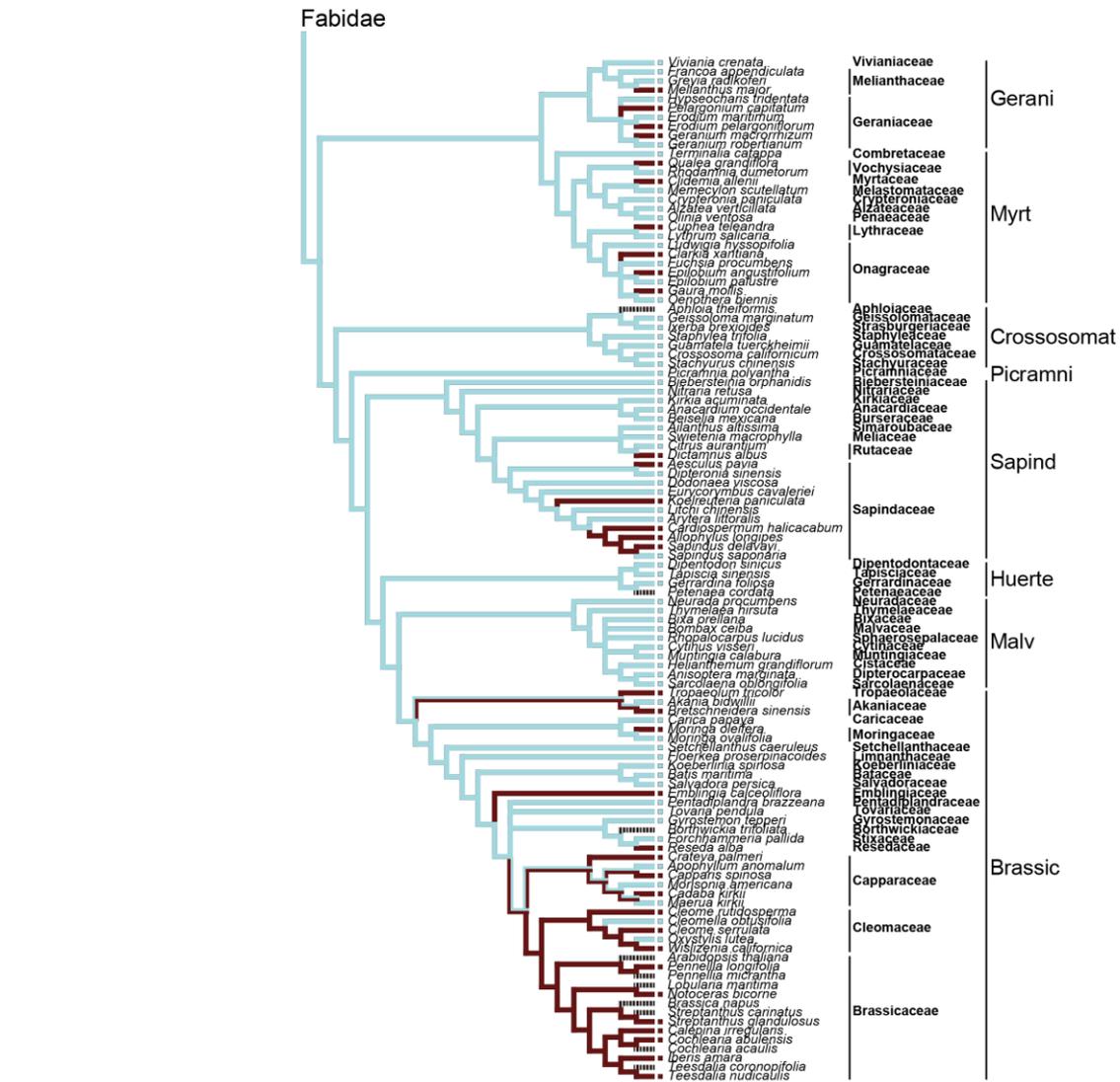
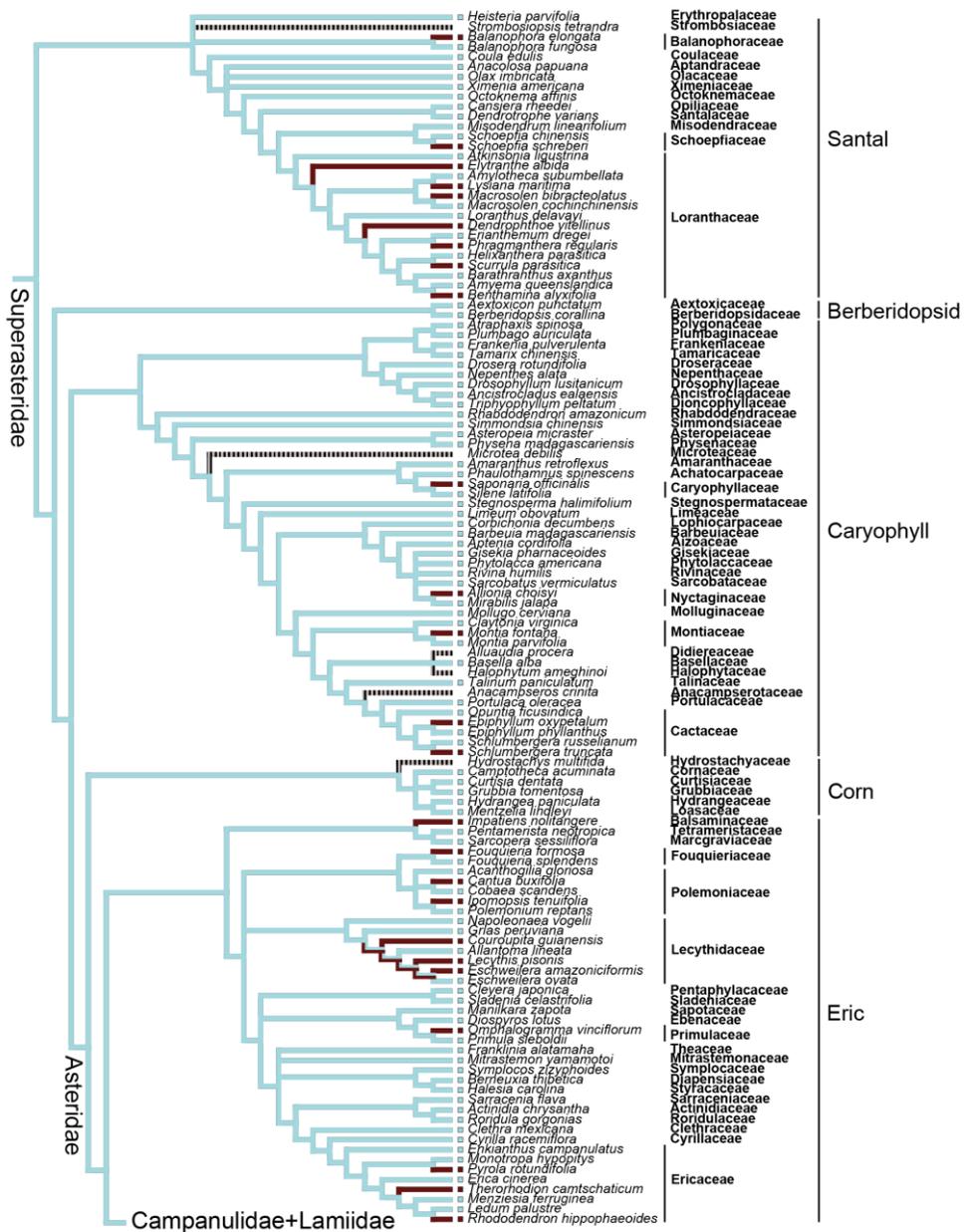
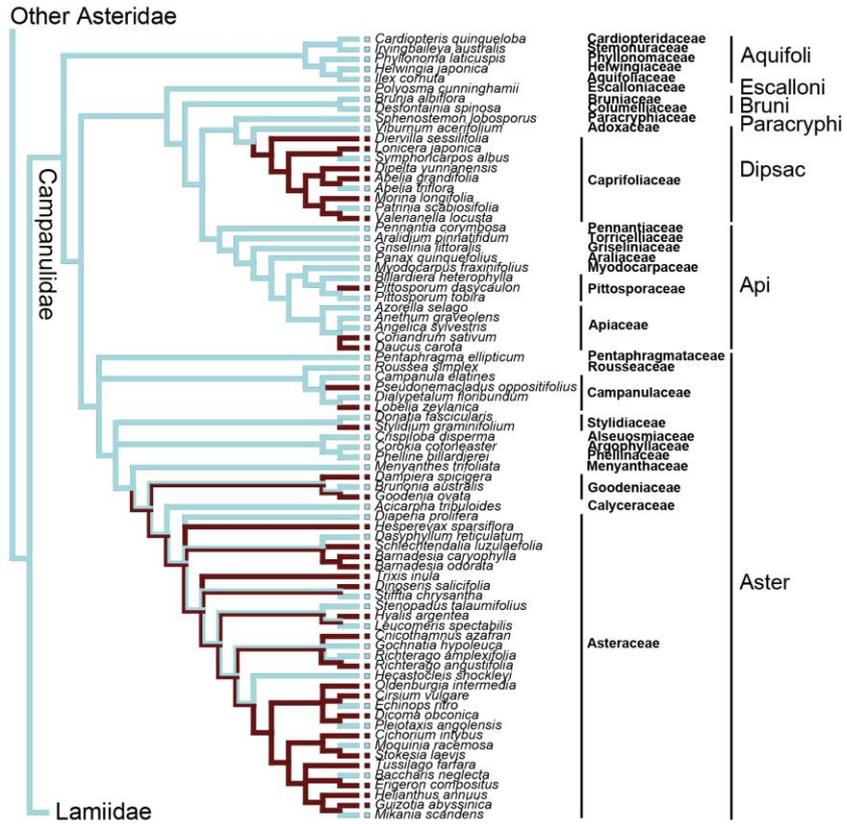


Figure III.2E



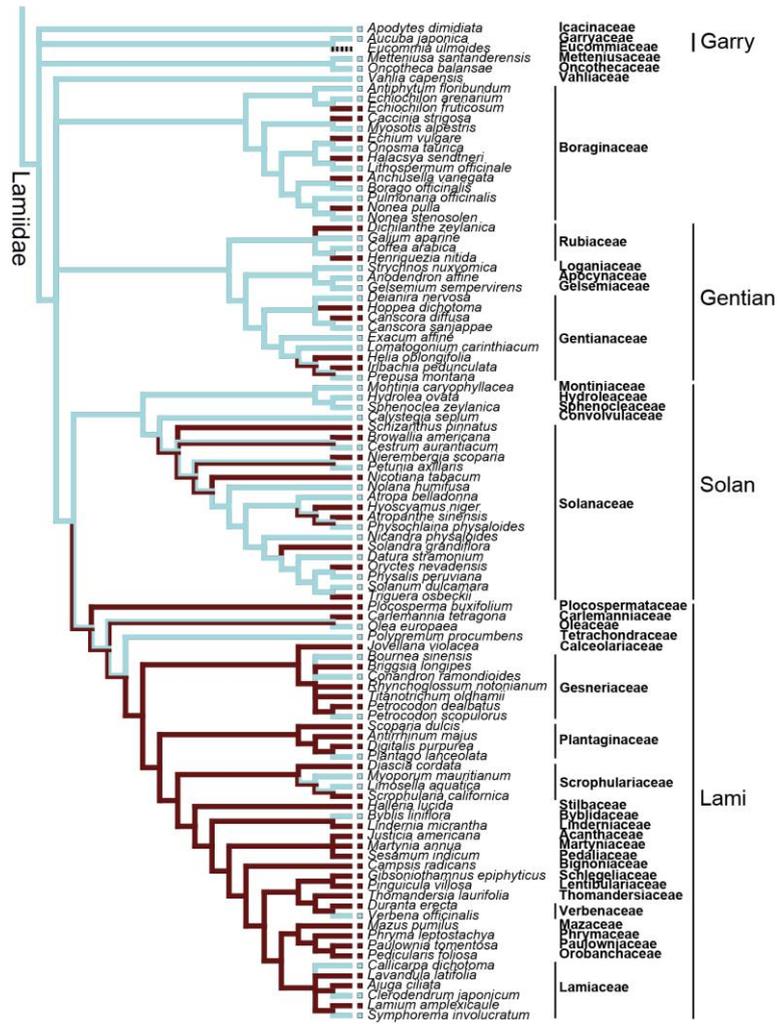
■ actinomorphic  
■ zygomorphic  
■ equivocal  
■ data missing or not applicable

Figure III.2F



- actinomorphic
- zygomorphic
- equivocal
- data missing or not applicable

Figure III.2G



■ actinomorphic  
■ zygomorphic  
■ equivocal  
■ data missing or not applicable

## DISCUSSION

### Number of origins

The minimum number of origins found in this study (130) is much higher than the most recent published estimate (Citerne et al. 2010), which was around 70. The estimate of Citerne et al. (2010) for basal angiosperms was of only one origin (*Aristolochia*). Here, we identified a second origin of zygomorphy in Magnoliidae, in *Glossocalyx* (Fig. III.2A), a monospecific genus of Siparunaceae (Staedler and Endress 2009). The estimate of Citerne et al. (2010) for monocots was of 23 origins, which is only a little lower than our estimate of 29. The major discrepancy concerns eudicots, for which Citerne et al. (2010) found 46 origins, whereas we reconstructed here at least 99 origins. The estimates prior to Citerne et al (2010) tended to reflect the number of families in which zygomorphy was known to occur, neglecting the fact that zygomorphy could have originated several times in a given family (six in Boraginaceae, 13 in Proteaceae: Figs. III.2B and III.2G) and often overlooking very small families (e.g. Donoghue et al. 1998; Sargent 2004). Citerne et al. (2010) used the Angiosperm Phylogeny Group classification available at the time (APG III, 2009) to include all recognized families in their analysis and took the possibility of multiple origins within families in account by basing their tree on published phylogenies, and using descriptions covering lower taxonomic levels than family. The present study used a more recent phylogenetic consensus tree for the angiosperms, used a higher number of family-level phylogenies, and counted reversals along with origins, allowing us to detect transitions that so far had been overlooked. We do note, however, that due to the treatment of hard polytomies by Mesquite, that considers each species in a non-ancestral state to be a different origin, the total number of changes may have been slightly overestimated here.

Here, we have used parsimony to reconstruct the number of transitions of floral symmetry in angiosperms. We note that parsimony is more suitable when the rate of change is low and may underestimate the number of transitions when the rate of change is higher (Cunningham et al. 1998). To test how maximum likelihood would impact the results presented here, we built an ultrametric tree using the topology presented here and the `bladj` function of `phylocom` v.4.2 (Webb et al. 2008) with node ages drawn from the recent study by Magallón et al. (2015). However, we obtained unreasonably high transition rate estimates, resulting in complete uncertainty of ancestral states at all nodes of the tree (results not shown) (Schluter et al. 1997;

Pagel 1999; Hsiang et al. 2015). We interpret this result as a direct consequence of the sampling bias inherent to the construction of our dataset, which aimed specifically to capture all transitions of the character without sampling clades proportionately to their extant number of species. As a result, our sample built comparatively more contrasts between terminal species than exist in the actual, 300,000-species tree of angiosperms, explaining why transition rates were considerably overestimated in this experiment. These results suggest that inferring the complete scenario of floral symmetry evolution across angiosperms using maximum likelihood will require a substantially larger tree and sample of species than presented here.

#### *Developmental evidence on perianth symmetry evolution*

Many studies have addressed the issue of floral symmetry during floral development. They show that zygomorphy can range from being visible very early in floral development (before stamen initiation; early zygomorphy) to being visible only through the later stages of floral development, after all organs have been initiated (late zygomorphy; reviewed in Tucker, 1999).

Early zygomorphy appears to be widespread in angiosperms and can become manifest in various ways. The most commonly reported expression of early zygomorphy is unidirectional initiation of perianth parts: the organs start initiating on one end of the meristem, followed by lateral organs, which are themselves followed by the organs on the side opposite from that of the first organ that initiated. Such initiation has been reported for at least one of the perianth whorls in several superrosid families: papilionoid Fabaceae (Tucker 1984, 1989, 1994; Tucker and Stirton 1991; Sampaio et al. 2013; Leite et al. 2014), caesalpinoid Fabaceae (Tucker 1984; Tucker et al. 1985), Violaceae (Mayers and Lord 1984), and Resedaceae (Tucker 1999). This pattern of initiation has also been found in superasterids: Lamiaceae (Tucker 1999; Naghiloo, Esmailou, et al. 2014; Naghiloo, Khodaverdi, et al. 2014; Naghiloo, Gohari, et al. 2015), Orobanchaceae (Tucker 1999), Plantaginaceae (Bello et al. 2004), Paulowniaceae (Erbar and Glden 2011), Calceolariaceae (Mayr and Weber 2006), Gesneriaceae (Haston and Ronse De Craene 2007), Asteraceae (Dadpour et al. 2012), Lecythidaceae (Tsou and Mori 2007) and Caprifoliaceae (Roels and Smets 1996).

A second pattern of early zygomorphy expression is bidirectional initiation, in which the dorsal and ventral organ(s) are initiated before the lateral ones. Among superrosids, this has

been reported for papilionoid Fabaceae (Movafeghi et al. 2011), Violaceae (Mayers and Lord 1984), and Melianthaceae (Ronse De Craene et al. 2001). This pattern has also been observed in some superasterids: Lamiaceae (Naghiloo, Khodaverdi, et al. 2014) and Lecythidaceae (Tsou and Mori 2007). Another initiation order has also been reported, whereby the order of initiation is the exact opposite of that of bidirectional initiation: the lateral organs emerge first, to be followed by the dorsal and ventral organs. To our knowledge, reverse bidirectional initiation so far has only been reported in superasterids, specifically in the corolla of Asteraceae (Oraei et al. 2013) and the calyx of Balsaminaceae (Yu et al. 2010).

These modes of initiation can coexist in the same family or even the same species; for example, *Viola odorata* L. has bidirectional sepal initiation and unidirectional petal initiation (Mayers and Lord 1984). It should be noted that the presence of strictly lateral organs is necessary to make a distinction between unidirectional, bidirectional and reverse bidirectional; these three modes are essentially the same for trimerous whorls (e.g. the Orchidaceae species described by or tetramerous whorls that initiate organs two by two (e.g. the sepals of *Veronica speciosa* R.Cunn. ex A.Cunn. as reported by Tucker, 1999).

For other species, the first manifestation of zygomorphy appears to be very early differentiation among organs. This has been observed in monocots (Commelinaceae: Hardy et al. 2000, 2004), basal eudicots (Proteaceae: Douglas 1997), superrosids (Moringaceae: Olson 2003; Cleomaceae: Patchell et al. 2011), and superasterids (Asteraceae: Harris 1995; Ren and Guo 2015).

In other cases, zygomorphy is due to a missing perianth part, which either was suppressed very early or did not emerge at all (Asteraceae: Harris 1995; Sapindaceae: Ronse De Craene et al. 2000; Menispermaceae: Meng et al. 2012).

Late zygomorphy also appears to be widely distributed in angiosperms. It has been observed in basal eudicots (Ranunculaceae: Tucker 1999; Jabbour et al. 2009; Papaveraceae: Damerval et al. 2013), superrosids (Fabaceae, Polygalaceae: Tucker 1999; Tropaeolaceae: Ronse De Craene and Smets 2001; Brassicaceae: Busch and Zachgo 2007; Capparaceae: Naghiloo, Fathollahi, et al. 2015) and superasterids (Asteraceae: Harris 1995; Bignoniaceae, Lamiaceae: Tucker 1999). The symmetry of the flower may also vary throughout development, as observed in *Couroupita* Aubl. and *Cariniana* Casar., two of the basalmost zygomorphic

genera of Lecythidaceae (superasterids): both are zygomorphic at calyx initiation, go through an actinomorphic (Endress 1999)/disymmetric (Tsou and Mori 2007) phase, then become zygomorphic again.

Among families in which we know the stage at which zygomorphy appears, most cases of early zygomorphy are in large families with many known zygomorphic members or which are ancestrally zygomorphic. This applies to Fabaceae (Tucker 1997). Many occurrences of late zygomorphy tend to be in clades in which symmetry changes frequently, regardless of their apparent ancestral symmetry on our tree. It would be interesting to see if there is any overall tendency in angiosperms for families in which zygomorphy is fixed to have early zygomorphy and families with late zygomorphy to also have labile zygomorphy.

Both “early” and “late” zygomorphy appear to co-exist in some orders such as Lamiales (Lamiaceae: early and late; Bignoniaceae: late; Plantaginaceae: early; Tucker 1999; Bello et al. 2004; Naghiloo, Esmailou, et al. 2014; Naghiloo, Khodaverdi, et al. 2014; Naghiloo, Gohari, et al. 2015) and Brassicales (Cleomaceae: early; Brassicaceae: late; Capparaceae: late; Busch and Zachgo 2007; Patchell et al. 2011; Naghiloo, Fathollahi, et al. 2015).

Last, flowers may also be zygomorphic in early development but actinomorphic at maturity. Such “late actinomorphy” has been reported in Nymphaeaceae (Endress 1999), monocots (Potamogetonaceae: Poslusny and Sattler, 1974; Xanthorrhoeaceae, Melanthiaceae, Araceae: Endress 1999), basal eudicots (Trochodendraceae: Endress 1999), superrosids (Cistaceae: Nandi 1998; Saxifragaceae: Ronse De Craene et al., 1998), and superasterids (Adoxaceae: Endress 1999; Plantaginaceae: Bello et al., 2004; Lecythidaceae: Tsou and Mori, 2007; Gesneriaceae: Zhou et al., 2008). The relatedness of these species to species that are zygomorphic at anthesis varies greatly. Those of Plantaginaceae, Gesneriaceae and Lecythidaceae are more or less nested in zygomorphic clades, making them potential (Lecythidaceae: Fig. III.2E) or certain (Plantaginaceae and Gesneriaceae: Fig. III.2G) reversals. Those of Adoxaceae, Saxifragaceae and Xanthorrhoeaceae are in a clade that is ancestrally actinomorphic on our tree but have zygomorphic species as relatives within the same family (Saxifragaceae and Xanthorrhoeaceae: Figs. III.2A and III.2C), the same order (Potamogetonaceae: Fig. III.2A) or a sister order (Adoxaceae: Fig. III.2F). Nymphaeaceae, Trochodendraceae and Cistaceae are part of entirely actinomorphic families with no obvious zygomorphic relatives (Figs. III.2B. and III.2D). Endress (1999) suggested that such early

zygomorphy may be due to space constraints and/or the growth of a subtending bract that causes a side of the early floral meristem to have fewer resources for growth than the other.

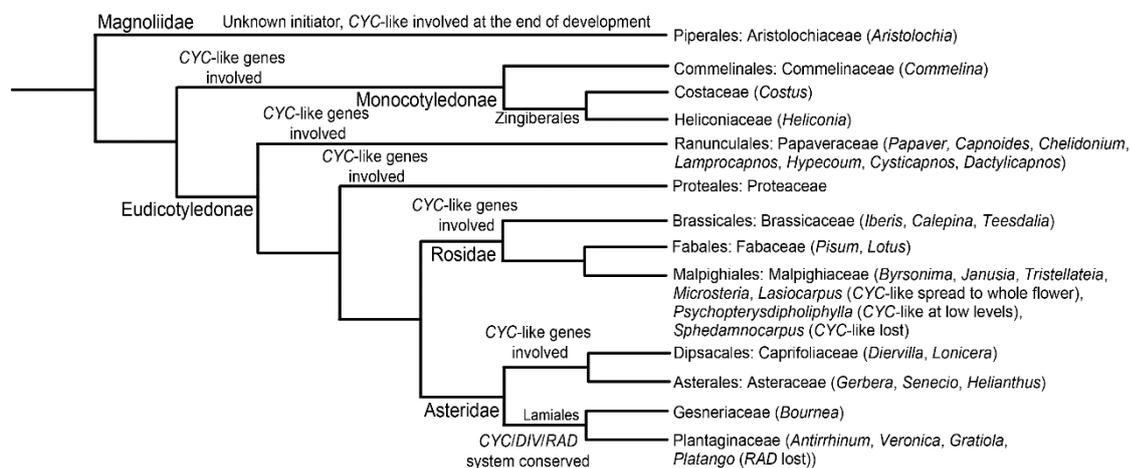
Collectively, structural ontogenetic studies of zygomorphic flowers thus suggest that timing of the onset of zygomorphy during development is highly labile across angiosperms. Although such studies bring critical evidence to better understand the variation of floral symmetry among closely related families, they also suggest that categorization of perianth zygomorphy into distinct, phylogenetically meaningful developmental character states at the angiosperm level is not a straightforward task.

#### Genetic bases of changes in perianth symmetry

Even though different clades might express zygomorphy in various ways, each clade seems to have recruited the same genes for controlling the mechanisms involved in the establishment of zygomorphy (Fig. III.3). This suggests that relatively few genes are involved in the genetic control of zygomorphy.

Considerable knowledge has accumulated on the genetic bases of changes in floral symmetry in monocots and eudicots in the past few years (Hileman 2014). Floral zygomorphy has been found to be linked to two transcription factors from the TCP family, *CYCLOIDEA* (*CYC*) and *DICHOTOMA* (*DICH*), which are paralogues with partially redundant functions that specify dorsal floral identity. Other influencing genes have been found in the MYB family of transcription factors: *DIVARICATA* (*DIV*) and *RADIALIS* (*RAD*). *DIVARICATA* (*DIV*) specifies ventral identity during late floral development, but is also expressed in the dorsal domain in the flower during early development (Almeida et al. 1997). *CYC* and *DICH*, which are expressed in the dorsal domain, positively regulate *RAD*. The role of *RAD* is to post-translationally restrict the *DIV* function to the ventral domain. In asterids, the *CYC/DIV/RAD* developmental programme, first identified in *Antirrhinum majus* L., seems to have been conserved among the other Lamiales (Fig. III.3) (Zhou et al. 2008; Preston and Hileman 2009; Preston et al. 2009). The transitions to zygomorphy in Dipsacales (Howarth et al. 2011) and Asterales (Broholm & al., 2008; Kim & al., 2008), both independent from those in Lamiales (Fig. III.2F and III.2G), also involve *CYC*-like genes. In rosids, at least three instances of zygomorphy evolution are associated with a *CYC*-dependent developmental programme (Fig. III.3) (Fabaceae: Feng et al., 2006; Wang et al., 2008, Mapighiales: Zhang et al., 2010,

Brassicaceae: Busch et al., 2012). Among the basal eudicots, *CYC*-like genes have been found to play a role in the zygomorphy of Papaveraceae in Ranunculales (Damerval et al. 2007; Jabbour et al. 2009). Two *CYC*-like genes were found to co-occur in many species of Proteaceae sampled across the family (Hélène Citerne, pers. comm.). In monocots, the expression of *CYC*-like genes, which has been investigated in Commelinales (Preston and Hileman 2012) and Zingiberales (Bartlett and Specht 2011) (Fig. III.3), has been found to concentrate in the ventral side of the flower, contrary to what occurs in eudicots. Horn et al. (2014) recently showed that *CYC*-like genes were found to be expressed in developing flowers of the magnoliid genus *Aristolochia*. However, their enhanced expression in the ventral side of the perianth occurs long after the initiation of the size differentiation between tepals, suggesting that they may not be involved in zygomorphy initiation but rather in the final growth processes in this genus.



**Fig. III. 3.** Simplified phylogeny of angiosperms showing the families in which the genetic bases of zygomorphy have been explored.

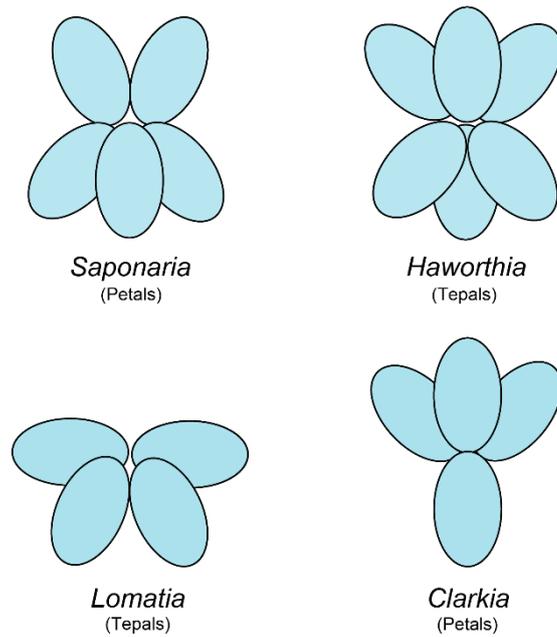
According to Hileman (2014), there is a recurring pattern found when comparing the expression of *CYC*-like paralogues in secondarily actinomorphic species to those of their zygomorphic relatives. One of the paralogues is expressed across the dorso-ventral axis while the other has been lost or is no longer expressed in flowers. For instance, in *Plantago lanceolata* L. (actinomorphic), a close relative of *Antirrhinum majus* (zygomorphic) in the Plantaginaceae (Fig. III.2G), the *DIV* ortholog is expressed in the entire flower and the *RAD* ortholog is not found (Reardon & al., 2014). There are also cases in which the flower has a zygomorphic perianth during most of its development and late actinomorphy is caused by a late regulation of the *CYC*-like paralogue responsible for the zygomorphy, such as in *Bournea* Oliv. in Gesneriaceae (Zhou et al. 2008). Two different reversals in Malpighiaceae (Fig. III.2C) have

either outright lost the expression of both paralogues or maintained both at very low levels (Zhang et al. 2013). The interesting element of these systems is that in some of them, the programme inducing zygomorphy is still present (regulated relatively late in *Bournea*, maintained at very low levels in Malpighiaceae); such systems could account for the secondary origins of zygomorphy within secondarily actinomorphic clades and clades in which floral symmetry is labile.

#### *Spatial distribution of perianth parts in zygomorphic flowers*

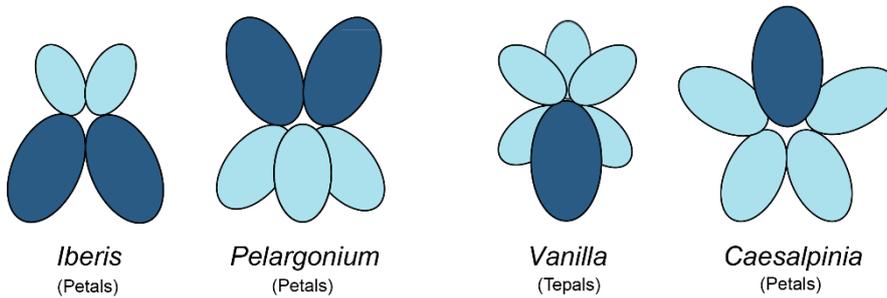
There is a wide range of possible morphological differences between zygomorphic flowers and their closest actinomorphic relatives. On one extreme of the spectrum, the only or most visible difference is an uneven distribution of perianth parts around the flower. Perianth parts can be attracted either towards one side of the perianth or conspicuously sorted between two opposite sides (dorsal and ventral) of the perianth (Fig. III.4A). The phenomenon is called positional zygomorphy (Endress 1999), in that the individual parts are identical within each whorl and the perianth would be actinomorphic if they were evenly distributed. In the species closest to this extreme, perianth parts concentrating toward one side of the flower can be found, for example, in some species of *Lomatia* R. Br. (Proteaceae). Tetramerous flowers can also have a similar arrangement, with three upper and one lower, such as in some species of *Clarkia* Pursh. (Onagraceae). Sorting between two sides tends to present two upper petals and three lower petals in pentamerous flowers, as in *Saponaria* L. (Caryophyllaceae).

**A) Zygomorphy mostly due to perianth part displacement**

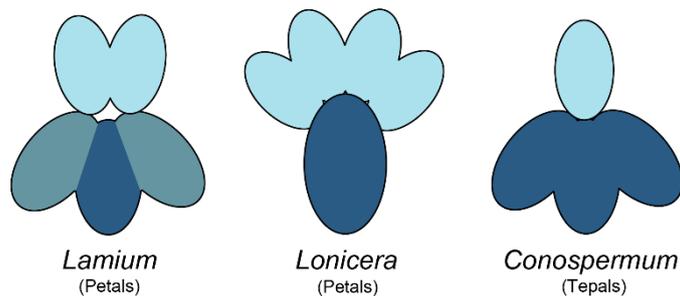


**B) Zygomorphy mostly due to perianth part differentiation**

Free perianth parts



Fused perianth parts



**Fig. III.4.** Illustration of the various forms of floral symmetry. A, Zygomorphy mostly due to perianth part displacement. B, Zygomorphy mostly due to perianth part differentiation; darker blue can mean both size and morphological differentiation.

The other extreme of zygomorphy expression at anthesis is perianth part differentiation in size or shape, which may combine with perianth part fusion and/or displacement in some

clades (Fig. III.4B). Trimerous flowers, such as *Vanilla* Mill. (Orchidaceae) and *Aristolochia* (Aristolochiaceae), have one petal or tepal that is different from the other two. Two situations are found in tetramerous flowers: one perianth part may differ from the other three (e.g., the upper tepal of *Conospermum* Sm., Proteaceae), or two perianth parts undergo the same type of differentiation (e.g., the lower petals of *Iberis* L., Brassicaceae). In pentamerous flowers, there can be one petal that is different from all the others (*Caesalpinia* L., Fabaceae; *Lonicera* L., Caprifoliaceae). Cases in which three of the contiguous petals resemble each other, but are different from the two other petals can be found as well (*Lobelia* L., Campanulaceae; *Saxifraga* L., Saxifragaceae). There are also cases in which the dorsal, lateral and ventral sets of perianth parts look distinctly different from each other (*Pisum* L., Fabaceae; *Lamium*, Lamiaceae).

Thus, perianth zygomorphy at anthesis may be achieved in various ways, some of them appearing recurrently in distantly related families.

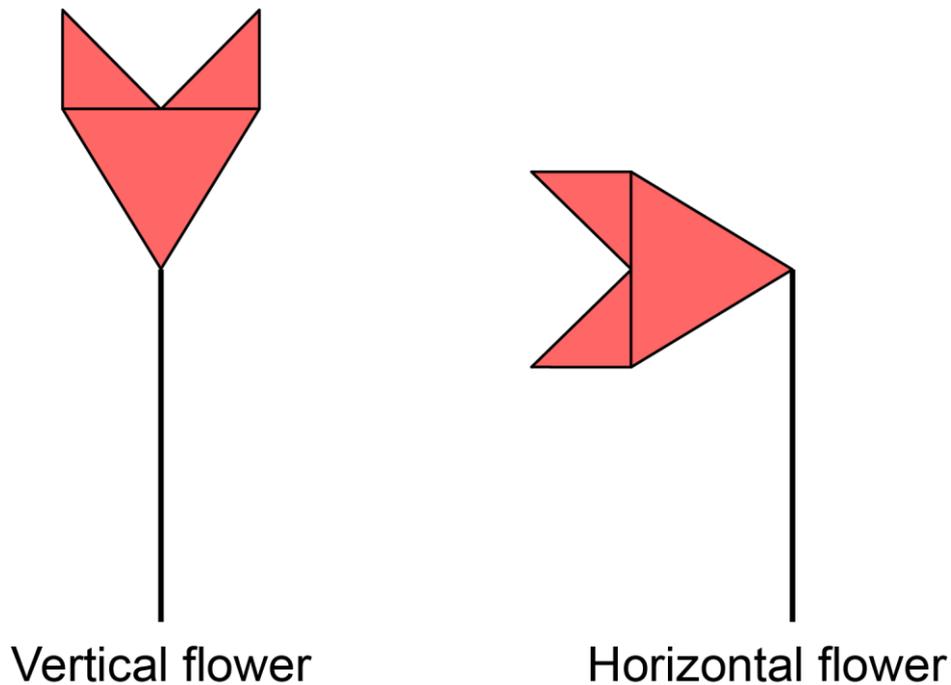
#### Selective advantages and constraints of zygomorphy

The quasi-absence of zygomorphy in basal angiosperms and its uneven distribution in the clades in which it is frequent may be due to the fact that it can be a selective advantage in some circumstances, but not in others. In this section, we review the advantages given by zygomorphy, but also the factors that can make it an evolutionary dead-end.

*Advantages.* Zygomorphy has some features that promote outcrossing. Fenster et al. (2009) found horizontal orientation (Fig. III.5) to have an influence on pollinator approach even in the case of an actinomorphic flower, leading to speculation that horizontal orientation was a first step toward zygomorphy. Three hypotheses discussed in Neal et al. (1998) give weight to the idea of zygomorphic flowers first appearing as favorably selected variants of actinomorphic horizontally oriented flowers (Fig. III.6A). The “Inflorescence type-flower orientation hypothesis” stipulates that pollinators do not land as well in horizontally-oriented actinomorphic flowers as in flowers whose lower perianth margin is expanded in such a way that it is usable as a landing platform. Selection would hence favor expansion of the lower margin of the perianth, which may also be used as advertising, such as in *Viola* (Violaceae) and *Lamium* L. (Lamiaceae) (Figs. III.2C and III.2G). The “marginal flower-landing platform hypothesis” (Fig III.6B) could be the equivalent of the “Inflorescence type-flower orientation hypothesis” for pseudanthia (condensed inflorescences that look like flowers, many of which

have peripheral flowers that look like petals). Provided that the inflorescence is rounded and flat-topped, an alternative solution is to have only the peripheral flowers expand into a platform while the others can remain actinomorphic and yet benefit from the pollinator landing on the inflorescence. More biomass tends to be allocated to zygomorphic flowers (Herrera 2009), so dedicating the “landing platform” role to a specific part of the inflorescence rather than having each flower have its own could be a better investment of limited resources. We speculate that flower size in relation to the pollinator may also be a factor: inflorescences with flowers that are individually about the same size as their pollinator would probably benefit more from an individual “landing platform”, while a platform made only of the inflorescence’s marginal flowers could be a better strategy for cases where individual flowers are smaller than the pollinator. This system is found in many Asteraceae, such as *Helianthus* L. (Fig. III.2F). The “dangerous lower margin hypothesis” (Fig. III.6C) is the complete opposite of the two previous ones: a species pollinated by flying vertebrates (most likely birds according to Leins and Erbar (2010), but rare cases involve bats (Tripp and Manos 2008), could benefit from having its lower margin *smaller* than the upper one as it avoids visits from insects (some of which may be nectar thieves) and makes pollination easier for larger pollinators that cannot land on the flower. Flowers with such a configuration include *Chasmanthe* N. E. Br. (Iridaceae) and *Musa* L. (Zingiberaceae) (Fig. III.2A and III.2B). Neal et al. (1998) also hypothesized that horizontally-oriented flowers whose higher margins are longer could have been selected because of better protection of pollen and nectar from rain.

## Flower orientation vocabulary



**Fig. III.5.** Clarification of the floral orientation terminology used in this paper.

The oldest known zygomorphic flower fossil (Early Cenozoic) is younger than the earliest known bee fossil (Late Cretaceous) (Citerne et al. 2010). This is compatible with the idea that floral zygomorphy only became widespread after the appearance of bees about 123 Ma years ago (Cardinal and Danforth 2013). Zygomorphic flowers are pollinated by bees, butterflies, birds (Tripp and Manos 2008; Leins and Erbar 2010; Mast et al. 2012), hawkmoths (van der Pijl 1961; Tripp and Manos 2008) and bats (Tripp and Manos 2008). The pollination syndromes associated with zygomorphy seem to be flexible in terms of pollinator preferences. For instance, shifts from bird to insect pollination have been documented in both *Hakea* Schrad. & J.C.Wendl. (Proteaceae; Mast et al., 2012) and *Ruellia* L. (Acanthaceae; Tripp and Manos, 2008). The opposite transition has also been inferred, for instance in *Penstemon* (Plantaginaceae; Castellanos et al., 2003, 2004; Wilson et al., 2007) and *Aquilegia* (Ranunculaceae; Whittall and Hodges, 2007). In some species, polymorphism in perianth symmetry may be maintained because both morphs present advantages. Gómez et al (2006), who focused on *Erysimum mediohispanicum* Polatschek (Brassicaceae), a species that can be actinomorphic, disymmetric or zygomorphic, found that the actinomorphic and zygomorphic forms were favored in different ways: actinomorphic flowers produced more seeds, but

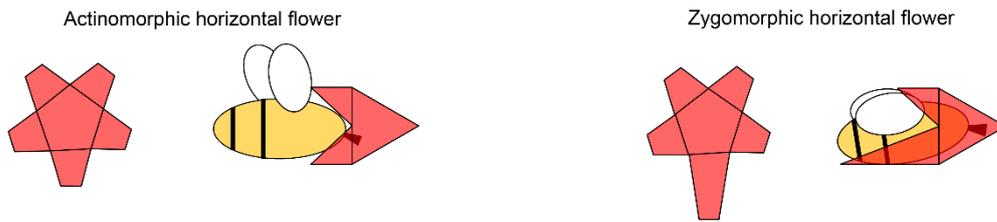
zygomorphic ones were those who produced most offspring. Gómez et al. (2009) showed that the same species displayed different types of symmetry depending on the local pollinators.

*Constraints.* Evolutionary, ecological and developmental constraints can prevent symmetry transitions from happening or make zygomorphy a disadvantageous trait, even though perianth zygomorphy has been proven to promote outcrossing.

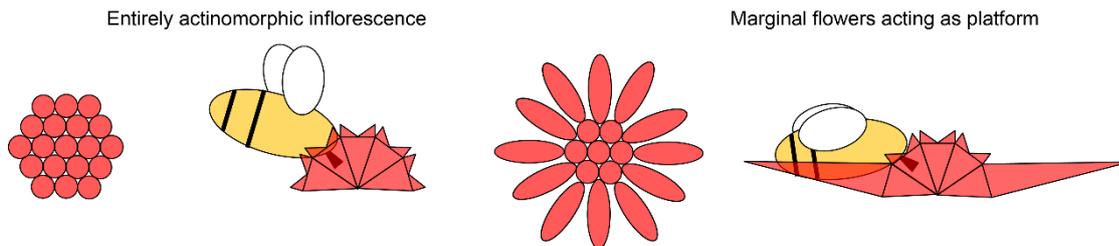
In monocots and asterids, zygomorphy and polyandry are almost mutually exclusive (Jabbour et al. 2008; Citerne et al. 2010), that is, possessing a number of stamens more than twice as large as the perianth merism. One of the reasons for this is that *CYC* and *DICH*, while inducing perianth zygomorphy, also inhibit stamen growth (Howarth and Donoghue 2006; Ronse De Craene 2010). This reduces the chances for perianth zygomorphy to appear in clades that tend to be polyandrous, and zygomorphic flowers from developing many stamens, which prevents adaptation possibilities in both cases.

The fact that zygomorphy limits the pollinators that have access to the flowers can be a disadvantage in itself. The study of Tripp and Manos (2008) on *Ruellia* (Acanthaceae) found that some lineages pollinated by bats and hawkmoths were potentially an evolutionary dead-end. Extremely specialized plant-pollinator relationships (Leins and Erbar 2010) may also be a constraint in that the plant species totally depends on a single species of pollinator for its survival. It is at risk to go extinct should the pollinator go extinct or change its distribution in such a way that it no longer overlaps with that of the plant.

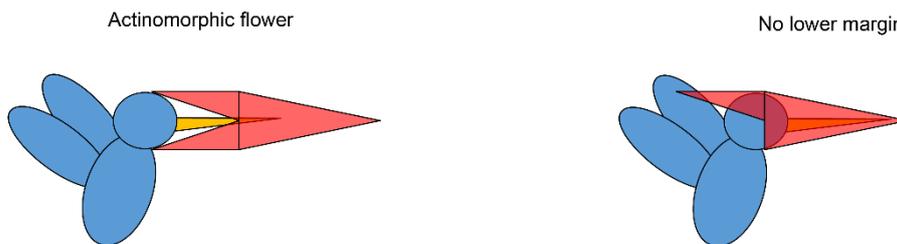
### A) Inflorescence type-flower orientation hypothesis



### B) Marginal landing platform hypothesis



### C) Dangerous lower margin hypothesis



**Fig. III.6.** Illustration of three hypotheses by Neal & al. (1998) to explain the relationship between floral symmetry, floral orientation, inflorescence type, and type of pollinator. A, Flower lacking a landing platform (actinomorphic) versus a flower whose lower margin is extended into a platform (zygomorphic). B, Inflorescence whose flowers are actinomorphic and smaller than the pollinator vs. inflorescence whose marginal flowers develop into a platform. C, Accessibility of an actinomorphic flower to a pollinating bird vs. that of a flower lacking a lower margin (zygomorphic).

Perianth zygomorphy has some advantages in terms of pollination efficiency, but species in which it appears are on the specialist side of the generalist-specialist trade-off. The advantages can last for a given species only as long as compatible pollinators share its environment. If these pollinators move or decline in population density, the strategy-changing possibilities of the plant species are limited because of constraints in the floral shape. This also works the other way around, as some features present in actinomorphic flowers can prevent them from becoming zygomorphic in a context where the latter state could present an advantage.

### *Symmetry and species diversity*

Zygomorphic species are subject to two opposing trends. On the one hand, they favor reproductive isolation via plant-pollinator specialization, and hence favor speciation. On the other hand, specialization may be so extreme that the high dependency on a limited number of pollinators may ultimately lead to extinction. A key question thus becomes the relative effect of these two factors on diversification, and how this effect depends on other clade-specific traits. This study suggests that Proteaceae (ca. 1700 species) is the family with the highest number of origins of perianth zygomorphy across angiosperms (at least 13: Fig. III.2B). In a previous study, we found that perianth zygomorphy does not appear to have an impact on diversification rates in this family, in contrast to climate (Reyes et al. 2015). Similarly, Vamosi and Vamosi (2011) showed that having a large distribution area can be more favorable to species richness than floral zygomorphy and other putative key innovations. These studies highlight that some presumed key innovations have no impact on species richness, and suggest that they are “hitchhiking” on other traits that have an impact on species diversification. Furthermore, Maddison and FitzJohn (2015) and Rabosky and Goldberg (2015) point out that such a hitchhiking phenomenon causes potential problems in interpreting the results of methods that are intended to see if a character state is correlated with species diversification; an effect can be found when there is in reality none. Thus, more work needs to be done that takes into account these potentially confounding factors to further test whether or not zygomorphy may be viewed as a key innovation in angiosperms as a whole (Sargent 2004). Our new framework of zygomorphy evolution across angiosperms, including the characterization of multiple origins and reversals within families, is a first step towards more detailed study and tests of zygomorphy-dependent diversification in angiosperms.

### *Future directions*

Here, we have shown that perianth zygomorphy is derived in angiosperms and is a highly homoplastic trait distributed throughout angiosperms (Fig. III.2). Zygomorphy can occur in many forms (Fig. III.4), but the developmental programmes behind these diverse forms are only starting to be deciphered (Fig. III.3). We reviewed the reasons why flowers with a zygomorphic perianth could have been selected and why reversals to actinomorphy are comparatively less frequent. Our results highlight the families in which detailed studies of the development of floral symmetry or pollination could be interesting. Many studies have been made in the

ontogeny of zygomorphic species across angiosperms, but many clades containing zygomorphic species of great interest have yet to be explored or have only been explored via their actinomorphic species: Iridaceae (Figure III.2A), Saxifragaceae, Malpighiaceae, Geraniaceae, Sapindaceae (Figure III.2C and III.2C) and several non-Lamiales families of Lamiidae (Boraginaceae, Rubiaceae and Gentianaceae) (Figure III.2G).

Efforts have been made to study the genetic pathways linked with perianth zygomorphy in several clades (Fig. III.3), but much work remains to be done in this domain also. So far, most efforts have concentrated on a few families of the superrosids and superasterids. A study on magnoliids was published very recently (Horn et al. 2014), and some clades to our knowledge still remain unexplored, such as Orchidaceae and Geraniaceae. Orchidaceae as a whole and *Pelargonium* L'Hér. ex Aiton (Geraniaceae) each display their zygomorphy in various forms (e.g., Struck 1997), which makes them excellent candidate clades for studying the mechanics behind various perianth part modifications. More work needs to be done in the orders in which the exploration of zygomorphy-inducing pathways has already begun. In some cases (Asterales, Fabales, Commelinales), the exploration so far has focused on a single family, sometimes a single species (Fig. III.3). However, the variability of zygomorphy within families such as the Lamiaceae, Caprifoliaceae or Proteaceae, suggests that investigation of intra-familial variation in genetic pathways could be just as informative as inter-familial variation. Last, it will also be important to test how frequently these origins of floral zygomorphy are associated with higher species diversity across angiosperms, and to look for evolutionary correlates between perianth zygomorphy and other floral traits.

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# **CHAPTER 3: Does heterogeneity of rates of morphological evolution affect ancestral state reconstructions? An empirical test with five floral characters**

*Written by Elisabeth Reyes, Jürg Schönenberger, Maria von Balthazar, Sophie Nadot, Hervé Sauquet. In preparation, planned for a submission to Nature: Scientific Reports*

In this chapter, I address the issue of whether reconstruction methods and models (e.g. parsimony, Mk1 or Mk2 in maximum likelihood) and transition rate differences between various portions of a phylogenetic tree affect the ancestral states that are reconstructed and if so, to what extent? To find this out, I used the same methods as the previous chapter, except with five perianth characters instead of just one: perianth symmetry, fusion, phyllotaxy, pentamery and differentiation (Figure I.5). Four of the five perianth characters had a natural dichotomy in their forms, which could be easily used to produce binary states: actinomorphy or zygomorphy for symmetry, free or united organs for fusion, whorled or spiral organization of organs for phyllotaxy, and undifferentiated or differentiated perianth for differentiation (Figure I.5). Merism is by nature a multistate character, which can be made binary in various ways. I decided to separate species in which pentamery is present (pentamerous) from those in which it is absent (non-pentamerous), as pentamery is the merism most commonly found in angiosperms. My initial species samples were those represented in my symmetry tree from Chapter 2 and those present in a tree combining all the angiosperm species for which the state of these characters had been entered in PROTEUS, hereafter called the PROTEUS species tree. To construct the so-called five-character tree, I used the following procedure. I optimized one of the four non-symmetry characters on the tree made with the symmetry species selection and compared it to an optimization on the PROTEUS species tree. For each change in character state that was present in the PROTEUS species tree, but not the symmetry tree, the species necessary to show the changes were added to the symmetry tree, thus making it a tree that showed all the changes for two characters. The third character was then mapped on the two-character tree and compared to its mapping on the tree made with the PROTEUS data, with

species being added as needed. The procedure was repeated until I arrived to a five-character tree. Once this was done, I checked the character states present in each family against the family-level descriptions for changes that could have been absent from PROTEUS. Species were added to the sample as needed. Species that had data for all five characters were favored during every step. The end sample was made of 1232 species.

APG IV (APG 2016) had recently been published at the time, bringing changes to the circumscription of some of the families; the differences it brought to the optimizations compared to one with the APG III (APG 2009) definitions were fortunately very minor. The `bladj` function from the Phylocom program (Webb et al. 2008) enabled me to give branch lengths to my tree; it is the same function as the one used to date the symmetry tree, hence it involves the same process as the one described in the introduction to Chapter 2.

Trying to record all the changes of five characters instead of just one on a single tree proved to have several benefits. The addition of species needed to show the state change in non-symmetry character breaks branches from the symmetry-only tree. As a consequence, a family with one change in perianth symmetry and many changes in and out of the fused state will be represented with more species in the tree than if sampled based on symmetry only. This changes state distribution and enhances the resolution of ancestral state reconstruction as well. This factor lowered the transition rates estimated by maximum likelihood compared to the symmetry-only tree and allowed me to use it for ancestral state reconstruction taking branch lengths into account.

To evaluate the extent of rate heterogeneity within angiosperms, I compared the rates estimated when the same model applied to the entire angiosperm tree to those that were estimated when different subsections of the tree had a different model applied to them. For this, the tree was divided into five partitions, on which the evolution of all five characters was optimized separately: a basal angiosperm grade (ANA grade and Magnoliidae), Monocotyledoneae, basal eudicots (Ranunculales to Buxales), superrosids (incl. Dilleniales), and superasterids (Figure IV.1). Parsimony, Mk1 and Mk2 were used to reconstruct ancestral states on both the full angiosperm tree and the partitions (collectively the partitioned tree). Here, most of the inferred transition rates were slow enough for each node to have distinct state probabilities, unlike the high transition rate induced artefact that appeared in the study presented in Chapter 2.

To test the impact of changing optimization models between Mk1 and Mk2 and optimizing on the full or partitioned trees, I compared the inferred ancestral states of the various optimizations on two sets of nodes. One set consisted of 15 nodes that defined the larger commonly accepted subdivisions of the tree (Cantino et al. 2007): Angiospermae, Mesangiospermae (all angiosperms except the ANA grade), Magnoliidae, Monocotyledoneae, Commelinidae, Eudicotyledoneae, Pentapetalae, Superrosidae, Rosidae, Malvidae, Fabidae, Superasteridae, Asteridae, Campanulidae and Lamiidae. These are the same 15 key nodes used in the first eFLOWER paper (H. Sauquet et al., in prep.), allowing for immediate comparison of results between the two studies. The other set was intended to comprise the ancestral crown-group nodes of all 64 orders. In reality, only 54 nodes were examined due to ten orders being monospecific on the tree. The transition rate heterogeneity between the angiosperm partitions was shown to be strong; for a given character, partitions always had transition rates different from that of the full tree and very frequently from each other. Despite this, in both sets of nodes, a majority of them kept the same state despite the change of maximum likelihood model, but a minority of them were extremely sensitive to the model used. The most-probable ancestral states inferred using the MK2 model were prone to be different from those inferred in comparable circumstances: an Mk1 model used on a full tree and an Mk2 model used on a partitioned tree. By comparison, Mk1 used on a full tree, Mk1 used on a partitioned tree and Mk2 used on a partitioned tree had more similarities between each other in terms of inferred most-probable ancestral states.

I also performed a series of Mk1 constrained rate tests on the full tree on a small logarithmic scale going from 0.0001 changes per Ma (the order of magnitude of the lowest estimated rates from the previous test) to 0.1. Ancestral states were overall insensitive to variations in transition rates, but a minority of nodes had a state that was variable as a function of the transition rate for at least one character. Others were quite visibly approaching the equivocal state probabilities characteristic of extremely high transition rates as the transition rate increased. All nodes had equivocal probabilities when the transition rate was 0.1.

**Does heterogeneity of morphological evolutionary rates affect  
ancestral state reconstruction? An empirical test with five floral  
characters**

Elisabeth Reyes<sup>1</sup>, Jürg Schönenberger<sup>2</sup>, Maria von Balthazar<sup>2</sup>, Sophie Nadot<sup>1</sup>, Hervé Sauquet<sup>1</sup>

<sup>1</sup>Ecologie Systématique Evolution, Univ. Paris-Sud, CNRS, AgroParisTech, Université Paris-Saclay, 91400, Orsay, France

<sup>2</sup>University of Vienna, Department of Botany and Biodiversity Research, Rennweg 14, Vienna, A-1030, Austria

In preparation

## ABSTRACT

Ancestral state reconstruction is an important part of the study of morphological evolution. Reconstructing ancestral states using model-based methods often involves estimating transition rates among character states. However, the estimated value of these transition rates may change according to the conditions in which they are estimated, such as taxon sampling and taxonomic scale. These variations may in turn impact the probability of the state at a given node. Here, we test the influence of maximum likelihood methods on five binary floral characters in which one of the states is a candidate key innovation (perianth symmetry, fusion, phyllotaxy, pentamery and differentiation) optimized on a phylogenetic tree of angiosperms including 1232 species sampled from all 424 currently recognized families. We compare the states reconstructed by an equal-rate (Mk1) and two-rate (Mk2) models fitted either with a single set of rates for the whole tree or as a partitioned model, with different rates for each of five partitions of the tree. We also test the impact of four different arbitrary transition rates under the Mk1 model. We focus our comparisons on both a set of 15 key nodes and a set of 54 nodes corresponding to the most recent common ancestors of all orders with more than one species. We found a strong signal for rate heterogeneity among the five subdivisions for all five characters. However, our results suggest that the choice of a model has little overall impact on reconstructed ancestral states for these characters in angiosperms, albeit with some variation depending on the character. Our results also show that the derived states from our characters are usually absent at both key node and order node level and evolved many times independently, except for pentamery and differentiation in Pentapetalae and fusion in Superasteridae.

## INTRODUCTION

Studying the evolution of morphological characters requires establishing the evolutionary history of organisms. One way to do this is to use a phylogenetic tree of the set of species examined and reconstruct the ancestral states. Maximum likelihood using the continuous-time Markov model (Pagel 1994, 1999; Schluter et al. 1997; Lewis 2001) is one of the most common methods available to reconstruct ancestral states for discrete morphological characters. The method can be used to calculate the probability (proportional marginal likelihood) of each node to be in each state. To do so, instantaneous transition rates among states must first be estimated by finding the set of parameter values that maximize the overall likelihood of the data, given a phylogenetic tree with branch lengths and a distribution of observed values at the tips (Schluter et al. 1997). However, the transition rate in itself can affect the probabilities of states. One of the main advantages of maximum likelihood over parsimony, which simply infers the minimum number of changes necessary to explain the distribution of tip states, is that it takes into account the time that was available for the character to change. Under low transition rates, the common ancestor of two sister species with the same state will have a high probability of being in the same state as the two sister species and an extremely low probability of being in any other state. Under higher rates (or longer divergence time between species), the probability of a state change happening between a node and its descendant or tip increases. This leads to increasing probability that node and tip have a different state.

Transition rates on trees using the maximum likelihood approach are conditioned by a given model. Moreover, the estimated rates may be different depending on the size of the tree on which they are assumed to remain homogenous; the larger the tree, the more likely it is that it contains several regions with different “true” transition rates (King and Lee 2015). While transition rates may certainly impact reconstructed ancestral states, it remains unknown to what extent transition rates need vary to have a noticeable impact. However, these issues have so far only been very little explored (Beaulieu et al. 2013; King and Lee 2015).

Angiosperms, commonly known as flowering plants, comprise an estimated 300,000 extant species. Flowers are the most distinctive attribute of the clade and, despite a well-conserved groundplan, they are remarkably variable. Due to the size of angiosperms, two main approaches have so far been used to study character evolution across the clade: an angiosperm-

wide approach with a very limited number of representative taxa sampled from each clade (Zanis et al. 2003; Hileman and Irish 2009) or an examination of smaller clades with more complete sampling (Knapp 2010; Busch et al. 2012; Reyes et al. 2015; Sauquet et al. 2015) The first approach inevitably conceals the true extent to which some characters change across angiosperms. The second approach, while more likely to capture most if not all of the character evolution of the clade of focus, reveals only part of a much bigger picture. One of the factors making both these approaches possible is that the phylogeny of angiosperms is well-known (Stevens 2016). This, combined with the existing record of flower morphology, makes angiosperms an ideal framework for reconstructing the evolution of floral characters across the clade and using this framework to evaluate the impact of various assumptions and modifications of the model on reconstructed ancestral states.

Perianth symmetry, fusion, phyllotaxy, pentamery and differentiation are important structural characters of the flower that can be identified and compared across all angiosperms. Symmetry and fusion both contribute to plant-pollinator specialization by restricting flower access to specific animal pollinators. Phyllotaxy has an important impact on perianth organization, with some floral innovations being possible only on whorled flowers, but not spiral ones. Pentamery is the most frequent merism in angiosperms, for reasons that have yet to be discovered. Differentiation lets different sets of perianth parts specialize in different roles, including the most well-known division between protective but inconspicuous sepals and showy but fragile petals. Because of their functional significance, these characters have received much attention in previous works and they have been suggested as potential key innovations at the scale of angiosperms (Sargent 2004; Endress 2011). Confirming the key innovation status of a given character state (e.g., perianth fusion) requires showing that its origins are correlated with a higher rate of species diversification, which in turn necessitates a clear understanding of where in the tree that state originates. This study focuses on providing the necessary background framework for the evolution of these five perianth characters in angiosperms. Although previous work has clarified some patterns in early-diverging angiosperms using parsimony, we here provide the largest dataset assembled to date to address this question, use maximum likelihood methods, and explore the sensitivity of our results to various assumptions of the models, in particular among-lineage rate constancy.

The first studies of models that took into account the possibility of among-lineage evolutionary rate variation were published in the 1990s (Yang and Roberts 1995; Galtier and

Gouy 1998). Early applications of such models on non-molecular data include those of Collar et al. (2005) and O'Meara et al. (2006) for continuous traits, and Thomas et al. (2006) for discrete traits. Beaulieu et al. (2013) proposed a model to take hidden transition rate changes into account and applied it to plant habit in Campanulidae. As part of exploring the evolution of traits that enable plants to survive freezing temperatures, Zanne et al. (2014) compared a set of transition rate estimates at the level of the angiosperms to those estimated for Magnoliidae, Monocotyledoneae, Superrosidae and Superasteridae. These four subdivisions had noticeably different transition rates from each other and from angiosperms as a whole, as well as different frequencies of the occurrence of combinations of character states.

We previously reconstructed ancestral states for perianth symmetry across all angiosperms, using a 761-species dataset sampling all putative transitions of this character and reconstructing these transitions using parsimony (Reyes et al. 2016). In this study, we expand the floral symmetry dataset into a new one comprising five floral (perianth) traits recorded in 1232 species of angiosperms, representing all 424 families and 64 orders recognized by APG IV (APG IV, 2016). We use this new dataset to test the impact of various assumptions of models on estimated transition rates and ancestral states. First, we test the impact of symmetric (Mk1) vs. asymmetric (Mk2) rate models on discrete binary character evolution (Dunn et al. 2005; Vanderpoorten and Goffinet 2006; Webster et al. 2012; King and Lee 2015). Further, we compare both best-fit models and estimated transition rates among five distinct characters in order to measure among-character rate heterogeneity (Vanderpoorten and Goffinet 2006; Couvreur et al. 2008; O'Meara 2012; Berv and Prum 2014; Zanne et al. 2014; Wright et al. 2016). Last, we test the impact on ancestral state reconstruction of whole-tree rate constancy by relaxing this assumption and allowing five partitions of the angiosperm tree to evolve at their own rates (Zanne et al. 2014).

## **MATERIALS AND METHODS**

We recorded five perianth characters (see below) in a large sample of angiosperm species using an exemplar approach. We included at least one species from each currently recognized family (see details below). Families lacking a perianth, such as Piperaceae and Ceratophyllaceae, were treated as missing data, but maintained in our sample in order to present a complete overview of floral trait evolution on mapped phylogenetic trees (Reyes et al., 2016).

All data were scored in the PROTEUS database (Sauquet 2016). APG IV (APG 2016) was followed for order and family delimitations. Higher-level clades above the rank of order follow the definitions given by Cantino et al. (2007) and Soltis et al. (2011).

### Choice of characters

To measure the impact of transition rate heterogeneity on ancestral state reconstruction, we selected five important perianth characters, chosen because of their broad applicability to all flowering plants, their biological significance, and their variability in most larger clades.

*Symmetry.* Perianth symmetry has two main states: actinomorphy, or radial symmetry, which is ancestral in angiosperms (Endress 2011; Reyes et al. 2016), and the derived state known as zygomorphy, or bilateral symmetry. Zygomorphy is easy to identify and is found across all major clades of angiosperms (Citerne et al. 2010; Reyes et al. 2016). Rare cases of asymmetry and disymmetry are treated here as missing data, as in Reyes et al. (2016).

*Fusion.* All floral organs are considered to have been free from each other in early flowers (Endress 2011). Sympetaly, the fusion of petals, appears several times across angiosperms and is considered to be a key innovation in Asteridae (Endress 2006, 2011). Here we considered within-whorl fusion for the perianth as a whole, scored on a continuous scale of 0 to 1, and then transformed in PROTEUS as a binary character using a threshold of 5%. This means that flowers with free sepals and petals were scored as 0 and treated here as flowers with a free perianth, while those with entirely fused sepals and petals were scored as 1 and treated here as fused (even if the calyx remained free from the corolla), whereas flowers with, for instance, a fused calyx and a free corolla were scored as 0-1 and treated as polymorphic in PROTEUS.

*Phyllotaxy.* Phyllotaxy of perianth organs exhibits two main states: whorled and spiral. Spiral perianth phyllotaxy is much more frequent in basal angiosperms (the ANA grade and Magnoliidae) than in the rest of the group and has been hypothesized to represent an obstacle to floral elaboration (synorganization) that evolved in many whorled flowers (Endress 1990; Endress and Doyle 2007). Some characteristics of spiral flowers have led many authors to

consider that it is the ancestral state in angiosperms (Arber and Parkin 1907; Endress 1990; Soltis et al. 2000, 2005 and references within Soltis et al. 2005). However, most recent parsimony ancestral state reconstructions find the two states to be equivocal at the root of angiosperms (Ronse De Craene et al. 2003; Zanis et al. 2003; Soltis et al. 2005; Endress and Doyle 2007, 2009; Doyle and Endress 2011).

*Pentamery.* Flowers with a pentamerous perianth are characteristic of one of the largest clades in angiosperms, Pentapetalae (Cantino et al. 2007). This group has more species than all remaining angiosperms taken together, representing 75% of all angiosperms, making pentamery the most frequent form of perianth merism found across angiosperms (Ronse De Craene 2010). However, pentamery is present in other parts of the tree, such as Ranunculaceae in basal eudicots and Siparunaceae in Magnoliidae (Ronse De Craene and Smets 1994; Endress 2011; Ronse De Craene 2016), and the reason why it is so predominant in Pentapetalae remains unknown.

*Differentiation.* A perianth made entirely of parts identical to each other, i.e., an undifferentiated perianth, is the most common type in basal angiosperms. By contrast, most flowers of angiosperms are characterized by a differentiated perianth, comprising at least two whorls of parts different from each other in color, size and/or shape, or of spiral series displaying different type of perianth parts such as members of the genus *Camellia* (Theaceae). Some studies have suggested that the undifferentiated perianth is ancestral in angiosperms (Doyle and Endress 2000; Ronse De Craene et al. 2003; Zanis et al. 2003; Soltis et al. 2005; Hileman and Irish 2009), while others have interpreted the ancestral state as equivocal (Endress and Doyle 2009; Doyle and Endress 2011).

Following initial recording in PROTEUS, the matrix of five characters and 1232 species for this study was exported as a NEXUS file from the database and transformed in Mesquite 3.04 (Maddison and Maddison 2015) in such a way that all polymorphic cells were converted to the presumed derived state. In the case of phyllotaxy, the derived state was assumed to be spiral, as suggested by Bayesian analyses in a currently conducted study (Sauquet et al., unpubl. data). The transformation affected the symmetry state inferred for 22 species, the fusion state for 136 species, the phyllotaxy for seven species, the merism for 102 species and the differentiation state for 13 species. This was done because we were interested in the presence of the derived state rather than whether or not it appeared concurrently with the ancestral state.

All analyses were then conducted again with the original, untransformed matrix (see Appendix IV.7).

### Character coding and taxon sampling

Family-level descriptions were used to identify families presenting variation in the studied characters. A total of 661 sources were then used to score the perianth traits of particular species, including a large number of scientific articles, *The Families of Flowering Plants* (Watson and Dallwitz 2016), and the online *Flora of North America*, *Flora of China* and *Flora of Pakistan*; the last four sources were also those used for family-level descriptions. Drawings linked to taxonomic descriptions and photos from *Encyclopedia of Life* (ww.eol.org) that had been marked as trusted were used to determine the state of perianth symmetry, merism, differentiation, and fusion when it was not mentioned in the descriptions themselves.

Families that presented no variation in any of the five characters were represented by a single species. For families that were polymorphic for any of the traits, we included as many species as necessary to represent the variation in character states, including multiple transitions and/or reversals, as in our previous study of perianth symmetry (Reyes et al. 2016). We favored species in which the state of all five characters had been reported in PROTEUS. When no such species were available, we chose a species in which the state of four of the characters were known. If possible, the state of the missing character was informed by the addition of an extra species, with preference for those for which the state of several characters was known. This method resulted in a taxon sample of 1232 species.

### Phylogenetic tree

The backbone tree used here is based on the consensus tree from the Angiosperm Phylogeny Website (Stevens 2016), with the internal phylogenetic structure of families resolved according to the same studies as in Reyes et al. (2016: Appendix II.1). Although it would be desirable to use molecular data to estimate branch lengths, our sampling strategy, designed specifically to capture the most of the variation in the focal perianth traits, did not allow us to do so because many of the species sampled do not have sufficient data in public DNA sequence databases (e.g., GenBank) and it was beyond the scope of this study to generate new sequence data for these species. Therefore, we used an alternative method to approximate branch times for this study. We employed the `bladj` function of `phylocom` (Webb et al. 2008) to

transform the consensus tree topology into an ultrametric tree, via constraining 398 nodes defining families and deeper clades (Appendix IV.1) to be the same age as they are in the recent study by Magallón et al. (2015).

#### Ancestral state reconstruction and transition rate estimation

Ancestral state reconstruction was performed using parsimony and maximum likelihood in Mesquite 3.04 (Maddison and Maddison 2015). We used two models for maximum likelihood character optimization: an equal-rate model (Mk1; i.e., single rate of transition for both forward and backward change) and a two-rate model (Mk2; i.e., a model allowing asymmetric rates of forward and backward change). To test and account for among-lineage rate heterogeneity, we used a similar approach as Zanne et al. (2014) and subdivided the tree into five partitions consisting of two grades and three large clades, each with its own model and transition rates (Figure IV.1). The first partition comprises the ANA grade, Chloranthales, and Magnoliidae, the second is Monocotyledoneae, the third includes the basal eudicots (incl. Ranunculales, Proteales, Trochodendrales, and Buxales), the fourth is Superrosidae (incl. Dilleniales), and the fifth is Superasteridae. The rationale for including two paraphyletic grades in our tree partitioning scheme is that members of these grades may have inherited ancestral transition rates, while the large clades nested in them may have shifted to distinct evolutionary regimes characterized by different transition rates. Two orders were excluded from all analyses due to a lack of both overall morphological information and state diversity in the characters studied: Ceratophyllales, the perianthless sister order to all Eudicotyledoneae; and Gunnerales, which comprises two families, one (Gunneraceae) that is poorly described and monomorphic in regard to our study characters, another (Myrothamnaceae) that is perianthless.

In practice, we pruned the tree to isolate the taxa from a given partition and performed ancestral state reconstruction on the resulting subtree. The likelihood of a tree-wide model in which each partition has its own rate is proportional to the product of the likelihoods of the model fitting each of the isolated partitions for a given character (Zanne et al. 2014). The Akaike Information Criterion (AIC) was then used to compare the relative fit of the data of the partitioned model to those of the default unpartitioned model.

Finally, we also tested the impact of four arbitrary transition rate values on the inferred character states and their probabilities under the Mk1 model on the full tree. Values for each

node were recorded under transition rates ( $q$ ) of 0.0001, 0.001, 0.01, and 0.1. From tests done for Reyes et al. (2016), we know that a binary character under a Mk1 model always reaches a 0.5 probability for both states under extremely high rates; we refer to this phenomenon as the high transition rate equilibrium. This equilibrium is possible for all our test nodes under the higher test rates, whether or not the value of the transition rate affects which state is the most-probable. Because of this, an observed change or absence of change in the most-probable state is the only criterion that we can use to distinguish nodes sensitive to arbitrary change in transition rate from those that are not. If a state was observed to become less probable as the transition rate increased, but never became less probable than the other state, it was considered simply to be converging towards the high transition rate equilibrium.

We also tested for transition rate asymmetry by fitting the Mk1 and the Mk2 models to each trait, both for angiosperms as a whole and for each partition of the tree. The relative fits of the two models were evaluated using the AIC. Further, to compare the relative magnitudes of transition rate asymmetry in Mk2 implementations, we here report on the transition rate symmetry ratio, calculated by dividing the lowest rate by the highest, creating a scale from 0 (unidirectional evolution) to 1 (both rates almost identical, virtually a Mk1 model). In the context of this ratio, “symmetry” is unrelated to perianth symmetry but rather the similarity between the forward and backward rates of Mk2. We also used the AIC to test which arbitrary rate best fitted the evolution of each character.

## RESULTS

### *Rate differences between full tree and partition*

We estimated the transition rates on an angiosperm-wide tree using Mk1 and Mk2 models. For each model, one set of estimates corresponds to the entire tree under the same model (which we will refer to as the full tree) while the other corresponds to different models for the five partitions of the tree (which we will refer to as the partitioned tree). The Mk1 transition rates of the five characters, which are plotted on Figure IV.2 for both the full and the partitioned tree, are used here for comparison.

Some partitions have much higher transition rates compared to the full tree version. We excluded unreasonably high rates from the histogram (see below). The [ANA+Magnoliidae]

(Chloranthales not mentioned to keep name short in future references to it) and Monocotyledoneae partitions are the most noticeable cases of having a higher transition rate than the full tree: both have a differentiation transition rate close to 0.013, while the character is found to have a transition rate of 0.0042 when optimized on the full tree, suggesting that this trait evolved faster in these two parts of the tree. In contrast, some partition estimates are found to have much lower transition rates than full tree estimates. For instance, pentamery has a transition rate of 0.0001 in Monocotyledoneae, while having a transition rate of 0.0069 at the level of angiosperms.

Five combinations are absent from the graph from Figure IV.2: fusion in [ANA+Magnoliidae] and Superrosidae (incl. Dilleniales), and symmetry in Monocotyledoneae and basal eudicots, all of which have transition rates so high (0.7-7) that the ancestral states of all nodes examined are equivocal. We interpret these unrealistically high rates as artefacts of ML ancestral state reconstruction due to our sampling approach (see Discussion). In any case, there is no change of perianth phyllotaxy in Monocotyledoneae in our dataset, rendering the inclusion of their estimated Mk1 rate ( $1.03 \times 10^{-10}$ ) in the histogram irrelevant to the test.

### Best-fit tree models

Four out of five full-tree character tree mappings are better fitted by Mk2 than by Mk1 (Figure IV.3; Appendix IV.6), suggesting strong rate asymmetry for most traits. Assuming that all partitions follow the same model (i.e., five Mk1 or five Mk2 models), a combination of five partition models is always much better fit to the data than a single model for the entire angiosperm tree (Appendix IV.6), suggesting a strong signal for among-lineage rate heterogeneity in the evolution of these characters. The smallest AIC difference between the full tree and the combination of partition models is 10 (Mk2 in symmetry and phyllotaxy), which is high enough for the models to be considered very different. When partitions are taken separately, each of them is best fitted by Mk1 or Mk2, or no better fitted by one model than the other (Figure IV.3; Appendix IV.6). If these best-fitted models are combined for a given character, they will fit the entire angiosperm tree better than five Mk1 models or five Mk2 models. For instance, according to Figure IV.3, the best composite model for differentiation would consist of the Mk1 models for [ANA+Magnoliidae], Monocotyledoneae and basal eudicots, and the MK2 models for Superrosidae and Superasteridae.

There is a general tendency for full trees and partitions that have a high symmetry ratio under Mk2 to be best fitted by Mk1 or have neither model fit better than the other, and for those that have a high symmetry ratio to be best fitted by Mk2. When Mk1 rates are imposed on the tree, fusion, symmetry, pentamery and differentiation have their lowest AIC at  $q = 0.0100$ . Phyllotaxy has its lowest AIC at  $q = 0.0010$ .

### Character optimization results

Appendix IV.8 shows the character state data that we have for all the species of our tree. Overall, most of the nodes tested are not very sensitive to model changes, although we observed more differences in shallow nodes (such as most recent common ancestors of orders; Appendices IV.3 and IV.5) than in deep nodes (the 15 key clades; Appendices IV.2 and IV.4) of the phylogeny. Moreover, model sensitivity was greater for perianth differentiation than for the other four characters considered in this study.

*Full tree and partitions under Mk1 and Mk2.* Changes in the most-likely state induced by going from the full tree model to a partition model are more frequent under Mk2 than under Mk1. The partitioned tree is less affected by a change from Mk1 to Mk2 than the full tree.

To simplify the interpretation of results, we chose to classify the proportional marginal likelihoods of character states in three categories. Those with a probability of 0.90 or higher (the default threshold used by Mesquite) are considered to have strong support and are indicated by two asterisks in the appendices in which the results are compiled (Appendices IV.2 and IV.3) and Table IV.1. Values higher than 0.60 and lower than 0.90 are considered to have moderate support and are indicated by a single asterisk in the appendices. Those not exceeding 0.60 are considered too close to 0.50 and are thus regarded to have weak support (no asterisk). While the term “equivocal” is commonly used for probabilities that are covered by weak and moderate support in this study, we use it for state probabilities of exactly 0.5. Table IV.1 summarizes the results for Caryophyllales, as an example of a node subject to changes in both fusion state and differentiation state.

**Table IV.1: Ancestral state reconstruction for Caryophyllales; most parsimonious character state; most-likely character state of full tree and partitions under Mk1 and Mk2** (full = full-tree model; part = partitioned-tree model). Asterisks in the maximum likelihood columns: none = weak support (0.51-0.60), one = moderate support (0.61-0.89), two = strong support (0.90-1).

<b>Caryophyllales</b>	Parsimony	Mk1 (full)	Mk1 (part)	Mk2 (full)	Mk2 (part)
fusion	fused	free*	fused*	free*	fused
symmetry	actinomorphic	actinomorphic**	actinomorphic**	actinomorphic**	actinomorphic**
phyllotaxy	whorled	whorled**	whorled**	whorled**	whorled**
pentamery	pentamerous	pentamerous* *	pentamerous* *	pentamerous* *	pentamerous*
differentiation	undifferentiated	differentiated**	differentiated**	differentiated* *	undifferentiated

The results for the full tree and the five partitions, both under Mk1 and Mk2, for the 15 key nodes were assembled in Appendix IV.2. Table IV.2 shows the number of nodes that change for each pair of tests under Mk1 vs Mk2 and the full versus partitioned tree. The most frequent changes of most-likely state are between the Mk2 full tree and the Mk2 partitioned tree and between the Mk1 full tree and the Mk2 full tree.

**Table IV.2: Number of changes in most-likely estimated ancestral state between models and tree types.** The method used to partition the tree reduced the reconstructable key node number to 13 rather than 15. The nodes that were lost have their names written in italics in Figure IV.1. Each column compares the results of two of the model and tree type combinations and reports the number of nodes that change their most-likely state in each case. Mk1 = one rate ML model; Mk2 = two-rate ML model; full = full tree; part = partitioned tree.

15 nodes (full) 13 nodes (part)	<b>Mk1</b> (full) ≠ (part)	<b>Mk2</b> (full) ≠ (part)	<b>(full)</b> Mk1 ≠ Mk2	<b>(part)</b> Mk1 ≠ Mk2
fusion	1	1	0	0
symmetry	0	0	1	0
phyllotaxy	0	4	5	0
pentamery	0	0	0	0
differentiation	1	4	6	0

The results for the full tree and the five partitions, both under Mk1 and Mk2, for the 54 tested order nodes, were assembled in Appendix IV.3. Table IV.3 shows the number of nodes that change for each pair of tests under Mk1 vs Mk2 and the full versus partitioned tree.

**Table IV.3: Number of changes in most-likely estimated ancestral state between models and tree types.** Each column compares the results of two of the model and tree type combinations and reports the number of nodes that change most-likely state in each case. Mk1 = one rate ML model; Mk2 = two-rate ML model; full = full tree; part = partitioned tree.

54 nodes	Mk1 (full) ≠ (part)	Mk2 (full) ≠ (part)	(full) Mk1 ≠ Mk2	(part) Mk1 ≠ Mk2
fusion	1	1	0	0
symmetry	0	2	5	1
phyllotaxy	1	8	7	0
pentamery	0	0	0	0
differentiation	1	12	17	3

It should be noted that in many of the above cases, the order nodes in which fusion and differentiation states change between models are directly descended from key nodes that exhibit a similar behavior. For instance, the only nodes to change fusion state were Superasteridae and Caryophyllales. Concerning differentiation, all key nodes and the vast majority of order nodes that change states between models belong to the three basalmost partitions. This indicates that sensitivity of most-likely states to model changes tends to affect clusters of closely related nodes.

Most states reconstructed by maximum likelihood are identical to those inferred by parsimony. At the key node level (13-15 nodes), there are a few cases of conflict between the two methods: there are three such cases in fusion, one in symmetry, four for phyllotaxy, five for differentiation. For instance, the ancestral state for Superrosidae, Rosidae and Malvidae is fused according to parsimony, but free according to the Mk1 and Mk2 full tree models. At the order node level, there are four cases in fusion (three are orders of Malvidae), four in symmetry, seven in phyllotaxy, one in merism and 13 in differentiation.

*Constrained Mk1 rates.* All tested nodes for all characters have their high transition rate equilibrium at  $q = 0.1$ , suggesting that such a rate of evolution is too high to make any inference on ancestral states in angiosperms. Fusion and differentiation were found to be more sensitive to these arbitrary rate changes than symmetry, phyllotaxy and merism. Table IV.4 summarizes the results for Caryophyllales, as an example of a node subject to change depending on the transition rate. The results for the constrained rates for the 15 key nodes were assembled in Appendix IV.4 and those for the order nodes in Appendix IV.5.

**Table IV.4: Most-likely state and state probability under constrained rates.**  $q$  = imposed Mk1 transition rate. The indicated state is the most-likely, followed by its marginal probability. (hr) = high rate equilibrium.

<b>Caryophyllales</b>	Parsimony	$q=0.0001$	$q=0.0010$	$q=0.0100$	$q=0.1000$
fusion	fused	fused (0.99)	fused (0.92)	free (0.65)	equivocal (hr)
symmetry	actinomorphic	actinomorphic (1)	actinomorphic (1)	actinomorphic (1)	equivocal (hr)
phyllotaxy	whorled	whorled (1)	whorled (1)	whorled (1)	equivocal (hr)
pentamery	pentamerous	pentamerous (1)	pentamerous (1)	pentamerous (1)	equivocal (hr)
differentiation	differentiated	differentiated (1)	differentiated (1)	differentiated (0.90)	equivocal (hr)

Two main types of results were observed. Figure IV.4 shows, for the Caryophyllales node, the evolution of the state probability of a fused and a differentiated perianth as a function of the transition rate with denser sampling than with all other nodes. The probability of a fused perianth shows the general pattern of a node that can change most-probable states. Its lowest point is a 0.35 chance of being differentiated, but the lowest point from other nodes on which we tested intermediate transition rates was observed to become extremely close to 0. The probability of a differentiated perianth presents the pattern of a node that does not change state, with a gradual probability decrease that eventually reaches the high rate equilibrium (0.5) and does not leave it. The most noticeable difference between the two curves is the slump formed by the fusion curve as the result of going under 0.5, then later converging towards it. In some nodes that kept the same most-probable state from 0.0001 to 0.01, the probabilities followed a pattern that resembled this slump or its mirror image rather than the pattern from the differentiation curve. They are indicated in italics in Appendices IV.4 and IV.5 and treated as nodes suspected to be subject to state change. Tables IV.5 and 6 show how many confirmed and suspected state change nodes are in each set.

**Table IV.5: Number of nodes confirmed or suspected to state changes under different Mk1 rates (Key nodes).** Confirmed: observed change in most-probable state. Suspected: state change not observed, but pattern includes a slump or a bump.

<b>15 nodes</b>	<b>Total</b>	<b>Confirmed</b>	<b>Suspected</b>
fusion	5	1	4
symmetry	0	0	0
phyllotaxy	0	0	0
pentamery	0	0	0
differentiation	5	4	1

**Table IV.6: Number of nodes confirmed or suspected to state changes under different Mk1 rates (Order nodes).** Confirmed: observed change in most-probable state. Suspected: state change not observed, but pattern includes a slump or a bump.

<b>54 nodes</b>	<b>Total</b>	<b>Confirmed</b>	<b>Suspected</b>
fusion	8	2	6
symmetry	0	0	0
phyllotaxy	1	1	0
pentamery	1	1	0
differentiation	12	10	2

## DISCUSSION

Here, we have shown that reconstructed ancestral states were robust to the different models we tested. We showed that a change in the most-likely state for a given character was possible from altering transition rates while keeping all other parameters unchanged. We also showed that the probability of a given character state as a function of the Mk1 transition rate could form at least two different types of curves, which always converge towards an equilibrium of 0.5 at high transition rates.

Our choice of focal nodes to evaluate the impact of models and rates in this study consisted entirely of relatively deep nodes in the angiosperm phylogeny tree. While present in the tree, the derived states of the characters tested were sometimes virtually absent from these nodes, indicating that most of the origins of these states took place in clades nested within orders. For instance, our symmetry results show that zygomorphy appeared mostly within orders, consistent with our previous study of this character (Reyes et al. 2016). Zygomorphy is a potential most-likely ancestral state for only seven orders (Asparagales, Commelinales, Poales, Zingiberales, Boraginales, Lamiales, Solanales) and one deeper key node (Commelinidae). However, some characters behaved differently. Pentamery, for example, appears to have evolved early in the evolution of angiosperms and is reconstructed here as a synapomorphy of Pentapetalae as a whole (with many reversals), which comprises 75% of species diversity.

The model made of five partitions corresponding to five major angiosperm groups was always better fitted to the tree than a single model made to fit a single tree of flowering plants. A model based on even more tree partitions would probably fit the tree even better, but there would be a trade-off with the size of the partitions; the results could be biased if the partitions are too small. Here, we chose the strict minimum number of tree partitions we considered necessary. Our approach is similar to that used by Zanne et al. (2014), with one version of their model uniform across the tree and the other allowed to have different rates in different subdivisions.

There are four character partitions in which neither Mk1 nor Mk2 is preferred despite a low symmetry ratio. They all correspond to characters for which, in that specific partition, one of the states has very few origins with no reversals, yet the estimated reversal rate is a level of magnitude higher than that of the very low origin rate. We suggest that these high reversal rates are an artifact of ML optimization. Similar artifacts have been observed by us in the present study (phyllotaxy in the Pentapetalae partitions, pentamery in [ANA+Magnoliidae] and Monocotyledoneae respectively), ongoing studies (Sauquet et al., unpubl. data) and previous authors (Vanderpoorten and Goffinet 2006; Goldberg and Igc 2008), and appear to emerge when one particular state is rare and asymmetric rates are allowed such as in the Mk2 model. However, more work is clearly needed on characterizing and finding ways to prevent such reconstruction problems.

The effect of each test varied depending on the character. The differences between the full and partitioned tree under Mk1 and Mk2 only had an impact on fusion in Superasteridae and Caryophyllales, while the constrained rates test affected more nodes (5 key nodes and 8 order nodes). Differentiation was the character with the most nodes that were sensitive to all three means of changing the model. Pentamery was unaffected by Mk model and tree type changes, but an order node changed state under constrained rates. Symmetry and phyllotaxy showed similar sensitivity to all tests and always had many fewer sensitive nodes than differentiation.

In general, when mapping character evolution on the full tree, greater differences between the Mk1 and Mk2 trees were observed than when allowing among-lineage rate heterogeneity (i.e., partitioned tree analyses).

For phyllotaxy, one of the characters the most subject to this phenomenon, we suspect that the results from the ANA+Magnoliidae partition for phyllotaxy may be more accurate than the results of the Mk2 full tree. It may imply that the model that fits the phyllotaxy full tree best, Mk2, may actually be less accurate than the less well supported Mk1. Simulations may be required to confirm this observation. King and Lee (2015) performed their tests on simulated trees, and were able to see if the model that was used to generate the tree would be favored in a model fit test. In doing so, they discovered that the highest likelihood could be found for inaccurate models. The evolution of phyllotaxy in the Mk2 full tree may be a case of an inaccurate model being favored by AIC. More precisely, we suspect that the advantage of the Mk2 model over the Mk1 model for the full phyllotaxy tree may be an artifact due to the artificially high reversal rate of this character.

Our constrained rate results are consistent with the observation by Cunningham et al. (1998) that use of parsimony is only suitable when rates of character state change are low. While a convergence towards a probability of 0.5 without most-likely character state change and a change in most-likely character state were the most frequently found patterns in the constrained rate tests, other patterns were found as well (Figure IV.4).

Vanderpoorten and Goffinet (2006) reported that the inferred root state can change between Mk1 and Mk2 when one of the character states is rare or unique. This finding is similar to what we observed with phyllotaxy, and may be linked to the rarity of the spiral state and the artefact affecting states with few origins and no reversals discussed above. Zanne et al. (2014), when giving different transition rates to each subdivision of the tree for growth form and habitat climate, had similar transition rates between Superasteridae and Superrosidae. Such a proximity in transition rates between the two subdivisions was found here for phyllotaxy, pentamery and differentiation. However, Superrosidae have a much higher transition rate between fusion states than Superasteridae and the relation is inverted for symmetry.

Wu et al. (2015) mentioned that their maximum likelihood results were sometimes more equivocal than those for parsimony. This may be one of the two situations from Figure IV.4 in which the states probabilities are close to 0.5: the most-probable state change or of the high rate equilibrium. Soltis et al. (2013) found at least one node in which the parsimony inference and the maximum likelihood most-likely state were different. This is probably a case where the rate is high enough for the least parsimonious character to be the most-likely state.

We suspect spiral floral phyllotaxy to be a case of macroevolutionary self-destruction (Bromham et al. 2016), more specifically the type that causes lower diversification in groups in which it has appeared. Self-destructing traits are characterized by a tendency to be present in several small clades at the tip of the phylogenetic tree. This is true of spiral phyllotaxy, especially in Pentapetalae, where it appears only in a handful of relatively small clades, such as Paeoniaceae family and a subclade of Theaceae.

Doyle and Endress (2000) concluded that the perianth is free or basally fused in the common ancestor of all angiosperms, which is compatible with the free state found in our study.

Our results regarding phyllotaxy globally agree with the perianth phyllotaxy parsimony reconstructions by Ronse De Craene et al. (2003), Zanis et al. (2003), Endress and Doyle (2007), Doyle & Endress (2000), Endress and Doyle (2009), Doyle and Endress (2011) and Soltis et al. (2005). These reconstructions, however, leave the state of the common ancestors of angiosperms equivocal (all) and also that of Laurales either equivocal (Ronse De Craene et al. 2003; Soltis et al. 2005; Endress and Doyle 2007, 2009) or spiral (Doyle and Endress 2011). Most of our reconstructions find the common ancestor of angiosperms to be whorled. The common ancestor of Laurales is reconstructed as whorled, as in the reconstruction of Zanis et al. (2003). The only model among those we tested that reconstructs both Angiospermae and Laurales as ancestrally spiral, Mk2 fitted to the full tree, also reconstructs that state for the common ancestor of Monocotyledoneae, which do not contain any spiral species.

The larger regions of the tree in which the probability of pentamery is over 0.9 coincide with the origins of pentamery found by Ronse De Craene (2003), by Soltis et al. (2003), by Zanis (2003) and by Soltis et al. (2005) (Hernandiaceae from Magnoliidae, Ranunculaceae and Sabiaceae form basal eudicots, Pentapetalae themselves). All the extra pentamery occurrences we found were either isolated species or small families outside Pentapetalae (four in Magnoliidae, one in Monocotyledoneae, one in the basal eudicots). We also note that there are several potential or confirmed re-originations of pentamery within parts of Pentapetalae in which the state was lost, (such as the presence of pentamerous species nested within the non-pentamerous Fagales).

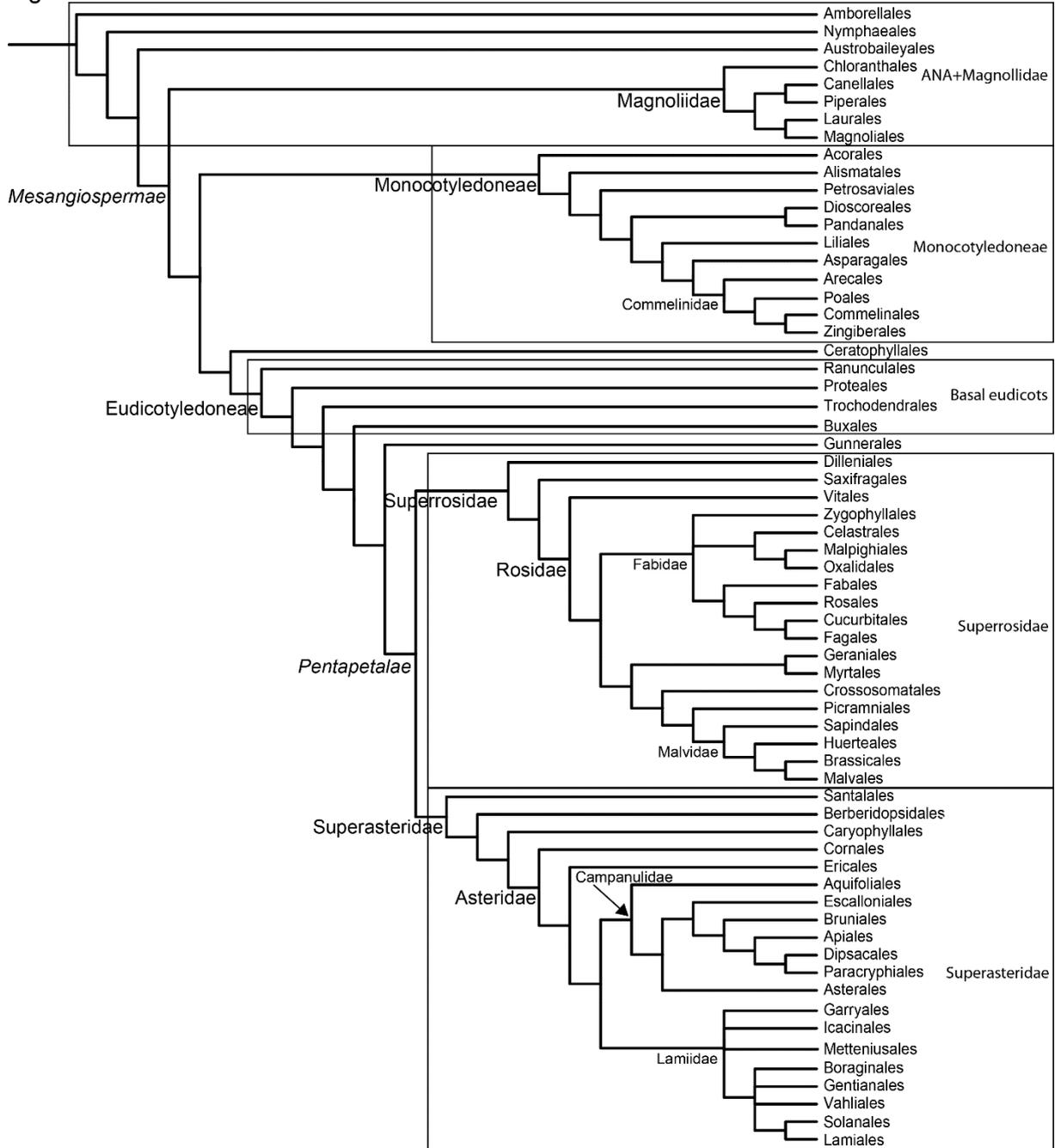
Most previous reconstructions of perianth differentiation (Doyle and Endress 2000; Ronse De Craene et al. 2003; Zanis et al. 2003; Hileman and Irish 2009) agree that angiosperms are ancestrally undifferentiated and that the ancestral state of this character in Eudicotyledoneae is equivocal (undifferentiated in Soltis et al. 2005). Results for nodes basal to Pentapetalae are very sensitive to model changes in the full tree and tend to have weak support (0.6 probability or under) in the five partitioned trees. Pentapetalae are mostly differentiated, with occasional undifferentiated shallow clades. The reconstructions of Ronse De Craene (2003) and Soltis et al. (2005) agree with the Mk2 version of the full tree in our study, while that of Doyle and Endress (2009) reconstruction is closer to our Mk1 full tree.

The as yet unpublished first eFLOWER paper by Sauquet et al. (H. Sauquet et al., in prep.) included a similar, complementary study focusing on 21 floral traits sampled from 792 species and using three reconstruction methods (parsimony, ML and Bayesian). The results for the four perianth traits shared with our study are, in general, very consistent with those presented here, despite important differences in the sampling approach and the source used for branch lengths. The set of key nodes we chose overlaps with those whose states are shown in the eFLOWER paper (H. Sauquet et al., in prep.). Among the reconstruction methods used in the latter, the results for maximum likelihood are very close to what we obtained in the Mk2 full tree, which we will use as the main basis of comparison (see below). The results for fusion and symmetry are similar, with only one node that has a different most-probable state between the two studies for each character; Commelinidae for symmetry (moderate support for zygomorphy instead of strong support for actinomorphy) and Asteridae for fusion (weak support for the fused state rather than strong support for free). The same ancestral states for phyllotaxy and differentiation are found in the two studies. We should note, however, that in the case of phyllotaxy, the ML results of Sauquet et al. (unpubl. data) and ours for Mk2 full-tree both reconstruct the root of angiosperms as spiral, but our partitioned-tree analyses and their Bayesian analyses showed that the root was probably whorled instead. In the case of differentiation, all the reconstruction of Sauquet et al. (unpubl. data) agree on early nodes being undifferentiated, while only our Mk2 full tree for differentiation yielded similar results. The transition rates were much higher in our study (about twice as high) but the symmetry ratios were about the same between comparable characters. The symmetry ratio for fusion was, however, higher here than in Sauquet et al. (unpubl. data).

### Conclusion and perspectives

In this study, we found that most ancestral states for deep and more shallow nodes of the angiosperm tree were unaffected by changes in models (number of transition rates in model, value of transition rates, taxonomic scale of the reconstruction). However, we found strong signal for among-lineage rate heterogeneity in perianth evolution, and we observed that accounting for this heterogeneity may influence some reconstructed ancestral states. We also observed that, for the equal-rate (Mk1) model, low rates lead to results most similar to the parsimony optimization, while very high rates lead to complete uncertainty, consistent with the predictions of the Markov model. These factors will need to be taken into account in future studies focusing on ancestral state reconstruction. Figure IV.4 shows the evolution of state probabilities as the Mk1 rate increases. It would be interesting to know how state probabilities evolve as Mk2 rates change. Such a test would have to factor in both the absolute value of the transition rates and their symmetry ratio, which will involve a protocol more complex than the one used to make these observations for Mk1.

Figure V.1



**Figure IV.1.** Order-level tree of angiosperms. The members of each partition/subdivision are contained in rectangles. The key nodes in italics are those not reconstructed in the partitioned tree.

Figure IV.2

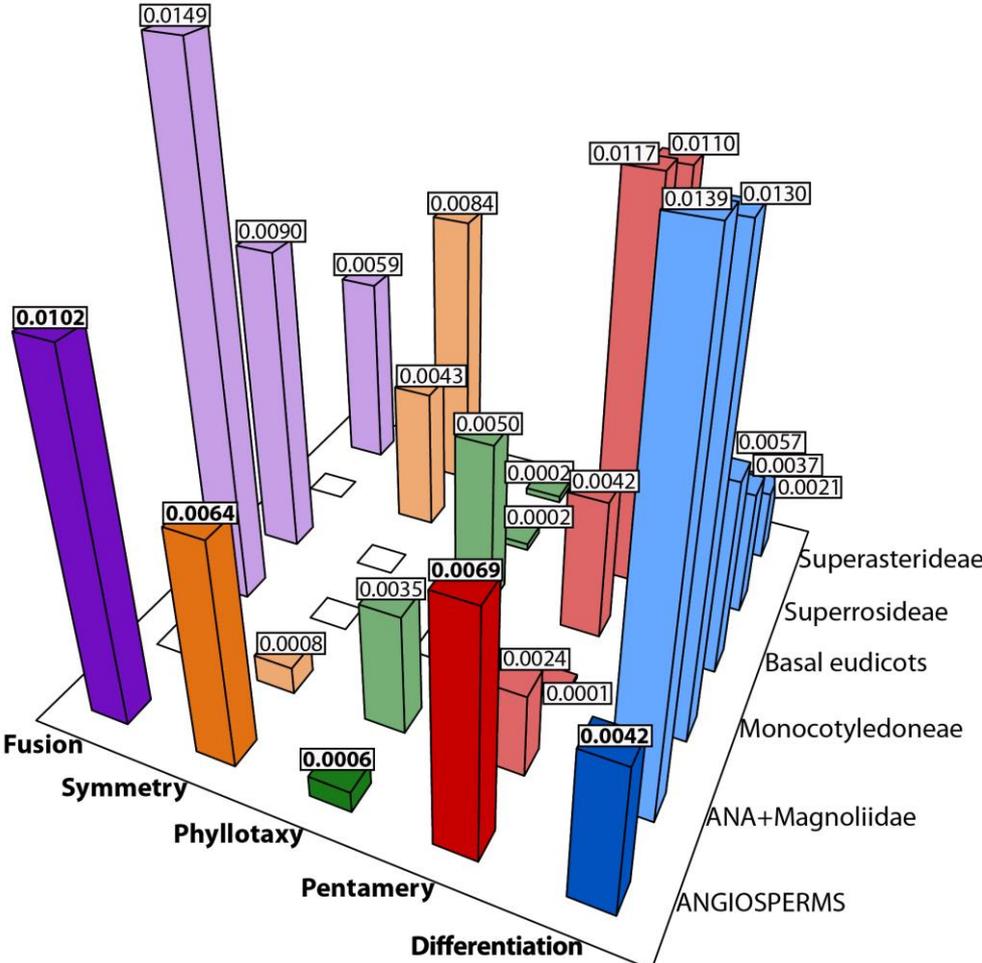
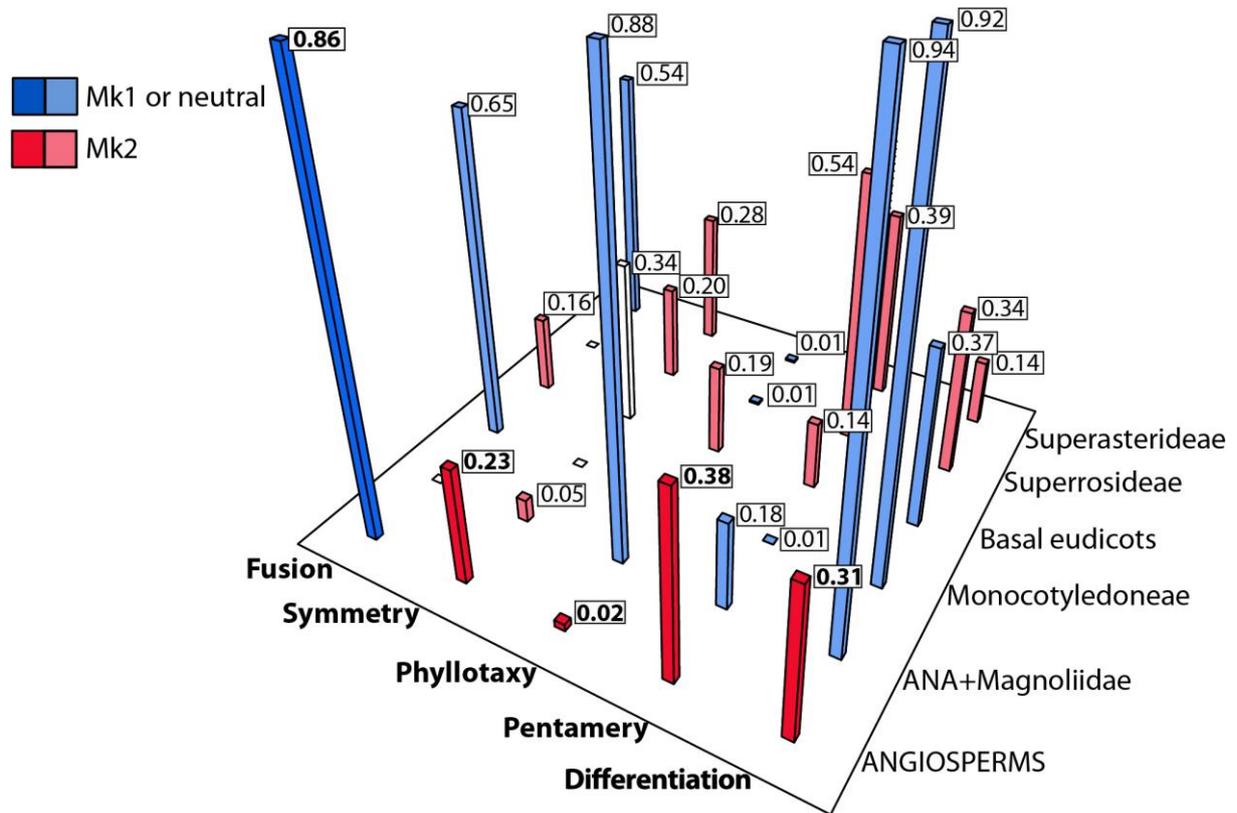


Figure IV.2. Histogram of the Mk1 transition rates for the full tree (dark colors) and partitions (light colors) for each of the five characters.

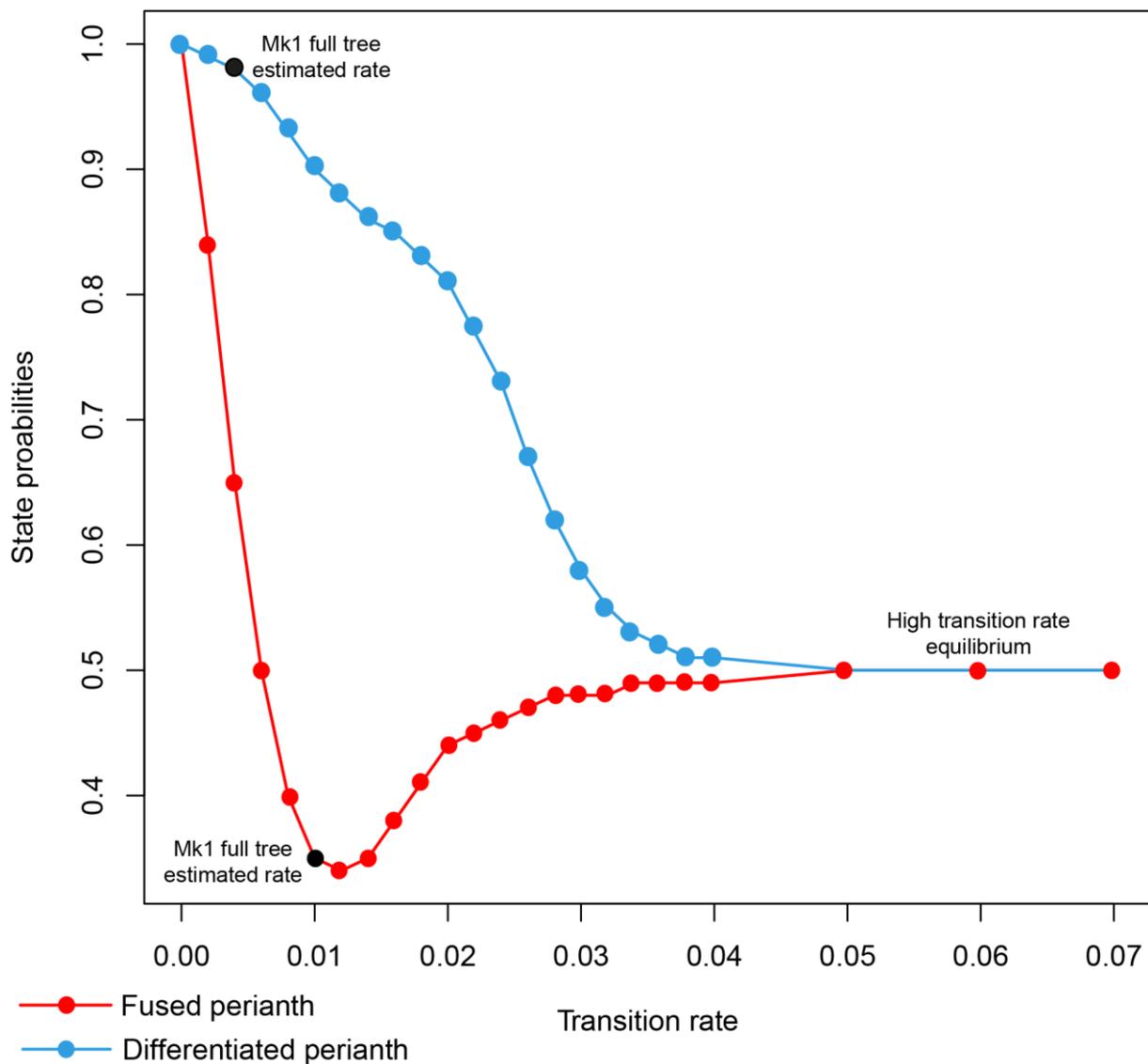
Figure IV.3



**Figure IV.3.** Histogram of Mk2 symmetry ratios for the full tree (dark colors) and partitions (light colors). Blue bars are best fitted by Mk1 or have no difference in fit between Mk1 and Mk2. Red bars are best fitted by Mk2. The white bar is from a partition in which the transition rate was exceptionally high under Mk1 but not Mk2, making the latter the only one available for comparison.

Figure IV.4

### Caryophyllales ancestral state probabilities in function of Mk1 transition rates



**Figure IV.4.** Caryophyllales state probabilities in function of fixed Mk1 transition rate. Black dots correspond to the points closest to the rate and probability obtained by estimation. Each of these states are also the one inferred by parsimony reconstruction. In both cases, the probability of the others state has the exact opposite evolution

# GENERAL DISCUSSION

The aim of this thesis was to explore the evolutionary patterns of floral characters and their impact on species diversification. In Chapter 1, I investigated the impact of perianth zygomorphy and presence in Mediterranean hotspots on diversification in Proteaceae. I found one such state, perianth zygomorphy, to not be correlated with any changes in diversification, whereas the presence in Mediterranean hotspots was found to possibly be the source to a macroevolutionary sink (Burin et al. 2016). In a derivative of that chapter, I optimized perianth symmetry on a species-level phylogeny of Proteaceae, using a maximum likelihood model and showed that there were 16 origins of zygomorphy and six reversals to actinomorphy (See Figure V.1). In Chapter 2, I investigated the number of times zygomorphy has appeared across the angiosperm tree, which resulted in finding at least 130 origins accompanied by 69 reversals to actinomorphy. I also conducted a review on what was currently known about floral zygomorphy in terms of ecology, evolution and development. In Chapter 3, I tested the influence of transition rate heterogeneity and the use of different reconstruction models on ancestral states, and found that rate heterogeneity within angiosperms was strong, but this heterogeneity and the change in reconstruction models only affected ancestral state reconstruction in a minority of nodes. All three chapters involved ancestral state reconstruction and were based on the assumption that the ancestral states reconstructed were robust as long as the same tree remained in use. Chapter 3 demonstrated that some ancestral state results were not robust to model changes and that one of the causes is rate heterogeneity among angiosperm clades, meaning that a change of the scale on which ancestral states are reconstructed can lead to changes in the inference of most-probable ancestral states.

Many evolutionary studies require some degree of prior knowledge of where in the phylogenetic tree and how many times a given character state has appeared. In addition, candidate key innovations now need to be proven to be recurrently correlated with higher diversity, as they may otherwise be “hitchhiking” on another character that is the actual cause of the higher diversity. The existence of self-destructive states and the possible late start in diversification of clades defined by derived states has made it important to know which state is the ancestral one and which is derived to be able to interpret test results accurately, including in comparisons between sister clades (Käfer and Mousset 2014; Bromham et al. 2016).

The species sampling method used in the second and third chapters, which focused on representing most changes in a character across the whole phylogeny of angiosperms, is somewhat of a novelty and by definition has a higher density of state changes than other sampling methods. The other sampling methods, such as random exemplars and using supraspecific taxa, inherently sample fewer changes in character state than the method I used, but result in character state distributions closer to reality. My trait-dependent exemplar approach will be hereafter referred to as “change-capture”. An extensive discussion of the properties I have been able to observe for trees built with this approach is necessary to evaluate their utility for future studies. Below, I will also discuss what seems to be missing to better understand species diversification and other existing studies on angiosperm diversification.

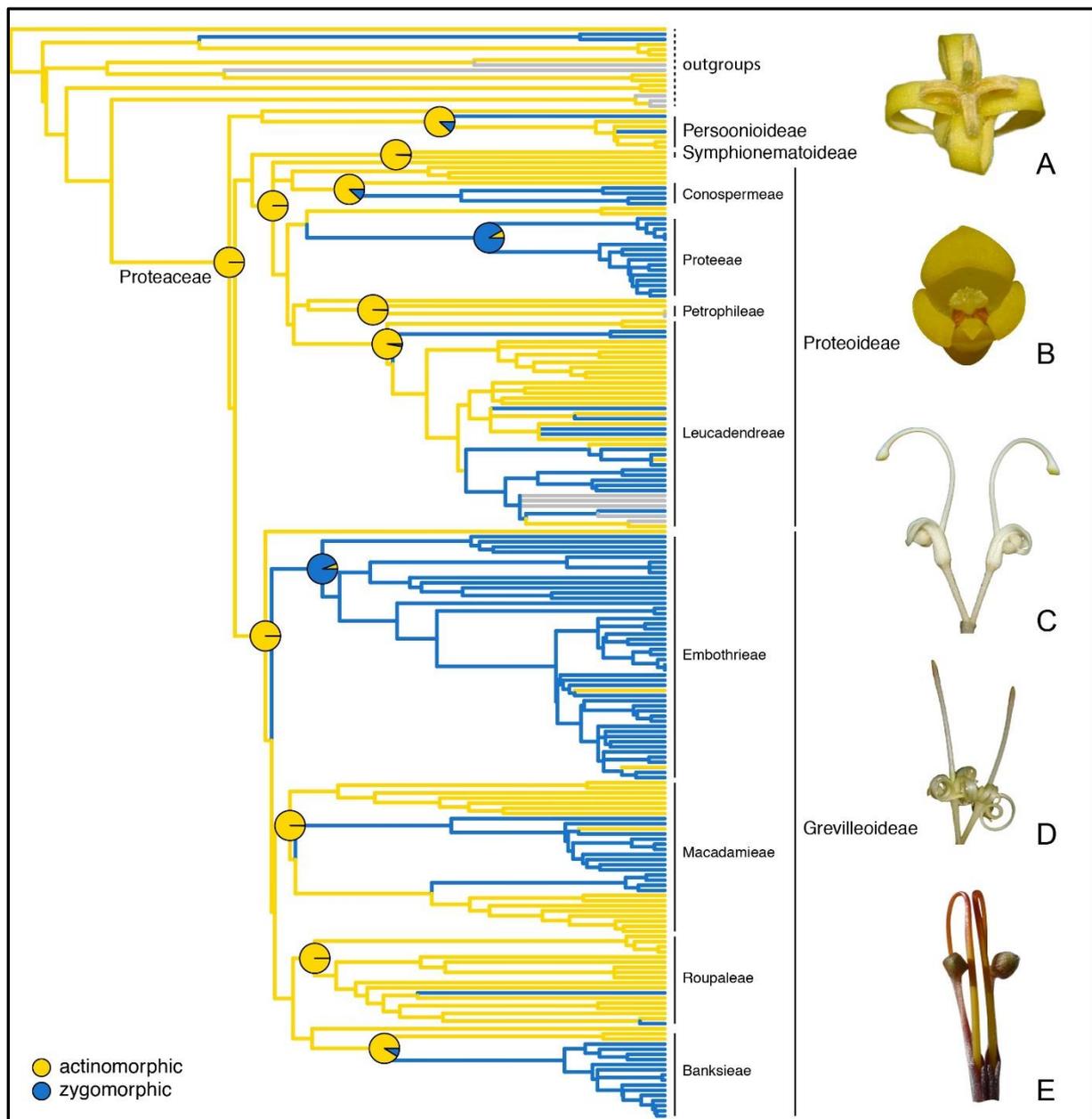
## **PROPERTIES OF TREES CREATED BY “CHANGE-CAPTURING” TRANSITIONS IN BINARY CHARACTERS**

### *1. What the reconstructed ancestral states of Proteaceae symmetry tell us*

The “change-capture” method is focused on sampling changes in a given character, and hence tends to produce trees with estimated transition rates that, even when low enough not to make the reconstruction identical in all nodes, are overestimated. My first attempt at “change-capture” sampling was done on a single character, perianth symmetry, with the purpose of having at least one species from each family of flowering plants. Maximum likelihood analyses inferred transition rates so high that all nodes were reconstructed as having the same state probabilities between each other, so only a parsimony reconstruction was used (Figure II.2). In Chapter 3 of this thesis, the “change-capture” method was used with five characters, by adding the species that were needed to accommodate the variation of other characters to the symmetry-based tree. I was able to use both parsimony and a maximum likelihood reconstruction that was not subject to a high transition rate induced artifact on that tree. It should be noted, however, that this artifact occurred again in four out of the 25 character and tree partition combinations. The parsimony reconstructions on these two “change-capture” trees for Proteaceae symmetry suggests that the family was ancestrally actinomorphic with several origins of zygomorphy and occasional reversals to actinomorphy. This pattern is the same as that seen in the genus-level maximum likelihood reconstruction presented in the supplementary material of Chapter 1 (Figure II.S7) and the species-level maximum likelihood reconstruction from Citerne et al.

(2016) (Figure V1). The maximum likelihood symmetry optimizations on the “change-capture” trees, on the other hand, reconstructed Proteaceae as ancestrally zygomorphic. This was the case on both the full angiosperm tree and the basal eudicots partition.

Transition rates estimated by “change capture” are much higher than those estimated in the first eFLOWER paper (H. Sauquet et al., in prep.), which uses random exemplar sampling. This is probably the cause of the difference in ancestral state reconstruction found between maximum likelihood on the “change-capture” trees and the other Proteaceae reconstructions. One additional reason why this difference is attributed to overestimated rates is the observation that in the Mk1 constrained rate test from Chapter 3, nodes that could change state depending on the transition rate had the same state as they did in parsimony under low transition rates and had the other state become more probable under higher rates. According to this pattern, the tree presented in Chapter 1 and that in from Citerne et al. (2016) would be the ones under lower transition rates and the tree presented in Chapter 3 the one under high rates. This idea was confirmed by an extra test done on a partition consisting only of the Proteaceae selection included in the study presented in Chapter 3: lower transition rate values for Mk1 (identical transition rate for origins and reversals) and Mk2 (two different rates for origin and reversal), with preserved symmetry ratio of rates for Mk2, make Proteaceae ancestrally actinomorphic with several origins of zygomorphy. An important element to note is that in the case of the analyses presented in Chapter 3, there is an extra factor besides the “change-capture” sampling method that has almost certainly led to overestimated transition rates in Proteaceae. Due to different dating sources (Sauquet et al. 2009 for Chapter 1, and Citerne et al. (2016) and Magallón et al. 2015 for Chapter 3), the estimated age of Proteaceae in Chapter 1 and in Citerne et al. (2016) is around 85 million years old, while they are estimated to be only 42.5 million years old in Chapter 3. I did not use the ages from the first chapter in Chapter 3 on the basis of considering the ages in Magallón et al. (2015) as relative to each other and equal treatment of all families that could have inaccurate ages in the tree. A possible additional factor is that there is a higher proportion of zygomorphic terminal taxa in the Proteaceae used in Chapter 3 compared to those from Chapter 1 and Citerne et al. (2016).



**Figure V.1:** Figure 1 from Citerne et al. (2016). Maximum likelihood reconstruction (equal rates model) of perianth symmetry evolution in Proteaceae on a new species-level tree (best-scoring RAxML tree dated with penalized likelihood). Branches are colored according to the state with highest marginal likelihood of their terminal node (or the tip state for terminal branches). Grey branches correspond to missing or inapplicable data (i.e. perianth absent). For full details, see Figs. S1 and S2. The photographs on the right-hand side illustrate some of the variation in perianth symmetry found across Proteaceae. (A) *Persoonia amaliae* (Persoonioideae), actinomorphic flower. (B) *Synaphea* sp. (Proteoideae: Conospermeae), zygomorphic flower. (C) *Buckinghamia celsissima* (Grevilleoideae: Embothrieae), pair of zygomorphic flowers. (D) *Viotia leptophylla* (Grevilleoideae: Macadamieae), pair of actinomorphic flowers. (E) *Banksia ericifolia* (Grevilleoideae: Banksieae), pair of zygomorphic flowers. Photograph credits: A-D, Peter Weston; E, Hervé Sauquet.

## 2. The consequence of overestimated transition rates on other ancestral state reconstructions

The above comparison of the maximum likelihood reconstruction for Chapter 1, Citerne et al. (2016) and the Proteaceae selection from Chapter 3 sheds light on the main weakness of the “change-capture” sampling method when ancestral states are reconstructed with maximum likelihood methods. It first seems as if it may be a reliable method as long as one produces a tree in which the estimated transition rates are low enough to avoid creating a reconstruction made entirely of nodes with identical state probabilities. Once that condition is fulfilled, one would simply need to remember to consider the rates on a relative scale rather than an absolute one. However, the constrained transition rate tests done on data used in Chapter 3 show that, even when not high enough to reach the high transition rate equilibrium of state probabilities, the absolute value of the transition rates actually does matter. As shown in Chapter 3, some nodes can have their most-probable state under maximum likelihood change solely from the transition rates being high enough, with all else being equal. The transition rate value needed for this to happen varies greatly among the few nodes that display this pattern. Some of these values are lower than the rates estimated by the first eFLOWER paper (H. Sauquet et al., in prep.) while others are higher. If transition rates are greatly overestimated, the number of state-variable nodes that are past the point at which they change states is artificially increased as well. This can cause the variable nodes of a “change-capture” phylogeny to have different most-likely states than an eventual pre-existing tree with character state sampling proportions closer to reality for the clade. On the other hand, this change in most-likely character state only affects nodes in which it can happen, which have been shown to be a minority in the five characters tested in Chapter 3. They are consistently in places in the tree in which the character state is more labile than elsewhere, such as pre-Pentapetalae grades for differentiation in Chapter 3. The majority of nodes simply keep the same most-probable character state, although even those start converging towards the 0.5 state probability characteristic of the high transition rate equilibrium when constrained rates are high enough. Nodes that don't change state at all or change most-probable states at extremely low transition rates will probably have a consistent most-probable state under most estimated transition rates. In future studies, however, it may be a good idea to conduct some constrained transition rate tests on the nodes of interest and see if one of them has a state affected by transition rates. Nodes in which the most-likely state changes a lot as the transition rate increases will be the ones whose inferred ancestral states will need to be taken with caution in a tree under a given set of estimated transition rates. Such nodes will probably be those most likely to have different states in different studies or simply be

interpreted as equivocal. Another solution could be to have the constrained rates test presented as part of the study's results and highlight those that may change according to the inferred transition rate. This could be an alternative to a long MCMC search to measure one aspect of the uncertainty in reconstructed maximum likelihood states.

### 3. Impact of the differences in reconstruction model

A similar caution needs to be applied concerning the nodes that can change state between the Mk1 and Mk2 models. In this case, there is the extra complication that relative model fit to the data seems to be a poor indicator of both the accuracy of the reconstruction (King and Lee 2015) and of the extent to which the likelihood reconstruction resembles that from the parsimony analyses. The latter is shown by the fact that both the phyllotaxy and differentiation full-tree reconstructions from Chapter 3 are better fitted by Mk2 than by Mk1. However, the Mk1 reconstruction is the one that resembles parsimony the most for phyllotaxy, while the greatest resemblance is found for Mk2 in the character of differentiation.

In comparison to the maximum likelihood reconstructions, the parsimony reconstructions did not present too many complications and may represent a more suitable use of trees constructed with “change-capture” sampling. It was possible to reconstruct relevant ancestral states on a tree that was built to optimize a single character, as in Chapter 2. While equivocal branches are frequent in these reconstructions, the possibilities they suggested are still far fewer than what is possible via changing likelihood transition rates; the ancestral state probabilities presented in the appendices for Chapter 3 include several nodes that have only one state under parsimony but can have the other possible state be the most-probable one under high enough transition rates. However, the very principle of parsimony, which is to reach the tree's state distribution with as few changes possible, means that using it instead of maximum likelihood is nearly identical to arbitrarily imposing an extremely low transition rate on the tree rather than estimating the value of the transition rate (Cunningham et al. 1998). However, if constrained transition rates are needed for a future study, they can be used on a “change-capture” sampled tree, as their main problem stems from high *estimated* rates.

#### 4. Change capture versus sampling of sequenced species

Another element of “change-capture” sampling is that the variation of a given character for a given clade can sometimes only be illustrated by including species in the sampling that have been described but have yet to be sequenced and included in molecular phylogenetic trees. For any ancestral state reconstruction method that requires a dated tree, including maximum likelihood, there will be a choice between dating via genes at the expense of ignoring some state changes or constructing a tree with all trait changes at the price of using “second-hand” tree dating (with Phylocom, for example). Avoiding this trade-off is possible by generating new sequences for species that are part of their sample but not represented in GenBank. If the dilemma is not avoidable, both solutions have their advantages and drawbacks. Using only species for which molecular data are available will give better support to the phylogenetic tree, but will underestimate the actual number of state changes in the clade. This may seem like a small loss, but doing so may contribute to maintaining in scientific obscurity the very species that would need to be sequenced to make character evolution optimizations more complete.

In summary, “change-capture” is recommended only for those who intend to use parsimony, trace the evolution of several characters, or use constrained transition rates. It is recommended to test the sensitivity of node state to transition rates if a method requiring branch lengths is used. Only using species from GenBank may result in a tree that lacks some of the changes in state, while strictly adhering to a protocol of sampling the character state variation of a clade may require adding species that have yet to be sequenced, which can make “second-hand dating” of the tree necessary to obtain branch lengths.

## **ELEMENTS MISSING TO UNDERSTAND BETTER THE DIVERSIFICATION OF ANGIOSPERMS**

### 1. The need for both more floral data and better models

Efforts that need to be made to understand the causal factors that are responsible for driving angiosperm diversification better are on two fronts, both of which are unlikely to reach an ideal situation if kept separate from one another: one is better species sampling and phylogenetic resolution, and the other is the flexibility of models regarding the available

species. Methods that link character states to diversification rates exist, but either assume complete sampling and full tree resolution (Maddison et al. 2007) or a limited number of missing species or unresolved clades (Alfaro et al. 2009; FitzJohn et al. 2009). However, approaching complete sampling and resolution is a realistic possibility only for a limited number of clades. The variety of species that can be used for any given phylogenetic study is thus limited by the data accessible on GenBank, their reliability in respect to the published sequences and the accurate identification of the voucher specimen (O'Meara et al. 2016) and the capacity to generate new data. In addition, availability on GenBank does not necessarily mean that all morphological and environmental characteristics of the species have been described. Obtaining the description of all characters of interest for all known angiosperm species would probably take a virtually infinite amount of time, and may actually be impossible, as species are regularly being discovered or, more rarely, going extinct. The PROTEUS database, which is designed to work towards this target by collecting floral data in a standardized format, is a step in the right direction concerning most of these issues. However, the morphological data that can be added to it must have been recorded in the form of a published flora treatment or scientific paper, which will leave gaps where nothing has been recorded. Filling those gaps will be hindered by determining which aspects of a flower are considered important (or trivial) by the person describing them (see the issue with underreporting below), vocabulary that varies over time or between people working in the same field, and the difficulty of accessing some species.

Because of this, it may take less time and be more efficient to come up with models that are more tolerant of sampling biases and lack of resolution in clades. As mentioned above, one version of BiSSE, which was used in Chapter 1, can handle small unresolved clades in otherwise completely resolved trees (FitzJohn et al. 2009). However, many clades are known to be polymorphic for various characters, but the number of each species expressing each state is not known. In addition, when there are several species exhibiting a derived state present in the clade, it is preferable to know whether they are closely related to each other: in a hypothetical clade of 10 species equally distributed between two different states, five pairs of different-state sister species have different implications than that of two clusters of five species, each cluster showing a different state. A version of BiSSE is currently implemented in Mesquite, which can handle polytomies, but it is not the one that can handle missing data. Another problem with BiSSE is that it loses power if there is high tip ratio bias, which means

that it should not be used if one of the states is found in less than 10% of the species in a clade (Davis et al. 2013). This prevents it from being used on clades where one of the states is particularly rare. A method is needed that can handle large trees, polytomies, unresolved clades larger than 200 species (the current limit of BiSSE), multiple characters simultaneously (which is currently approximated using MuSSE, see below), missing data, high tip ratio bias, and ideally at least two of these elements at the same time. In addition, “change-capture” sampling might become a more viable method if there were a model that could take its inherent sampling bias into account, as the main problem with using basic maximum likelihood is high estimated transition rates. Such a model could also be of use on trees in which the state distribution is closer to reality but have an extremely labile character optimized on them.

Sabath et al. (2015), in a study focused on the evolution of dioecy, mentioned the possibility of hermaphroditism being underreported due to it being the most frequent state and hence not always mentioned in descriptions, while dioecy would always (or at least more often) be reported due to its relative rarity. While making an exhaustive list of all articles in which I have observed this phenomenon was not the purpose of my work, I have noticed a similar situation during the character coding phase of this thesis. There were frequent instances of species whose floral symmetry was not mentioned probably because it was actinomorphic, merism not mentioned for Monocotyledoneae (a large majority of which have trimerous flowers) and for Pentapetalae (named after the fact that most of its members are pentamerous), or pollination-oriented flower descriptions that mentioned petals but not sepals, which nevertheless had to be present given the family to which the species belonged. Such omissions, if they were part of the only individual description I could find for the species, ended up being recorded as missing data. This is why I recommend that future descriptions of species contain as many elements noticed by the observer as possible, even those that seem trivial and/or are shared by all related species, as a reader that has never personally observed the species can not otherwise be entirely sure that a given character state is expressed. Conversely, if most but not all species of a group (family, genus etc.) share a common attribute, it is best to specify the exception(s) in the group’s description or in individual species descriptions if the latter exist. New descriptions should be ideally accompanied by images and illustrations, as some elements that get nonetheless omitted by descriptions may be visible to those looking for them.

Developmental studies of flowers in general are also needed, including in families in which a few species have already been studied. Understanding developmental pathways may help understand why some character states with an apparent impact on diversification are transitioned into or out of more often than others.

## 2. Other existing approaches

There are several other approaches that aim to find out more about the causes of diversification in angiosperms. This thesis is a branch of the eFLOWER project, the aims of which include reconstructing the morphology of flowers in the deep nodes of the angiosperm tree and finding out which floral innovations are linked with major increases in diversification rates. Its first implementation (H. Sauquet et al., in prep.) uses the tree of Magallón et al. (2015) that was mentioned in Chapter 3 as well as in the present discussion. That tree is based on 792 angiosperms that were sampled from GenBank. Only 374 families (87% of the 424 recognized by APG IV) are represented, but they together account for 99% of angiosperm species. Due to the tree being initially built for the purpose of studying species relationships regardless of character state, the methods of Magallón et al. (2015) were quite close to a version of the species selection method used in this thesis, which gave priority to sequence availability rather than morphological character state availability. The species sampled by Magallón et al. (2015) were chosen in GenBank by giving priority to species that had all five markers used in their study. If there were no species with all five, the marker of a species from the same genus was used as a surrogate, if one was available; my analogue was to add the species with the missing character to the sample. This tree was greatly enhanced by the eFLOWER project via a focus on coding as many character states as possible for the species that were selected for the tree and to use these data to optimize 27 characters on the tree itself. The eFLOWER study on the Magallón tree has four characters that are equivalent to those used in Chapter 3 of this thesis. For perianth symmetry, phyllotaxy and differentiation, the maximum likelihood transition rates are generally lower in value in the eFLOWER study than those presented in Chapter 3, but the Mk2 symmetry ratios remain extremely close to each other. In the case of perianth fusion, however, the results in Chapter 3 show a higher Mk2 symmetry ratio than in the eFLOWER study. This indicates that the number of character state changes in the eFLOWER study is somewhat proportional to those found in Chapter 3, but the origin to reversal ratio for perianth fusion is lower in the eFLOWER study. In order to include both a member of each family and all the changes in perianth symmetry, I was able to find that, for Chapter 2 of this thesis, a set

of 761 species was required, which is less than what was used for the Magallón tree. Adding four other perianth characters brought the sample to 1232 species. The only elements of this thesis that can be directly compared to those of the eFLOWER study are the parsimony and maximum likelihood full-tree optimizations for perianth fusion, symmetry, phyllotaxy and differentiation. The two studies otherwise use different approaches to taking rate heterogeneity and most-probable state uncertainty into account. In the case of perianth phyllotaxy, the eFLOWER study took rate heterogeneity into account via the use of a tree from which core monocots and core eudicots had been removed. This differs from the way partitions were done in Chapter 3, but gives the same ancestral state results as the ANA+Magnoliidae and basal eudicots partitions. In addition, the eFLOWER study takes phylogenetic uncertainty into account, whereas we do not, and it tests many more models while Chapter 3 included many more state transitions.

O'Meara et al. (2016) used another approach to studying character evolution. They selected 500 species first by calculating the proportion of the angiosperm species represented by each family, then had members of that family represent the same proportion in their sample. After this, they selected the required number of species among the members of that family that had at least the ITS marker sequenced. These species were scored for six floral characters: presence of petals, fusion of perianth parts, flower symmetry, number of stamens compared to perianth parts, carpel fusion, and ovary position. The 500 species set was reduced to 464 that covered 134 angiosperm families (31% of the total) because they could not score the character state for some species. The lack of missing data in the species retained enabled them to use MuSSE (FitzJohn 2012), a counterpart to BiSSE intended for multistate characters or combinations of binary characters. This latter variant was used to test the influence of combinations of the states of several characters by treating each combination as a different state of a single character; for example, a combination of two binary characters can be divided in four states that represent 00, 01, 10 and 11, respectively. They found that all combinations that included the presence of a corolla, a zygomorphic flower, and a small number of stamens diversified faster than the rest, but had a low origination rate. One of the problems of this method is an extremely low sampling fraction, which excludes most families that have less than 500 species.

In Soltis et al. (2005), an entire chapter is dedicated to floral diversification, in which whorled phyllotaxy, stabilizing merism, floral zygomorphy, perianth fusion and various adaptations to pollinators are singled out as possible causes of diversification. The chapter

presents a thorough review of what is known of various floral characters and how they can influence species diversification. It is, however, greatly lacking in terms of phylogenetic trees on which character evolution is optimized compared to the rest of the book, in which the trees presented, while relevant to the evolution of specific characters in specific clades, vary too much in terms of taxon sampling to give a clear visual picture of character evolution across the angiosperms.

Several studies of angiosperm evolution have included many more characters than used in this thesis, but focused almost exclusively on basal eudicots and only explored the results using parsimony (Doyle and Endress 2000; Ronse De Craene et al. 2003; Zanis et al. 2003; Endress and Doyle 2009).

In summary, there is need for a continued effort of DNA sampling and assembling as exhaustive as possible species descriptions of flowers to know the characteristics of as many species as possible. However, it is impossible to do this for every single angiosperm species, so new macroevolutionary analytical models that can account for and accommodate gaps in data need to be developed in the meantime. There are other studies seeking more information about the causes of character diversification, each with their strengths and weaknesses compared to this thesis.

# CONCLUSION AND PERSPECTIVES

I first went through the process of testing whether two character states were key innovations in the Proteaceae family. I found floral zygomorphy not to have any impact on diversification and presence in Mediterranean hotspots to be the source to a macroevolutionary sink. I also came to the conclusion that perianth zygomorphy is not a key innovation in the family, contrary to what seems to be the case in other families, such as Orchidaceae and Fabaceae; these families are two of the most species-rich among the angiosperms.

I found that my “change-capture” sampling method generates maximum likelihood transition rates too high to give conclusive ancestral state reconstruction results when used with a single character. This makes the use of parsimony a necessity for ancestral state reconstruction with this sampling strategy. The parsimony results showed that perianth symmetry was an extremely labile character. I found that perianth zygomorphy could contribute both to higher species diversification and species specialization, which can lead to extinction if the niche into which species were specialized disappears.

Chapter 3 showed that there was high transition rate heterogeneity among the five partitions into which I divided the angiosperms. It showed that using the “change-capturing” method on several characters instead of one could lead to transition rates that are lower than with only a single character, but possibly still high enough to produce biased results. The chapter also showed that diversification rates could have an impact on inferred diversification state and that this influence was strong enough to change the most-probable state of a node between a model adapted to the entire angiosperm tree and a model adapted to a partition of the tree.

Taken together, these chapters and my contribution to the article published by Citerne et al. (2016) show that there are several ways to sample species and several ways to reconstruct ancestral states. Combination of these factors can lead to a large variety of possible ancestral state reconstruction results in nodes that can have different states depending on the sampling method and ancestral state reconstruction method. Choices made in sampling methods will depend on what aspect of morphological evolution a study aims to put forward. One of the reasons these choices have to be made is that the ideal situation for studying floral morphology evolution, in which all angiosperm species are described and sequenced, is unlikely to happen,

at least in the near future. However, we need to continue getting as close to it as we can, as some little-known species or clades can potentially hold important information for floral evolution. After all, the basalmost known order and family of angiosperms consists of a single species, *Amborella trichopoda*. Its existence is the main reason why the root state is ambiguous in parsimony reconstructions of perianth phyllotaxy of angiosperms; without this species, the basalmost angiosperm order would be interpreted to be the whorled Nymphaeales, sister to the spiral Austrobaileyales and all other angiosperms. Building models that can account for various forms of biased sampling is necessary as well, as the content of GenBank and scientific literature as a whole is likely to remain biased for a very long time compared to what actually exists in nature. This thesis will hopefully help with future angiosperm diversification studies by providing a record of the clades in which each state is present and describing the properties of trees made with change-capture sampling.

Some of the ancestral state reconstruction for this thesis may be the first step of a study using the method of Käfer and Mousset (2014) of sister clade comparisons, as the method does not require clade resolution and attaches great importance to which of two character states displayed by sister clades is ancestral or derived. Interestingly, this method sums up two partially conflicting ideas of the level of phylogenetic resolution that is necessary to study character evolution. The need of two distinct sister clades points towards the need to resolve the phylogenetic tree as much as we can, because making such analyses on shallow clades will require resolving them to some extent. The lack of need for internal clade resolution, on the other hand, shows that the ideal situation in which the relationships of every single species to each other is known may not be strictly necessary to clarify the evolutionary history of some characters. These two aspects cohabitate via the fact that many large clades are still unresolved and even well-supported deeper nodes are not considered completely certain, thus making any model that can handle unresolved clades or take phylogenetic uncertainty into account extremely useful. Meanwhile, a level of phylogenetic certainty, in the form of largely accepted relationships represented by these very same deeper nodes, keeps the character evolution optimizations from becoming completely irrelevant. However, what constitutes a sufficient level of phylogenetic resolution to reconstruct most of the evolutionary history of an infrequently evolving character may not be sufficient for a more labile one. In addition, the need to detect both key innovations and self-destructive character states may involve demonstrating the existence of extremely small clades defined by the latter. Because of this,

character evolution will certainly continue, for a long time, to be one of the drivers of further phylogenetic resolution.

However, there are other factors besides floral character evolution that potentially contribute to angiosperm diversification. Non-floral characters, environmental factors, genomics, evo-devo and paleobotany could hold some of these answers as well.

# LITERATURE CITED

- Agnarsson I, Avilés L, Coddington JA, Maddison WP. 2006.** Sociality in theridiid spiders: repeated origins of an evolutionary dead end. *Evolution* **60**: 2342–2351.
- Alfaro ME, Santini F, Brock C, Alamillo H, Dornburg A, Rabosky DL, Carnevale G, Harmon LJ. 2009.** Nine exceptional radiations plus high turnover explain species diversity in jawed vertebrates. *Proceedings of the National Academy of Sciences of the United States of America* **106**: 13410–13414.
- Almeida J, Rocheta M, Galego L. 1997.** Genetic control of flower shape in *Antirrhinum majus*. *Development* **124**: 1387–1392.
- APG. 2009.** An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG III. *Botanical Journal of the Linnean Society* **161**: 105–121.
- APG. 2016.** An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants : APG IV. *Botanical Journal of the Linnean Society*.
- Arber EAN, Parkin J. 1907.** The origin of angiosperms. *Botanical Journal of the Linnean Society* **38**: 29–80.
- Armbruster WS. 2014.** Floral specialization and angiosperm diversity: phenotypic divergence, fitness trade-offs and realized pollination accuracy. *AoB Plants* **6**: plu003.
- Baker WJ, Couvreur TLP. 2013.** Global biogeography and diversification of palms sheds light on the evolution of tropical lineages. II. Diversification history and origin of regional assemblages. *Journal of Biogeography* **40**: 286–298.
- Barraclough TG. 2006.** What can phylogenetics tell us about speciation in the Cape flora? *Diversity and Distributions* **12**: 21–26.
- Bartlett ME, Specht CD. 2011.** Changes in expression pattern of the *TEOSINTE BRANCHEDI*- like genes in the Zingiberales provide a mechanism for evolutionary shifts in symmetry across the order. *American Journal of Botany* **98**: 227–243.
- Bateman RM, Hilton J, Rudall PJ. 2006.** Morphological and molecular phylogenetic context of the angiosperms: contrasting the “top-down” and “bottom-up” approaches used to infer the likely characteristics of the first flowers. *Journal of experimental botany* **57**: 3471–3503.
- Beaulieu JM, O’Meara BC, Donoghue MJ. 2013.** Identifying hidden rate changes in the evolution of a binary morphological character: the evolution of plant habit in campanulid Angiosperms. *Systematic Biology* **62**: 725–737
- Beck JB, Windham MD, Pryer KM. 2011.** Do asexual polyploid lineages lead short evolutionary lives? A case study from the fern genus *Astrolepis*. *Evolution* **65**: 3217–3229.

- Bello MA, Rudall PJ, González F, Fernández-Alonso JL. 2004.** Floral morphology and development in *Aragoa* (Plantaginaceae) and related members of the order Lamiales. *International Journal of Plant Sciences* **165**: 723–738.
- Berv JS, Prum RO. 2014.** Molecular phylogenetics and evolution a comprehensive multilocus phylogeny of the Neotropical cotingas (Cotingidae, Aves) with a comparative evolutionary analysis of breeding system and plumage dimorphism and a revised phylogenetic classification. *Molecular Phylogenetics and Evolution* **81**: 120–136.
- Broholm SK, Tähtiharju S, Laitinen RAE, Albert VA, Teeri TH, Elomaa P. 2008.** A TCP domain transcription factor controls flower type specification along the radial axis of the *Gerbera* (Asteraceae) inflorescence. *Proceedings of the National Academy of Sciences of the United States of America* **105**: 9117–9122.
- Bromham L, Hua X, Cardillo M. 2016.** Detecting Macroevolutionary Self-Destruction from Phylogenies. *Systematic Biology* **65**: 109–127.
- Burin G, Kissling WD, Guimara PR. 2016.** Omnivory in birds is a macroevolutionary sink. *Nature Communications* **7**: 11250.
- Busch A, Horn S, Mühlhausen A, Mummenhoff K, Zachgo S. 2012.** Corolla monosymmetry: evolution of a morphological novelty in the Brassicaceae family. *Molecular Biology and Evolution* **29**: 1241–1254.
- Busch A, Zachgo S. 2007.** Control of corolla monosymmetry in the Brassicaceae *Iberis amara*. *Proceedings of the National Academy of Sciences of the United States of America* **104**: 16714–16719.
- Cantino PD, Doyle JA, Graham SW, Judd WS, Olmstead RG, Soltis DE, Soltis PS, Donoghue MJ. 2007.** Towards a Phylogenetic Nomenclature of Tracheophyta. *Taxon* **56**: 822.
- Cardinal S, Danforth BN. 2013.** Bees diversified in the age of eudicots. *Proceedings of the Royal Society B* **280**: 20122686.
- Cardillo M, Pratt R. 2013.** Evolution of a hotspot genus: geographic variation in speciation and extinction rates in *Banksia* (Proteaceae). *BMC Evolutionary Biology* **13**: 155.
- Castellanos MC, Wilson P, Thomson JD. 2003.** Pollen transfer by hummingbirds and bumblebees, and the divergence of pollination modes in *Penstemon*. *Evolution* **57**: 2742–2752.
- Castellanos MC, Wilson P, Thomson JD. 2004.** “Anti-bee” and “pro-bird” changes during the evolution of hummingbird pollination in *Penstemon* flowers. *Journal of Evolutionary Biology* **17**: 876–885.
- Citerne H, Jabbour F, Nadot S, Damerval C. 2010.** The evolution of floral symmetry. *Advances in Botanical Research* **54**: 85–136.
- Citerne HL, Reyes E, Le Guilloux M, Delannoy E, Simmonet F, Sauquet H, Nadot S, Weston PH, Damerval C. 2016.** Characterization of *CYCLOIDEA*-like genes in Proteaceae, a basal eudicot family with multiple shifts in floral symmetry. *Annals of Botany*.

- Collar DC, Near TJ, Wainwright PC. 2005.** Comparative analysis of morphological diversity: does disparity accumulate at the same rate in two lineages of centrarchid fishes? *Evolution* **59**: 1783–1794.
- Colless DH. 1982.** Phylogenetics: the theory and practice of phylogenetic systematics, by E. O. Wiley. *Systematic Zoology* **31**: 100–104.
- Couvreur TLP, Richardson JE, Sosef MSM, Erkens RHJ, Chatrou LW. 2008.** Evolution of syncarpy and other morphological characters in African Annonaceae: A posterior mapping approach. *Molecular Biology and Evolution* **47**: 302–318.
- Cowling RM, Rundel PW, Lamont BB, Arroyo MK, Arianoutsou M. 1996.** Plant diversity in mediterranean-climate regions. *Trends in Ecology and Evolution* **11**: 362–366.
- Cunningham CW, Omland KE, Oakley TH. 1998.** Reconstructing ancestral character states: A critical reappraisal. *Trends in Ecology and Evolution* **13**: 361–366.
- Dadpour MR, Naghiloo S, Neycharan SF. 2012.** The development of pistillate and perfect florets in *Xeranthemum squarrosum* (Asteraceae). *Plant Biology* **14**: 234–243.
- Damerval C, Citerne H, Le Guilloux M, Domenichini S, Dutheil J, Ronse De Craene LP, Nadot S. 2013.** Asymmetric morphogenetic cues along the transverse plane : shift from disymmetry to zygomorphy in the flower of Fumariodeae. *American Journal of Botany* **100**: 391–402.
- Damerval C, Le Guilloux M, Jager M, Charon C. 2007.** Diversity and evolution of *CYCLOIDEA*-like TCP genes in relation to flower development in Papaveraceae. *Plant physiology* **143**: 759–772.
- Davis MP, Midford PE, Maddison W. 2013.** Exploring power and parameter estimation of the BiSSE method for analyzing species diversification. *BMC evolutionary biology* **13**: 38.
- Dettmann ME, Jarzen DM. 1998.** The early history of the Proteaceae in Australia: the pollen record. *Australian Systematic Botany* **11**: 401–438.
- Donoghue MJ, Ree RH, Baum DA. 1998.** Phylogeny and the evolution of flower symmetry in the Asteridae. *Trends in Plant Science* **3**: 311–317.
- Donoghue MJ, Sanderson MJ. 2015.** Confluence, synnovation, and depauperons in plant diversification. *New Phytologist* **207**: 260–274.
- Douglas AW. 1997.** The developmental basis of morphological diversification and in flowers of Conospermeae (*Stirlingia* and Conosperminae: Proteaceae). *International Journal of Plant Sciences* **158**: S13–S48.
- Doyle JA, Endress PK. 2000.** Morphological phylogenetic analysis of basal angiosperms: comparison and combination with molecular data. *International Journal of Plant Sciences* **161**: S121–S153.
- Doyle JA, Endress PK. 2011.** Tracing the early evolutionary diversification of the angiosperm flower. In: Wanntorp L, Ronse De Craene LP, eds. *Flowers on the Tree of Life*. Cambridge, UK: Cambridge University Press, 88–119.
- Drummond AJ, Rambaut A. 2007.** BEAST: Bayesian evolutionary analysis by sampling

trees. *BMC Evolutionary Biology* **7**: 214.

**Dunn CW, Pugh P, Haddock SHD. 2005.** Molecular phylogenetics of the Siphonophora (Cnidaria), with implications for the evolution of functional specialization. *Systematic Biology* **54**: 916–935.

**Endress PK. 1990.** Patterns of floral construction in ontogeny and phylogeny. *Biological journal of the Linnean Society* **39**: 153–175.

**Endress PK. 1992.** Evolution and floral diversity: the phylogenetic surroundings of *Arabidopsis* and *Antirrhinum*. *International Journal of Plant Sciences* **153**: S106–S122.

**Endress PK. 1999.** Symmetry in flowers: diversity and evolution. *International Journal of Plant Sciences* **160**: S3–S23.

**Endress PK. 2006.** Angiosperm floral evolution: morphological developmental framework. *Advances in Botanical Research* **44**: 1–61.

**Endress PK. 2011.** Evolutionary diversification of the flowers in angiosperms. *American Journal of Botany* **98**: 370–396.

**Endress PK, Doyle JA. 2007.** Floral phyllotaxis in basal angiosperms : development and evolution. *Current Opinion In Plant Biology* **10**: 52–57.

**Endress PK, Doyle JA. 2009.** Reconstructing the ancestral angiosperm flower and its initial specializations. *American journal of botany* **96**: 22–66.

**Erbar C, Gülden C. 2011.** Ontogeny of the flowers in *Paulownia tomentosa*: a contribution to the recognition of the resurrected monogeneric family Paulowniaceae. *Flora* **206**: 205–218.

**Feng X, Zhao Z, Tian Z, Xu S, Luo Y, Cai Z, Wang Y, Yang J, Wang Z, Weng L, Chen J, Zheng L, Guo X, Luo J, Sato S, Tabata S, Ma W, Cao X, Hu X, Sun C, Luo D. 2006.** Control of petal shape and floral zygomorphy in *Lotus japonicus*. *Proceedings of the National Academy of Sciences of the United States of America* **103**: 4970–4975.

**Fenster CB, Armbruster WS, Dudash MR. 2009.** Specialization of flowers: is floral orientation an overlooked first step? *New Phytologist* **183**: 502–506.

**FitzJohn RG. 2012.** Diversitree : comparative phylogenetic analyses of diversification in R. *Methods in Ecology and Evolution* **3**: 1084–1092.

**FitzJohn RG, Maddison WP, Otto SP. 2009.** Estimating trait-dependent speciation and extinction rates from incompletely resolved phylogenies. *Systematic Biology* **58**: 595–611.

**Friedman WE. 2009.** The meaning of Darwin’s “abominable mystery.” *American Journal of Botany* **96**: 5–21.

**Galtier N, Gouy M. 1998.** Inferring pattern and process : maximum-likelihood implementation of a nonhomogeneous model of DNA sequence evolution for phylogenetic analysis. *Molecular Biology and Evolution* **15**: 871–879.

**Goldberg EE, Igic B. 2008.** On phylogenetic tests of irreversible evolution. *Evolution* **62**:

2727–2741.

**Goldberg EE, Kohn JR, Lande R, Robertson KA. 2010.** Species selection maintains self-incompatibility. *Science* **330**: 493–495.

**Goldberg EE, Lancaster LT, Ree RH. 2011.** Phylogenetic inference of reciprocal effects between geographic range evolution and diversification. *Systematic Biology* **60**: 451–465.

**Gómez JM, Abdelaziz M, Camacho JPM, Muñoz-Pajares AJ, Perfectti F. 2009.** Local adaptation and maladaptation to pollinators in a generalist geographic mosaic. *Ecology Letters* **12**: 672–682.

**Gómez JM, Perfectti F, Camacho JPM. 2006.** Natural selection on *Erysimum mediohispanicum* flower shape: insights into the evolution of zygomorphy. *The American Naturalist* **168**: 531–545.

**Gyllenberg M, Parvinen K. 2001.** Necessary and sufficient conditions for evolutionary suicide. *Bulletin of Mathematical Biology* **63**: 981–993.

**Hardy CR, Davis JI, Stevenson DW. 2004.** Floral organogenesis in *Plowmanianthus* (Commelinaceae). *International Journal of Plant Sciences* **165**: 511–519.

**Hardy CR, Stevenson DW, Kiss HG. 2000.** Development of the gametophytes, flower and floral vasculature in *Dichosandra thyrsofolia* (Commelinaceae). *American Journal of Botany* **87**: 1228–1239.

**Harmon LJ. 2012.** An inordinate fondness for eukaryotic diversity. *PLOS Biology* **10**: e1001382.

**Harris EM. 1995.** Inflorescence and floral ontogeny in Asteraceae: a synthesis of historical and current concepts. *The Botanical Review* **61**: 93–278.

**Haston E, Ronse De Craene LP. 2007.** Inflorescence and floral development in *Streptocarpus* and *Saintpaulia* (Gesneriaceae) with particular reference to the impact of bracteole suppression. *Plant Systematics and Evolution* **265**: 13–25.

**He T, Lamont BB, Downes KS. 2011.** Banksia born to burn. *The New Phytologist* **191**: 184–196.

**Heard SB. 1996.** Patterns in phylogenetic tree balance with variable and evolving speciation rates. *Evolution* **50**: 2141–2148.

**Helanterä H, Strassmann JE, Carrillo J, Queller DC. 2009.** Uniclonal ants: where do they come from, what are they and where are they going? *Cell* **24**: 341–349.

**Herrera J. 2009.** Visibility vs. biomass in flowers: Exploring corolla allocation in Mediterranean entomophilous plants. *Annals of Botany* **103**: 1119–1127.

**Hileman LC. 2014.** Trends in flower symmetry evolution revealed through phylogenetic and developmental genetic advances. *Philosophical transactions of the Royal Society of London* **369**: 20130348.

- Hileman LC, Cubas P. 2009.** An expanded evolutionary role for flower symmetry genes. *Journal of biology* **8**: 90.
- Hileman LC, Irish VF. 2009.** More is better: the uses of developmental genetic data to reconstruct perianth evolution. *American Journal of Botany* **96**: 83–95.
- Hopper SD, Gioia P. 2004.** The Southwest Australian Floristic Region: evolution and conservation of a global hotspot of biodiversity. *Annual Review of Ecology, Evolution, and Systematics* **35**: 623–650.
- Horn S, Pabón-Mora N, Theuß VS, Busch A, Zachgo S. 2014.** Analysis of the *CYC/TBI* class of TCP transcription factors in basal angiosperms and magnoliids. *The Plant Journal* **81**: 559–571.
- Howarth DG, Donoghue MJ. 2006.** Phylogenetic analysis of the “ECE” (*CYC/TBI*) clade reveals duplications predating the core eudicots. *Proceedings of the National Academy of Sciences of the United States of America* **103**: 9101–9106.
- Howarth DG, Martins T, Chimney E, Donoghue MJ. 2011.** Diversification of *CYCLOIDEA* expression in the evolution of bilateral flower symmetry in Caprifoliaceae and *Lonicera* (Dipsacales). *Annals of Botany* **107**: 1521–1532.
- Hsiang AY, Field DJ, Webster TH, Behlke ADB, Davis MB, Racicot RA, Gauthier JA. 2015.** The origin of snakes: revealing the ecology, behavior, and evolutionary history of early snakes using genomics, phenomics, and the fossil record. *BMC Evolutionary Biology* **15**: 87.
- Hunter JP. 1998.** Key innovations and the ecology of macroevolution. *Trends in Ecology and Evolution* **13**: 31–36.
- Igic B, Busch JW. 2013.** Is self-fertilization an evolutionary dead end? *New Phytologist* **198**: 386–397.
- Igic B, Lande R, Kohn JR. 2008.** Loss of self-incompatibility and its evolutionary consequences. *International Journal of Plant Sciences* **169**: 93–104.
- Jabbour F, Damerval C, Nadot S. 2008.** Evolutionary trends in the flowers of asteridae: Is polyandry an alternative to zygomorphy? *Annals of Botany* **102**: 153–165.
- Jabbour F, Ronse De Craene LP, Nadot S, Damerval C. 2009.** Establishment of zygomorphy on an ontogenic spiral and evolution of perianth in the tribe Delphinieae (Ranunculaceae). *Annals of Botany* **104**: 809–822.
- Käfer J, Mousset S. 2014.** Standard sister clade comparison fails when testing derived character states. *Systematic Biology* **63**: 601–609.
- Kim M, Cui M-L, Cubas P, Gillies A, Lee K, Chapman MA, Abbott RJ, Coen E. 2008.** Regulatory genes control a key morphological and ecological trait transferred between species. *Science* **322**: 1116–1119.
- King B, Lee MSY. 2015.** Ancestral state reconstruction, rate heterogeneity, and the evolution of reptile viviparity. *Systematic Biology*.

- Knapp S. 2010.** On “various contrivances”: pollination, phylogeny and flower form in the Solanaceae. *Philosophical Transactions of the Royal Society of London* **365**: 449–460.
- Kokko H, Heubel KU. 2011.** Prudent males, group adaptation, and the tragedy of the commons. *Oikos* **120**: 641–656.
- Lambers H, Bishop JG, Hopper SD, Laliberté E, Zúñiga-Feest A. 2012.** Phosphorus-mobilization ecosystem engineering: the roles of cluster roots and carboxylate exudation in young P-limited ecosystems. *Annals of Botany* **110**: 329–348.
- Lambers H, Shane MW, Cramer MD, Pearse SJ, Veneklaas EJ. 2006.** Root structure and functioning for efficient acquisition of phosphorus: matching morphological and physiological traits. *Annals of Botany* **98**: 693–713.
- Leslie AB, Beaulieu JM, Crane PR, Donoghue MJ. 2013.** Explaining the distribution of breeding and dispersal syndromes in conifers. *Proceedings of the Royal Society B* **280**.
- Leins P, Erbar C. 2010.** *Flower and fruit: morphology, ontogeny, phylogeny, function and ecology*. Stuttgart: Schweizerbart Science Publishers.
- Leite VG, Mansano VF, Teixeira SP. 2014.** Floral ontogeny in Dipterygeae (Fabaceae) reveals new insights into one of the earliest branching tribes in papilionoid legumes. *Botanical Journal of the Linnean Society* **174**: 529–550.
- Lewis PO. 2001.** A likelihood approach to estimating phylogeny from discrete morphological character dat. *Systematic Biology* **50**: 913–925.
- Linder HP. 2003.** The radiation of the Cape flora, southern Africa. *Biological Reviews of the Cambridge Philosophical Society* **78**: 597–638.
- Linder HP. 2008.** Plant species radiations: where, when, why? *Philosophical Transactions of the Royal Society of London* **363**: 3097–3105.
- Maddison WP, FitzJohn RG. 2015.** The unsolved challenge to phylogenetic correlation tests for categorical characters. *Systematic Biology* **64**: 127–136.
- Maddison WP, Maddison DR. 2015.** Mesquite: a modular system for evolutionary analysis, version 3.04. <http://mesquiteproject.org>
- Maddison WP, Midford PE, Otto SP. 2007.** Estimating a binary character’s effect on speciation and extinction. *Systematic Biology* **56**: 701–710.
- Magallón S, Sánchez Reyes LL, Gómez-Acevedo SL, Hernández-Hernández T. 2015.** Major diversification shifts through angiosperm evolutionary history. *Botany Abstract* **5**.
- Magallón S, Gómez-Acevedo S, Sánchez-Reyes LL, Hernández-Hernández T. 2015.** A metacalibrated time-tree documents the early rise of flowering plant phylogenetic diversity. *New Phytologist* **207**: 437–453.
- Magnuson-Ford K, Otto SP. 2012.** Linking the investigations of character evolution and species diversification. *The American Naturalist* **180**: 225–245.

- Makinson RO. 2000.** *Flora of Australia volume 17A—Proteaceae 2 Grevillea*. Clayton, Australia: CSIRO Publishing.
- Mast AR, Milton EF, Jones EH, Barker RM, Barker WR, Weston PH. 2012.** Time-calibrated phylogeny of the woody Australian genus *Hakea* (Proteaceae) supports multiple origins of insect-pollination among bird-pollinated ancestors. *American Journal of Botany* **99**: 472–487.
- Mast AR, Willis CL, Jones EH, Downs KM, Weston PH. 2008.** A smaller *Macadamia* from a more vagile tribe: inference of phylogenetic relationships, divergence times, and diaspore evolution in *Macadamia* and relatives (tribe Macadamieae; Proteaceae). *American Journal of Botany* **95**: 843–870.
- Mayers AM, Lord EM. 1984.** Comparative flower development in the cleistogamous species *Viola odorata*. *Botanical Gazette* **145**: 83–91.
- Mayr EM, Weber A. 2006.** Calceolariaceae: floral development and systematic implications. *American Journal of Botany* **93**: 327–343.
- McDaniel SF, Atwood J, Burleigh JG. 2012.** Recurrent evolution of dioecy in bryophytes. *Evolution* **67**: 567–572.
- Meng A, Zhang Z, Li J, Ronse De Craene LP, Wang H. 2012.** Floral development of *Stephania* (Menispermaceae): impact of organ reduction on symmetry. *International Journal of Plant Sciences* **173**: 861–874.
- Moen D, Morlon H. 2014.** Why does diversification slow down? *Trends in Ecology and Evolution* **29**: 190–197.
- Mooers AØ, Heard SB. 1997.** Inferring evolutionary process from phylogenetic tree shape. *Quarterly Review of Biology* **72**: 31–54.
- Moore MJ, Soltis PS, Bell CD, Burleigh JG, Soltis DE. 2010.** Phylogenetic analysis of 83 plastid genes further resolves the early diversification of eudicots. *Proceedings of the National Academy of Sciences of the United States of America* **107**: 4623–4628.
- Moreau CS, Bell CD. 2013.** Testing the museum versus cradle tropical biological diversity hypothesis: phylogeny, diversification, and ancestral biogeographic range evolution of the ants. *Evolution* **67**: 2240–2257.
- Morlon H. 2014.** Phylogenetic approaches for studying diversification. *Ecology letters* **17**: 508–525.
- Morlon H, Parsons TL, Plotkin JB. 2011.** Reconciling molecular phylogenies with the fossil record. *Proceedings of the National Academy of Sciences of the United States of America* **108**: 16327–16332.
- Movafeghi A, Naghiloo S, Dadpour MR. 2011.** Inflorescence and floral development in *Astragalus lagopoides* Lam. (Leguminosae: Papilionoideae: Galegeae). *Flora* **206**: 219–226.
- Naghiloo S, Esmailou Z, Gohari G, Dadpour MR. 2014.** Comparative inflorescence and floral ontogeny in the genus *Mentha* (Mentheae: Nepetoideae: Lamiaceae): variable

sequences of organ appearance and random petal aestivation. *Plant Systematics and Evolution* **300**: 329–345.

**Naghiloo S, Fathollahi MR, Classen-Bockhoff R. 2015.** Flower ontogeny in *Capparis spinosa* (Capparaceae) with special emphasis on symmetry expression. *Nordic Journal of Botany* **33**: 754–760.

**Naghiloo S, Gohari GR, Siahkolae SN, Dadpour MR. 2015.** Floral development in *Scutellaria pinnatifida* (Lamiaceae): the ontogenetic basis for sepal reduction. *Plant Biology* **17**: 238–244.

**Naghiloo S, Khodaverdi M, Esmailou Z, Dadpour MR, Rudall PJ. 2014.** Comparative floral development in the tribe Mentheae (Nepetoideae: Lamiaceae) and its bearing on the evolution of floral patterns in asterids. *Journal of Systematics and Evolution* **52**: 195–214.

**Nandi OI. 1998.** Floral development and systematics of Cistaceae. *Plant Systematics and Evolution* **212**: 107–134.

**Neal PR, Dafni A, Giurfa M. 1998.** Floral symmetry and its role in plant-pollinator systems: terminology, distribution, and hypotheses. *Annual Review of Ecology and Systematics* **29**: 345–373.

**Nee S, May RM, Harvey PH. 1994.** The reconstructed evolutionary process. *Philosophical Transactions of the Royal Society of London* **344**: 305–311.

**Nickrent DL, Malécot V, Vidal-Russell R, Der JP. 2010.** A revised classification of Santalales. *Taxon* **59**: 538–558.

**Olson ME. 2003.** Ontogenetic origins of floral bilateral symmetry in Moringaceae (Brassicales). *American Journal of Botany* **90**: 49–71.

**O'Meara BC. 2012.** Evolutionary inferences from phylogenies: A review of methods. *Annual Review of Ecology, Evolution, and Systematics* **43**: 267–285.

**O'Meara BC, Ané C, Sanderson MJ, Wainwright PC. 2006.** Testing for different rates of continuous trait evolution using likelihood. *Evolution* **60**: 922–933.

**O'Meara BC, Smith SD, Armbruster WS, Harder D, Hardy CR, Hileman LC, Hufford L, Smith SA, Stevens PF, Litt A, Magallón S, Fenster CB, Diggle PK, Smith SD. 2016.** Non-equilibrium dynamics and floral trait interactions shape extant angiosperm diversity. *Proceedings of the Royal Society B* **283**: 20152304.

**Oraei M, Gohari G, Esmailou Z, Naghiloo S. 2013.** Comparative ontogeny of perfect and pistillate florets in *Senecio vernalis* (Asteraceae). *Flora* **208**: 285–292

**Pabón-Mora N, González F. 2008.** Floral ontogeny of *Telipogon* spp. (Orchidaceae) and insights on the perianth symmetry in the family. *International Journal of Plant Sciences* **169**: 1159–1173.

**Pagel M. 1994.** Detecting correlated evolution on phylogenies: a general method for the comparative analysis of discrete characters. *Proceedings Of The Royal Society* **255**: 37–45.

- Pagel M. 1999.** The maximum likelihood approach to reconstructing ancestral character states of discrete characters on phylogenies. *Systematic Biology* **48**: 612–622.
- Patchell MJ, Bolton MC, Mankowski P, Hall JC. 2011.** Comparative floral development in Cleomaceae reveals two distinct pathways leading to monosymmetry. *International Journal of Plant Sciences* **172**: 352–365.
- Pennell MW, Harmon LJ. 2013.** An integrative view of phylogenetic comparative methods: connections to population genetics, community ecology, and paleobiology. *Annals of the New York Academy of Sciences* **1289**: 90–105.
- Pigot AL, Phillimore AB, Owens IPF, Orme CDL. 2010.** The shape and temporal dynamics of phylogenetic trees arising from geographic speciation. *Systematic Biology* **59**: 660–673.
- Van der Pijl L. 1961.** Ecological aspects of flower evolution. II. Zoophilous flower classes. *Evolution* **15**: 44–59.
- Poslusny U, Sattler R. 1974.** Floral development of *Potamogeton richardsonii*. *American Journal of Botany* **61**: 209–216.
- Preston JC, Hileman LC. 2009.** Developmental genetics of floral symmetry evolution. *Trends in Plant Science* **14**: 147–154.
- Preston JC, Hileman LC. 2012.** Parallel evolution of TCP and B-class genes in Commelinaceae flower bilateral symmetry. *EvoDevo* **3**: 6.
- Preston JC, Kost MA, Hileman LC. 2009.** Conservation and diversification of the symmetry developmental program among close relatives of snapdragon with divergent floral morphologies. *New Phytologist* **182**: 751–762.
- Pruitt JN. 2013.** A real-time eco-evolutionary dead-end strategy is mediated by the traits of lineage progenitors and interactions with colony invaders. *Ecology letters* **16**: 879–886.
- Pulliam HR. 1988.** Sources, sinks and population regulation. *The American Naturalist* **132**: 652–661.
- Pyron RA, Burbrink FT. 2013.** Phylogenetic estimates of speciation and extinction rates for testing ecological and evolutionary hypotheses. *Trends in Ecology and Evolution* **28**: 729–736.
- Pyron RA, Wiens JJ. 2013.** Large-scale phylogenetic analyses reveal the causes of high tropical amphibian diversity. *Proceedings of the Royal Society B* **280**: 20131622.
- Quental TB, Marshall CR. 2010.** Diversity dynamics: molecular phylogenies need the fossil record. *Trends in Ecology and Evolution* **25**: 434–441.
- Rabosky DL. 2010.** Extinction rates should not be estimated from molecular phylogenies. *Evolution* **64**: 1816–1824.
- Rabosky DL. 2014.** Automatic detection of key innovations, rate shifts, and diversity-dependence on phylogenetic trees. *PLOS ONE* **9**: e89543.

- Rabosky DL, Donnellan SC, Talaba AL, Lovette IJ. 2007.** Exceptional among-lineage variation in diversification rates during the radiation of Australia's most diverse vertebrate clade. *Proceedings of the Royal Society B* **274**: 2915–2923.
- Rabosky DL, Goldberg EE. 2015.** Model inadequacy and mistaken inferences of trait-dependent speciation. *Systematic Biology* **64**: 340–355.
- Rabosky DL, Slater GJ, Alfaro ME. 2012.** Clade age and species richness are decoupled across the eukaryotic tree of life. *PLOS Biology* **10**: e1001381.
- Rankin DJ, López-Sepulcre A, Foster KR, Kokko H. 2007.** Species-level selection reduces selfishness through competitive exclusion. *Journal of Evolutionary Biology* **20**: 1459–1468.
- Reardon W, Gallagher P, Nolan KM, Wright H, Cardeñosa-Rubio MC, Bragalini C, Lee CS, Fitzpatrick DA, Corcoran K, Wolff K, Nugent JM. 2014.** Different outcomes for the MYB floral symmetry genes *DIVARICATA* and *RADIALIS* during the evolution of derived actinomorphy in *Plantago*. *New Phytologist* **202**: 716–725.
- Rebello TG. 2001.** *Proteas : A Field Guide to the Proteas of Southern Africa* (Vlaeberg, Ed.). Fernwood Press.
- Ren J-B, Guo Y-P. 2015.** Behind the diversity : Ontogenies of radiate, disciform, and discoid capitula of *Chrysanthemum* and its allies. **53**: 520–528.
- Reyes E, Morlon H, Sauquet H. 2015.** Presence in Mediterranean hotspots affects speciation and extinction rates in Proteaceae. *New Phytologist* **207**: 401–410.
- Reyes E, Sauquet H, Nadot S. 2016.** Perianth symmetry changed at least 199 times in angiosperm evolution. *Taxon* **65**: 945-964.
- Ricklefs RE, Renner SS. 1994.** Species richness within families of flowering plants. *Evolution* **48**: 1619–1636.
- Roels P, Smets E. 1996.** A floral ontogenetic study in the Dipsacales. *International Journal of Plant Sciences* **157**: 203–218.
- Rolland J, Condamine FL, Jiguet F, Morlon H. 2014.** Faster speciation and reduced extinction in the tropics contribute to the Mammalian latitudinal diversity gradient. *PLOS Biology* **12**: e1001775.
- Ronse De Craene LP. 2010.** *Floral diagrams: an aid to understanding flower morphology and evolution*. Cambridge: Cambridge University Press.
- Ronse De Craene LP. 2016.** Meristic changes in flowering plants : how flowers play with numbers. *Flora* **221**: 22–37.
- Ronse De Craene LP, Linder HP, Dlamini T, Smets EF. 2001.** Evolution and development of floral diversity of Melianthaceae, an enigmatic Southern African family. *International Journal of Plant Sciences* **162**: 59–82.
- Ronse De Craene LP, Roels P, Smets EF, Backlund A. 1998.** The floral development and floral anatomy of *Chrysosplenium alternifolium*, an unusual member of the Saxifragaceae.

*Journal of Plant Research* **111**: 573–580.

**Ronse De Craene LP, Smets EF. 1994.** Merosity in flowers: definition, origin, and taxonomic significance. *Plant Systematics and Evolution* **191**: 83–104.

**Ronse De Craene LP, Smets EF. 2001.** Floral developmental evidence for the systematic relationships of *Tropaeolum* (Tropaeolaceae). **88**: 879–892.

**Ronse De Craene LP, Smets E, Clinckemaiillie D. 2000.** Floral ontogeny and anatomy in *Koelreuteria* with special emphasis on monosymmetry and septal cavities. *Plant Systematics and Evolution* **223**: 91–107.

**Ronse De Craene LP, Soltis PS, Soltis DE. 2003.** Evolution of floral structures in basal angiosperms. **164**: S329–S363.

**Rudall PJ, Bateman RM. 2004.** Evolution of zygomorphy in monocot flowers : iterative patterns and developmental constraints. *New Phytologist* **162**: 25–44.

**Sabath N, Goldberg EE, Glick L, Einhorn M, Ashman T, Ming R, Otto SP, Vamosi JC, Mayrose I. 2015.** Dioecy does not consistently accelerate or slow lineage diversification across multiple genera of angiosperms. *New Phytologist* **209**: 1290–1300.

**Sampaio DS, de Chiara Moço MC, Mariath JEA. 2013.** Floral ontogeny of *Aeschynomene falcata* and *A. sensitiva* (Leguminosae: Papilionoideae) supports molecular phylogenetic data. *Plant Systematics and Evolution* **299**: 499–513.

**Sargent RD. 2004.** Floral symmetry affects speciation rates in angiosperms. *Proceedings of the Royal Society B* **271**: 603–608.

**Sauquet H. 2016.** PROTEUS: A database for recording morphological data and creating NEXUS matrices.

**Sauquet H, von Balthazar M, Magallón S, Doyle JA, Endress PK, Bailes E, Barroso de Morais E, Bull-Hereñu K, Carrive L, Chartier M, Chomicki G, Coiro M, El Ottra JHL, Epicoco C, Jabbour F, Haevermans T, Hernández R, Little SA, Löfstrand S, Luna JA, Massoni J, Nadot S, Prieu C, Reyes E, dos Santos P, Schoonderwoerd K, Soulebeau A, Städler Y, Tschan G, Wing-Sze Leung ASJ. 2015.** Fifteen clues to the early diversification of flowers: first results from the eFLOWER initiative. *Botany Abstract* **4**.

**Sauquet H, Carrive L, Poullain N, Sannier J, Damerval C, Nadot S. 2015.** Evolution of floral symmetry in Fumarioideae (Papaveraceae, Ranunculales): new evidence from an expanded molecular phylogenetic framework. *Annals of Botany*: In revision

**Sauquet H, Weston PH, Anderson CL, Barker NP, Cantrill DJ, Mast AR, Savolainen V. 2009.** Contrasted patterns of hyperdiversification in Mediterranean hotspots. *Proceedings of the National Academy of Sciences of the United States of America* **106**: 221–225.

**Schluter D, Price T, Mooers AØ, Ludwig D. 1997.** Likelihood of ancestor states in adaptive radiation. *Evolution* **51**: 1699–1711.

- Schnitzler J, Barraclough TG, Boatwright JS, Goldblatt P, Manning JC, Powell MP, Rebelo TG, Savolainen V. 2011.** Causes of plant diversification in the Cape biodiversity hotspot of South Africa. *Systematic Biology* **60**: 343–357.
- Schwander T, Crespi BJ. 2009.** Twigs on the tree of life? Neutral and selective models for integrating macroevolutionary patterns with microevolutionary processes in the analysis of asexuality. *Molecular Ecology* **18**: 28–42.
- Silvestro D, Schnitzler J, Zizka G. 2011.** A Bayesian framework to estimate diversification rates and their variation through time and space. *BMC Evolutionary Biology* **11**: 311–326.
- Soltis DE, Mort ME, Latvis M, Mavrodiev E V., O’meara BC, Soltis PS, Burleigh JG, de Casas RR. 2013.** Phylogenetic relationships and character evolution analysis of Saxifragales using a supermatrix approach. *American Journal of Botany* **100**: 916–929.
- Soltis DE, Sinters AE, Zanis MJ, Kim S, Thompson JD, Soltis PS, Ronse De Craene LP, Endress PK, Farris JS. 2003.** Gunnerales are sister to other core eudicots: Implications for the evolution of pentamery. *American Journal of Botany* **90**: 461–470.
- Soltis DE, Smith SA, Cellinese N, Wurdack KJ, Tank DC, Brockington SF, Refulio-Rodriguez NF, Walker JB, Moore MJ, Carlsward BS, Bell CD, Latvis M, Crawley S, Black C, Diouf D, Xi Z, Rushworth CA, Gitzendanner MA, Sytsma KJ, Qiu YL, Hilu KW, Davis CC, Sanderson MJ, Beaman RS, Olmstead RG, Judd WS, Donoghue MJ, Soltis PS. 2011.** Angiosperm phylogeny: 17 genes, 640 taxa. *American Journal of Botany* **98**: 704–730.
- Soltis DE, Soltis PS, Endress PK, Chase MW. 2005.** *Phylogeny and evolution of angiosperms*. Sunderland, Mass.: Sinauer Associates.
- Soltis PS, Soltis DE, Zanis MJ. 2000.** Basal lineages of angiosperms: relationships and implications for floral evolution. *International Journal of Plant Sciences* **161**: S97–S107.
- Stadler T. 2013.** Recovering speciation and extinction dynamics based on phylogenies. *Journal of Evolutionary Biology* **26**: 1203–1219.
- Staedler YM, Endress PK. 2009.** Diversity and lability of floral phyllotaxis in the pluricarpellate families of core Laurales (Gomortegaceae, Atherospermataceae, Siparunaceae, Monimiaceae). *International Journal of Plant Sciences* **170**: 522–550.
- Stevens P. 2001.** [last updated 14 Oct 2016]. Angiosperm Phylogeny Website. <http://www.mobot.org/MOBOT/research/APweb/> (accessed Apr 2016).
- Struck M. 1997.** *Pelargonium* (Geraniaceae) in southern Africa: ecological and evolutionary considerations. *Plant Systematics and Evolution* **208**: 71–97.
- Tank DC, Eastman JM, Pennell MW, Soltis PS, Soltis DE, Hinchliff CE, Brown JW, Sessa EB, Harmon LJ. 2015.** Nested radiations and the pulse of angiosperm diversification : increased diversification rates often follow whole genome duplications. *New Phytologist* **207**: 454–467.
- Thomas GH, Freckleton RP, Székely T. 2006.** Comparative analyses of the influence of developmental mode on phenotypic diversification rates in shorebirds. *Proceedings of the*

*Royal Society B* **273**: 1619–24.

**Tripp EA, Manos PS. 2008.** Is floral specialization an evolutionary dead-end? Pollination system transitions in *Ruellia* (Acanthaceae). *Evolution* **62**: 1712–1737.

**Tsou C-H, Mori SA. 2007.** Floral organogenesis and floral evolution of the Lecythidoideae (Lecythidaceae). *American Journal of Botany* **94**: 716–736.

**Tucker SC. 1984.** Unidirectional organ initiation in leguminous flowers. *American Journal of Botany* **71**: 1139–1148.

**Tucker SC. 1989.** Overlapping Organ Initiation and Common Primordia in Flowers of *Pisum sativum* (Leguminosae: Papilionoideae). *American Journal of Botany* **76**: 714–729.

**Tucker SC. 1994.** Floral ontogeny in Sophoreae (Leguminosae: Papilionoideae): II. *Sophora* sensu lato (*Sophora* group). *American Journal of Botany* **81**: 368–380.

**Tucker SC. 1997.** Floral Evolution, development, and convergence: the hierarchical-significance hypothesis. *International Journal of Plant Sciences* **158**: S143–S161.

**Tucker SC. 1999.** Evolutionary lability of symmetry in early floral development. *International Journal of Plant Sciences* **160**: S25–S39.

**Tucker SC, Stein OL, Derstine KS. 1985.** Floral development in *Caesalpinia* (Leguminosae). **72**: 1424–1434.

**Tucker SC, Stirton CH. 1991.** Development of the cymose inflorescence, cupulum and flower of *Psoralea pinnata* (Leguminosae: Papilionoideae: Psoraleeae). *Botanical Journal of the Linnean Society* **106**: 209–227.

**Valente LM, Reeves G, Schnitzler J, Mason IP, Fay MF, Rebelo TG, Chase MW, Barraclough TG. 2010.** Diversification of the African genus *Protea* (Proteaceae) in the Cape biodiversity hotspot and beyond: equal rates in different biomes. *Evolution* **64**: 745–760.

**Vanderpoorten A, Goffinet B. 2006.** Mapping uncertainty and phylogenetic uncertainty in ancestral character state reconstruction: an example in the moss genus *Brachytheciastrum*. *Systematic biology* **55**: 957–971.

**Vamosi JC, Vamosi SM. 2010.** Key innovations within a geographical context in flowering plants: towards resolving Darwin's abominable mystery. *Ecology letters* **13**: 1270–1279.

**Vamosi JC, Vamosi SM. 2011.** Factors influencing diversification in angiosperms: at the crossroads of intrinsic and extrinsic traits. *American Journal of Botany* **98**: 460–471.

**Verdú M, Pausas JG, Segarra-Moragues JG, Ojeda F. 2007.** Burning phylogenies: fire, molecular evolutionary rates, and diversification. *Evolution* **61**: 2195–2204.

**De Vos JM, Hughes CE, Schneeweiss GM, Moore BR, Conti E. 2014.** Heterostyly accelerates diversification via reduced extinction in primroses. *Proceedings of the Royal Society B* **281**: 20140075.

**Watson L, Dallwitz MJ. 1992.** [last updated 19 Oct 2016]. The families of flowering plants:

Descriptions, illustrations, identification, and information retrieval. <http://delta-intkey.com/angio/> (accessed Mar 2015).

**Webb CO, Ackerly DD, Kembel SW. 2008.** Phylocom: software for the analysis of phylogenetic community structure and trait evolution. *Bioinformatics* **24**: 2098–2100.

**Webster NB, Van Dooren TJM, Schilthuizen M. 2012.** Phylogenetic reconstruction and shell evolution of the Diplommatinidae (Gastropoda: Caenogastropoda). *Molecular Phylogenetics and Evolution* **63**: 625–638.

**Weston PH. 2006.** Proteaceae. In: Klaus K, ed. *The Families and Genera of Vascular Plants*. Leipzig, Germany.: Springer, 364–404.

**Whittall JB, Hodges SA. 2007.** Pollinator shifts drive increasingly long nectar spurs in columbine flowers. *Nature* **447**: 706–710.

**Wickett NJ, Mirarab S, Nguyen N, Warnow T, Carpenter E, Matasci N, Ayyampalayam S, Barker MS, Burleigh JG, Gitzendanner MA, Ruhfel BR, Wafula E, Der JP, Graham SW, Mathews S, Melkonian M, Soltis DE, Soltis PS, Miles NW, Rothfels CJ, Pokorny L, Shaw AJ, Degironimo L, Stevenson DW, Surek B, Villarreal JC, Chen T, Deyholos MK, Baucom RS, Kutchan TM, Augustin MM, Wang J, Zhang Y, Tian Z, Yan Z, Wu X, Sun X, Wong GK, Leebens-Mack J. 2014.** Phylotranscriptomic analysis of the origin and early diversification of land plants. *Proceedings of the National Academy of Sciences of the United States of America*: E4859–E4868.

**Wiens JJ. 2011.** Re-evolution of lost mandibular teeth in frogs after more than 200 million years, and re-evaluating Dollo's law. *Evolution* **65**: 1283–1296.

**Wilson P, Wolfe AD, Armbruster WS, Thomson JD. 2007.** Constrained lability in floral evolution: counting convergent origins of hummingbird pollination in *Penstemon* and *Keckiella*. *New Phytologist* **176**: 883–890.

**Wright SI, Kalisz S, Slotte T. 2013.** Evolutionary consequences of self-fertilization in plants. *Proceedings of the Royal Society B* **280**: 20130133.

**Wu ZY, Milne RI, Chen CJ, Liu J, Wang H, Li DZ. 2015.** Ancestral state reconstruction reveals rampant homoplasy of diagnostic morphological characters in urticaceae, conflicting with current classification schemes. *PLOS ONE* **10**: 1–20.

**Yang X, Kalluri UC, Jawdy S, Gunter LE, Yin T, Tschaplinski TJ, Weston DJ, Ranjan P, Tuskan GA. 2008.** The F-box gene family is expanded in herbaceous annual plants relative to woody perennial plants. *Plant Physiology* **148**: 1189–1200.

**Yang Z, Roberts D. 1995.** On the use of nucleic acid sequences to infer early branchings in the tree of life. *Molecular Biology and Evolution* **12**: 451–458.

**Yu W, Xu J, Wu Y, Yang G. 2012.** A comparative study of mammalian diversification pattern. *International Journal of Biological Sciences* **8**: 486–497.

**Yu SX, Zhou X-R, Xu G-F, Meng L, Bi H-Y. 2010.** Floral development in *Impatiens chishuiensis* (Balsaminaceae): formation of two well-developed anterolateral sepals and four carpels. *Plant Systematics and Evolution* **286**: 21–26.

- Xi Z, Ruhfel BR, Schaefer H, Amorim AM, Sugumaran M, Wurdack KJ, Endress PK, Matthews ML, Stevens PF, Mathews S, Davis CC. 2012.** Phylogenomics and a posteriori data partitioning resolve the Cretaceous angiosperm radiation Malpighiales. *Proceedings of the National Academy of Sciences of the United States of America* **109**: 17519–17524.
- Zanis MJ, Soltis PS, Qiu YL, Zimmer E, Soltis DE. 2003.** Phylogenetic analyses and perianth evolution in basal angiosperms. *Annals of the Missouri Botanical Garden* **90**: 129–150.
- Zanne AE, Tank DC, Cornwell WK, Eastman JM, Smith SA, FitzJohn RG, McGlenn DJ, O’Meara BC, Moles AT, Reich PB, Royer DL, Soltis DE, Stevens PF, Westoby M, Wright IJ, Aarssen L, Bertin RI, Calaminus A, Govaerts R, Hemmings F, Leishman MR, Oleksyn J, Soltis PS, Swenson NG, Warman L, Beaulieu JM. 2014.** Three keys to the radiation of angiosperms into freezing environments. *Nature* **506**: 89–92.
- Zeng L, Zhang Q, Sun R, Kong H, Zhang N, Ma H. 2014.** Resolution of deep angiosperm phylogeny using conserved nuclear genes and estimates of early divergence times. *Nature Communications* **5**: 4956.
- Zhang W, Kramer EM, Davis CC. 2010.** Floral symmetry genes and the origin and maintenance of zygomorphy in a plant-pollinator mutualism. *Proceedings of the National Academy of Sciences of the United States of America* **107**: 6388–6393.
- Zhang W, Steinmann VW, Nikolov L, Kramer EM, Davis CC. 2013.** Divergent genetic mechanisms underlie reversals to radial floral symmetry from diverse zygomorphic flowered ancestors. *Frontiers in Plant Science* **4**: 302.
- Zhou XR, Wang YZ, Smith JF, Chen R. 2008.** Altered expression patterns of TCP and MYB genes relating to the floral developmental transition from initial zygomorphy to actinomorphy in *Bournea* (Gesneriaceae). *New Phytologist* **178**: 532–543.

**SUPPORTING INFORMATION FOR CHAPTER 1: *PRESENCE IN  
MEDITERRANEAN HOTSPOTS AND FLORAL SYMMETRY AFFECT SPECIATION  
AND EXTINCTION RATES IN PROTEACEAE***

**Figure II.S1.** Additional parameter estimates obtained for the best BiSSE models selected for each trait of interest.

**Figure II.S2.** Parameter estimates obtained for the best BiSSE models selected for each trait of interest when nodes are spread evenly within *Grevillea*.

**Figure II.S3.** Maximum likelihood reconstruction of ancestral states for presence in Mediterranean hotspots.

**Figure II.S4.** Sensitivity of BiSSE analyses of floral symmetry to various hypothetical proportions of zygomorphic species in *Banksia* (net diversification rate estimates).

**Figure II.S5.** Sensitivity of BiSSE analyses of floral symmetry to various hypothetical proportions of zygomorphic species in *Banksia* (speciation rate estimates).

**Figure II.S6.** Sensitivity of BiSSE analyses of floral symmetry to various hypothetical proportions of zygomorphic species in *Banksia* (extinction rate estimates).

**Figure II.S7.** Maximum likelihood reconstruction of ancestral states for floral symmetry.

**Table II.S1.** Total number of species in each genus of Proteaceae, and number of species in each character state.

**Table II.S2.** AIC values and delta-AIC (in parentheses) obtained for each diversification model tested in the character-dependent analyses (BiSSE).

**Table II.S3.** Parameter estimates obtained from the BiSSE analysis testing the impact of occurring in Mediterranean hotspots on diversification rates in Proteaceae when nodes are spread evenly within *Grevillea*.

**Table II.S4.** Parameter estimates obtained in the BiSSE analysis of the impact of floral symmetry on diversification rates in Proteaceae when nodes are spread evenly within *Grevillea*.

**Table II.S5.** Results from the split BiSSE analyses on presence in Mediterranean hotspots.

**Table II.S6.** Results from the split BiSSE analyses on floral symmetry.

**Methods II.S1.** Ancestral state reconstruction.

**SUPPORTING INFORMATION FOR CHAPTER 2: *PERIANTH SYMMETRY*  
*CHANGED AT LEAST 199 TIMES IN ANGIOSPERM EVOLUTION***

**Table III.S1.** List of sources used to resolve relationships within families in the supertree used for this study.

**Table III.S2.** Summary of family-level states for floral symmetry and transitions reconstructed from this study (Fig. III.2).

**Appendix III.1.** Extraction of the PROTEUS database with complete data records and references used to assemble the perianth symmetry dataset. [Electronic]

**Appendix III.2.** NEXUS file for the tree from Figure III.2. [Electronic]

**SUPPORTING INFORMATION FOR CHAPTER 3: *DOES HETEROGENEITY OF  
MORPHOLOGICAL EVOLUTIONARY RATES AFFECT ANCESTRAL STATE  
RECONSTRUCTION? AN EMPIRICAL TEST WITH FIVE FLORAL CHARACTERS***

**Appendix IV.1.** Ultrametric tree used in the ML analyses, showing both constrained (red) and extrapolated (black) nodes ages (in Ma).[Electronic]

**Appendix IV.2.** Parsimony, Mk1 and Mk2 for 15 key nodes. full = full tree; part = partitioned tree; high rate= high rate equilibrium

**Appendix IV.3.** Parsimony, Mk1 and Mk2 for the 54 order nodes. full = full tree; part = partitioned tree; high rate= high rate equilibrium.

**Appendix IV.4.** Parsimony and Mk1 with imposed rates for 15 key nodes.

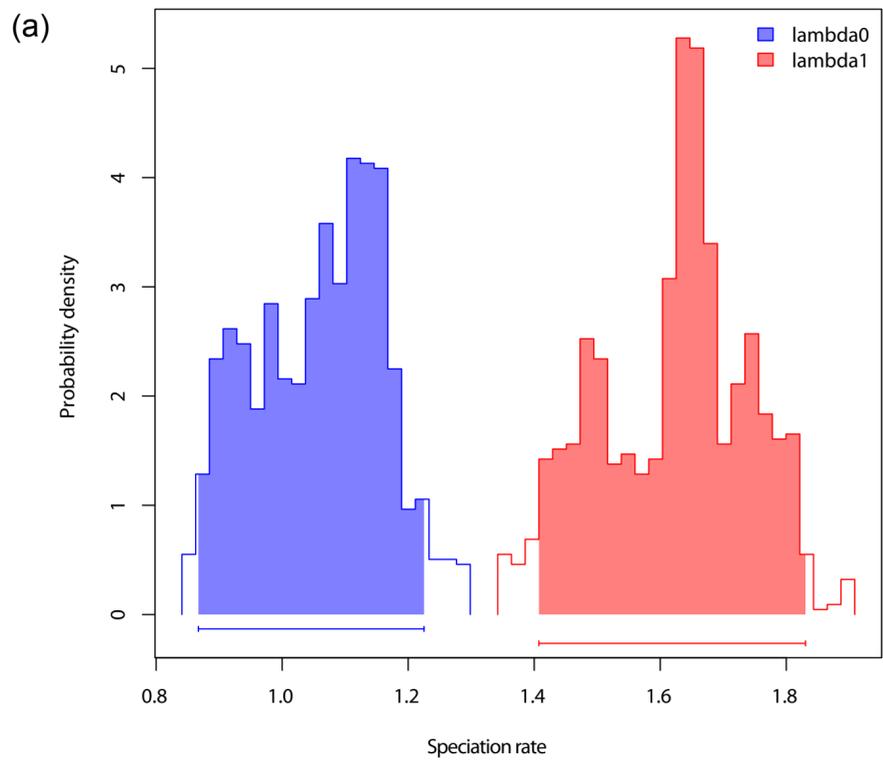
**Appendix IV.5.** Parsimony and Mk1 with imposed rates for the 54 order nodes.

**Appendix IV.6.** Parismony steps, Mk1 and Mk2 transition rates, Mk2 rate symmetry ratios, logLilkelihoods and AICs for the full tree an partitions.

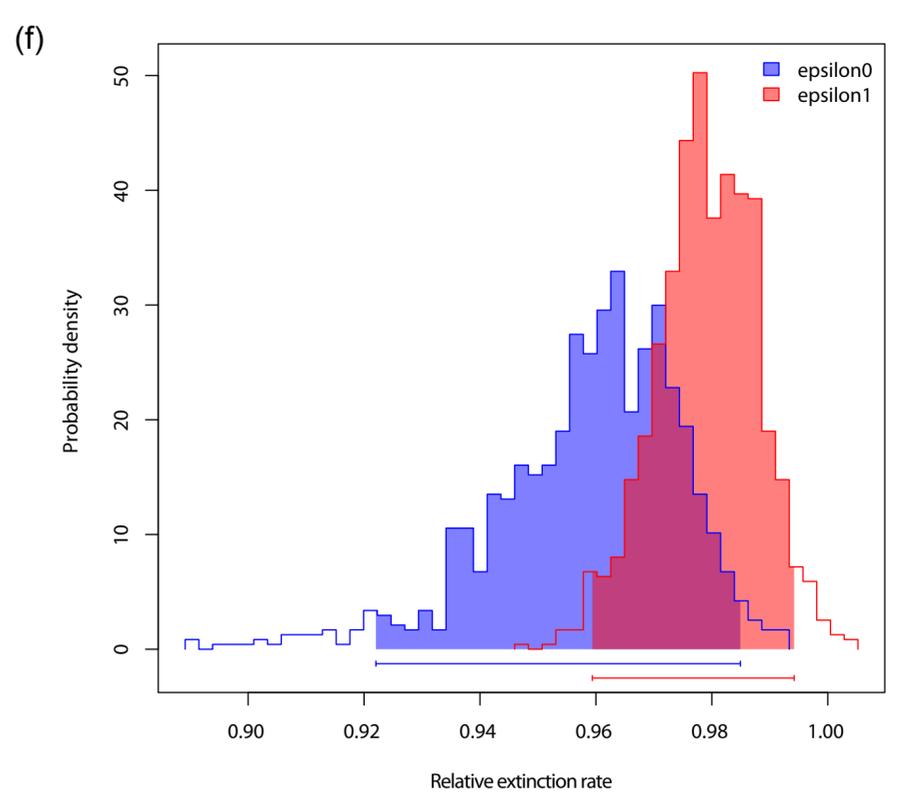
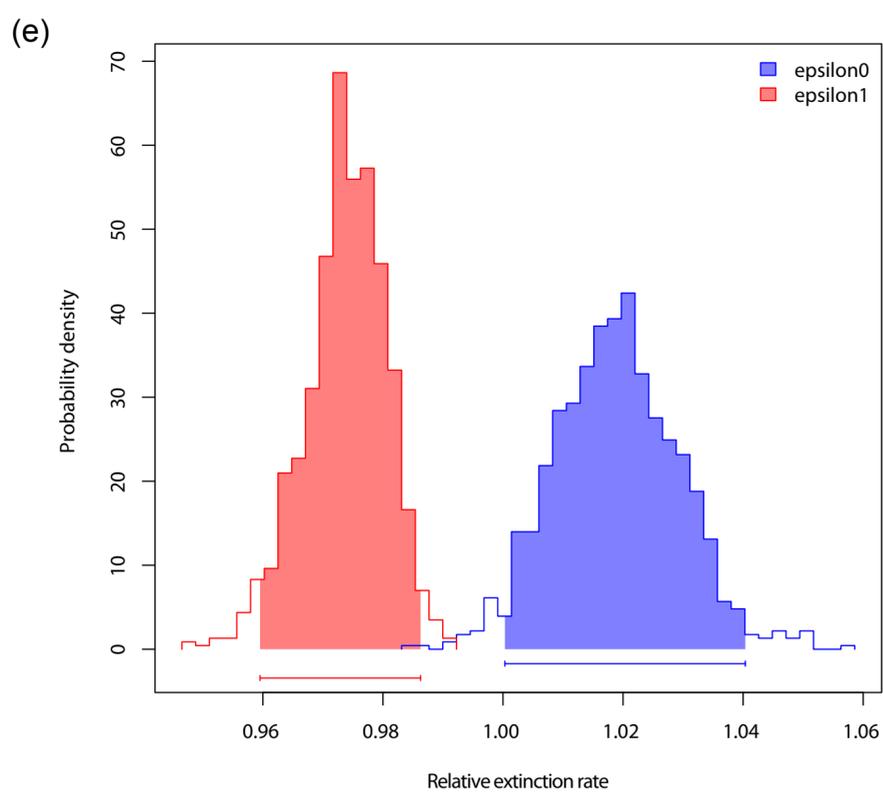
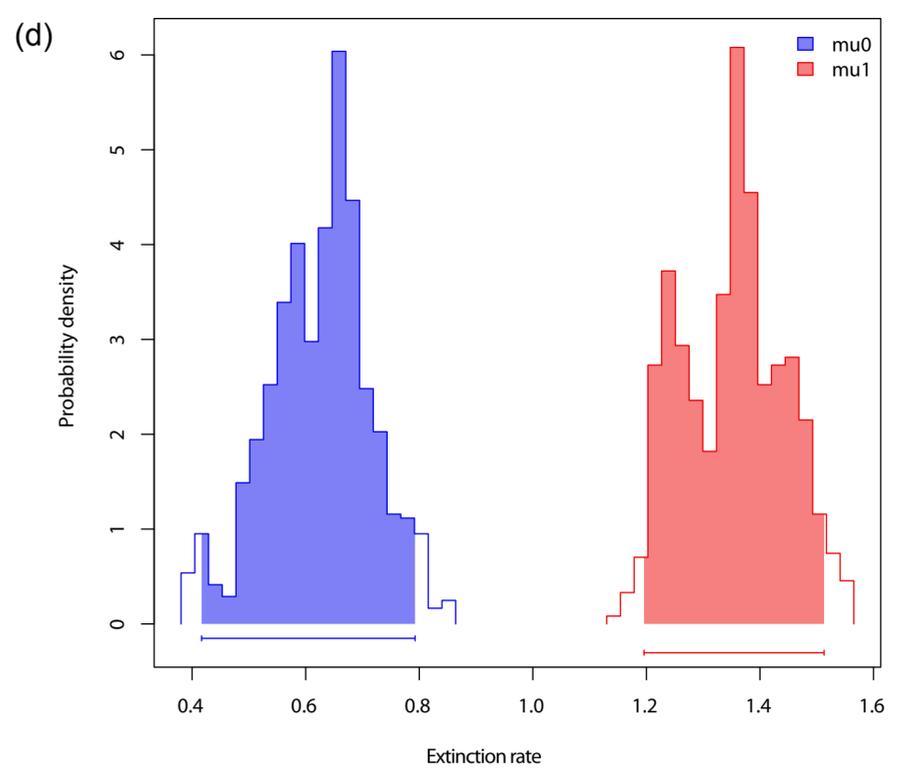
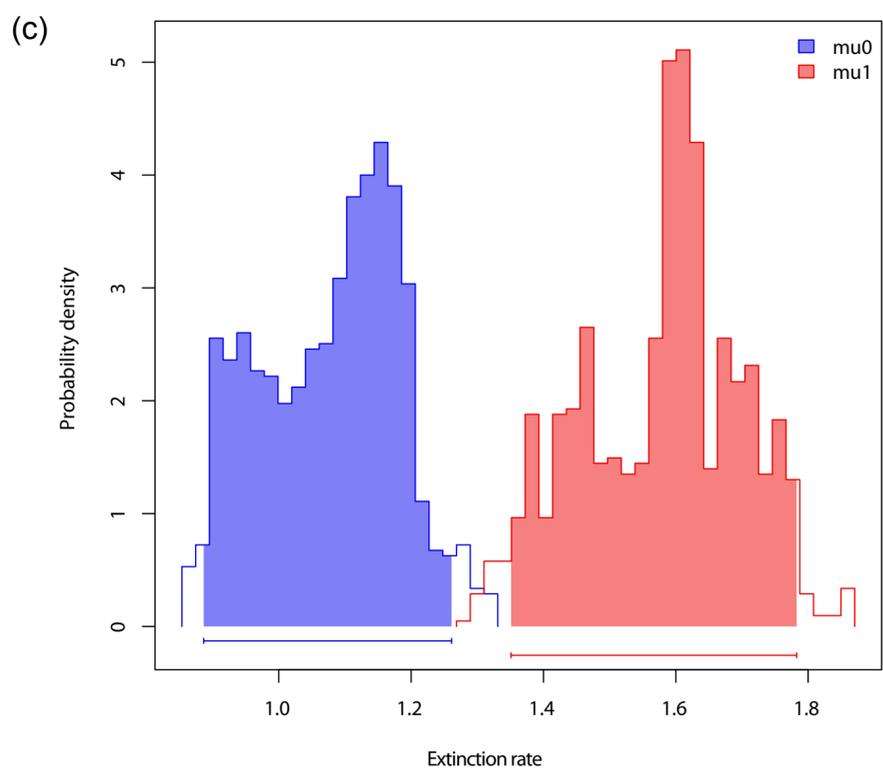
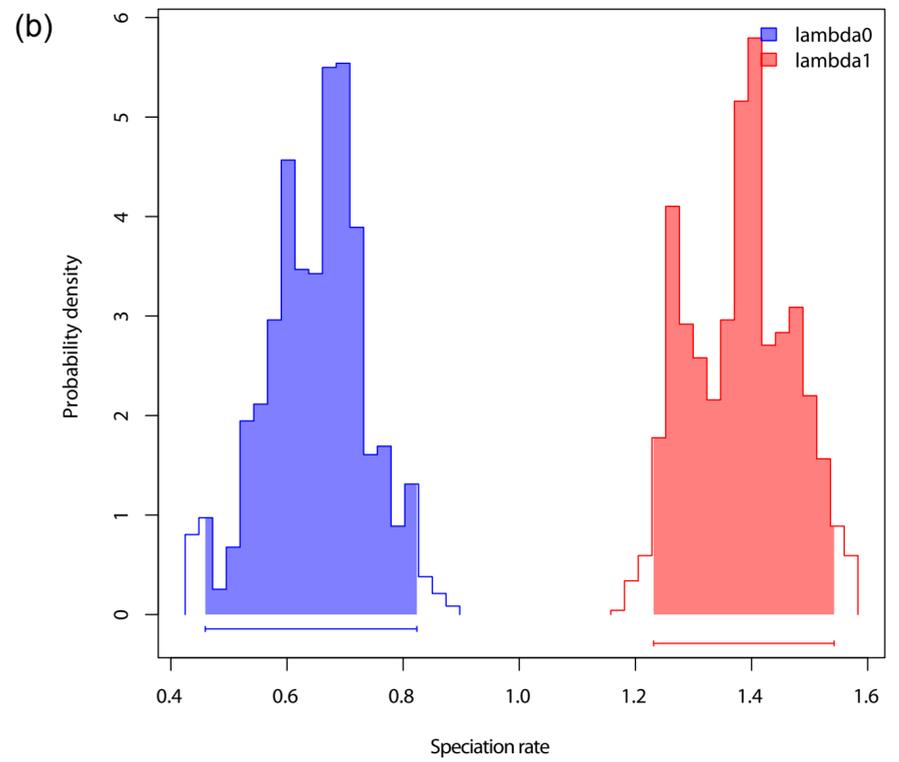
**Appendix IV.7.** Results and discussion for original data.

**Appendix IV.8.** Tree used for the reconstructions, with a breakdown of the character states that were available for each taxon at the tips.[Electronic]

Mediterranean hotspot (1) vs. other regions (0)

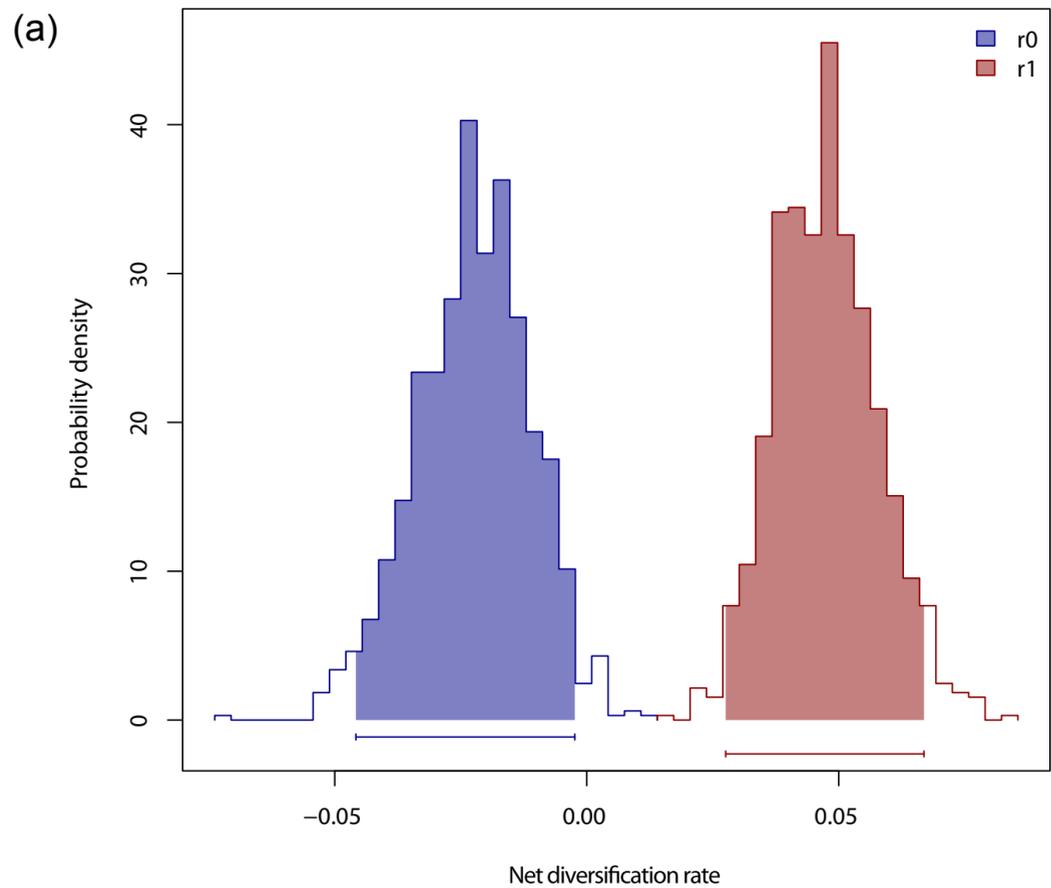


Zygomorphic (1) vs. actinomorphic (0)

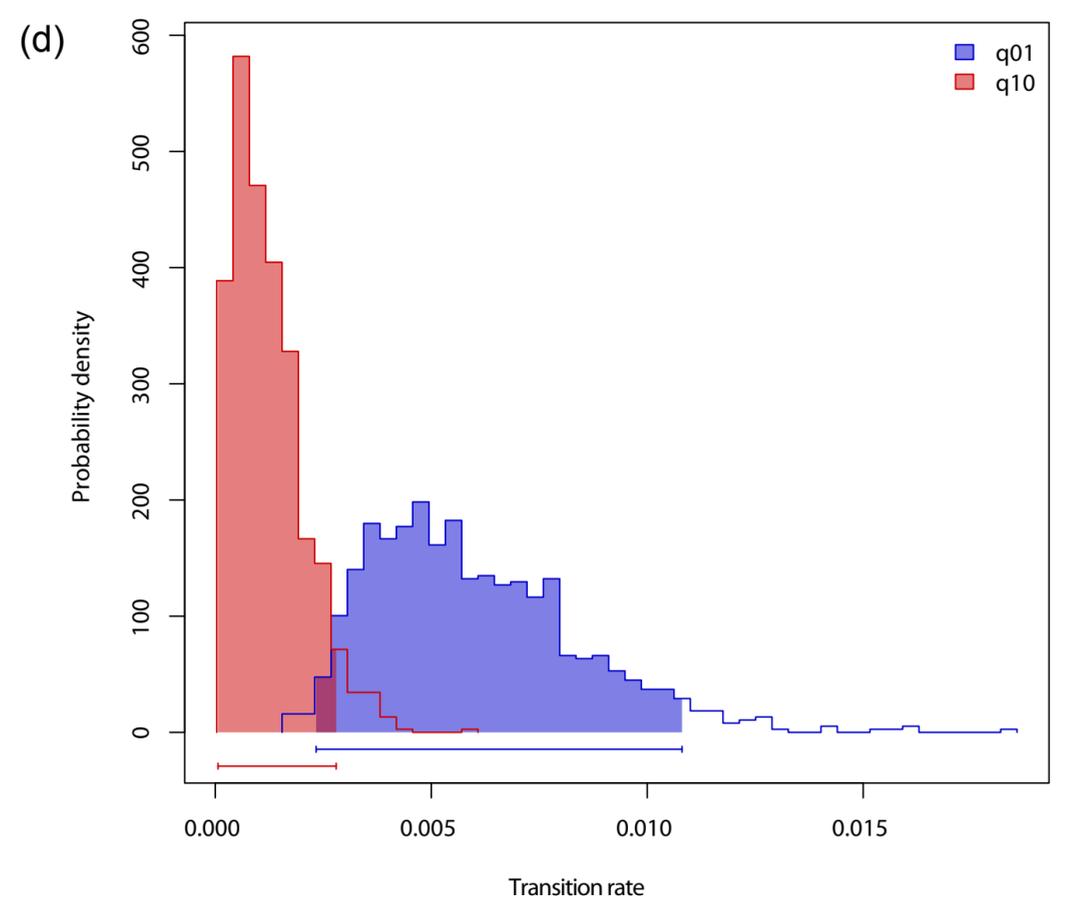
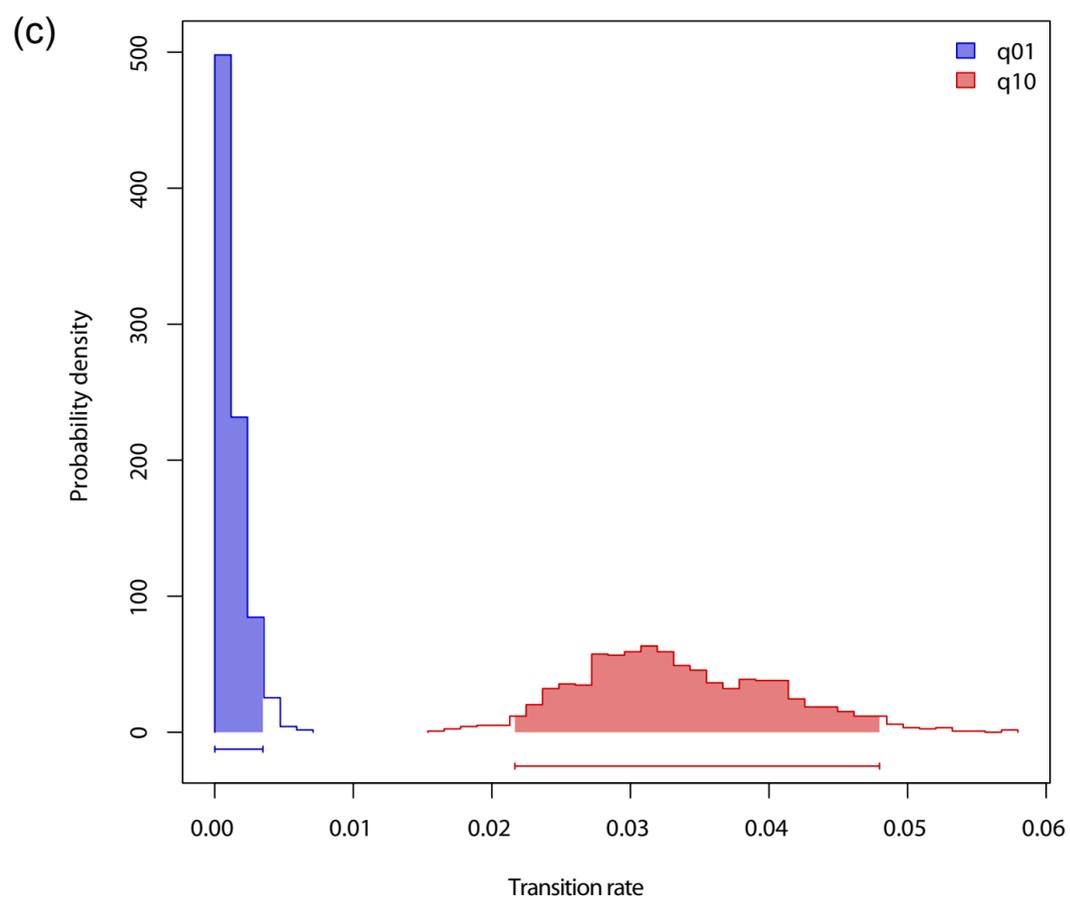
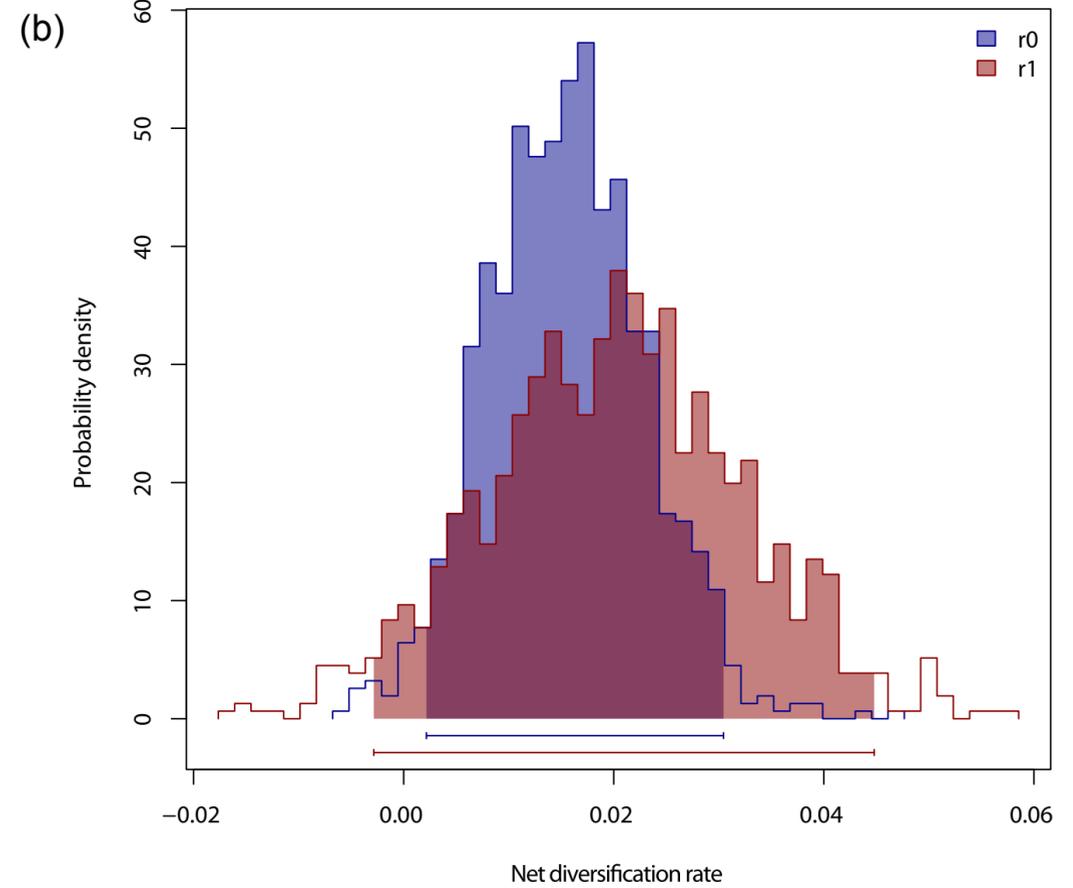


**Figure II.S1.** Additional parameter estimates obtained for the best BiSSE models selected for each trait of interest. (a,c,e) Presence in Mediterranean climate regions (red) vs. other regions (blue). (b,d,f) Floral zygomorphy (red) vs. actinomorphy (blue), assuming *Banksia* is unknown for this trait. (a,b) Speciation rates. (c,d) Extinction rates. (e,f) Relative extinction rates.

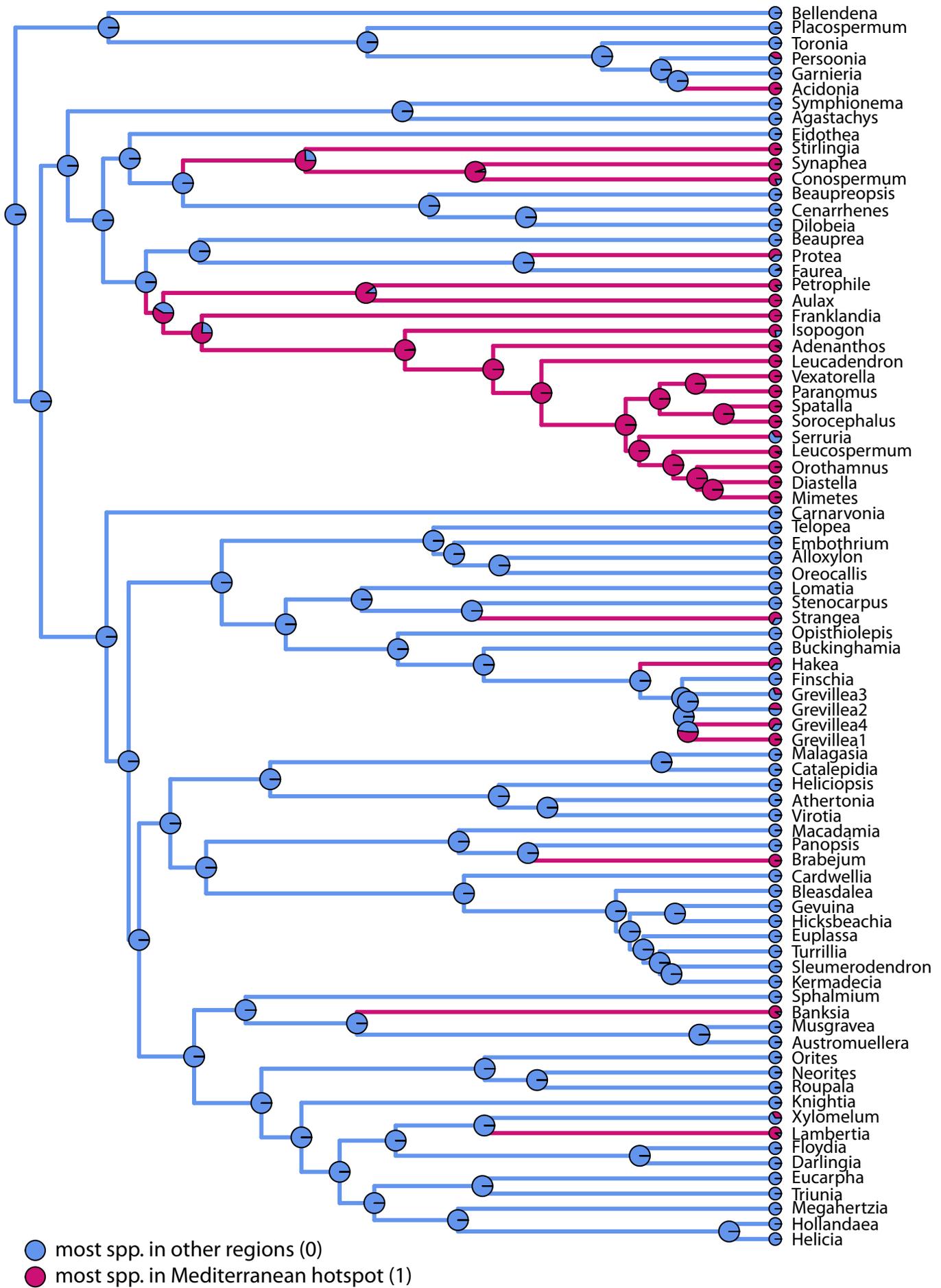
Mediterranean hotspot (1) vs. other regions (0)



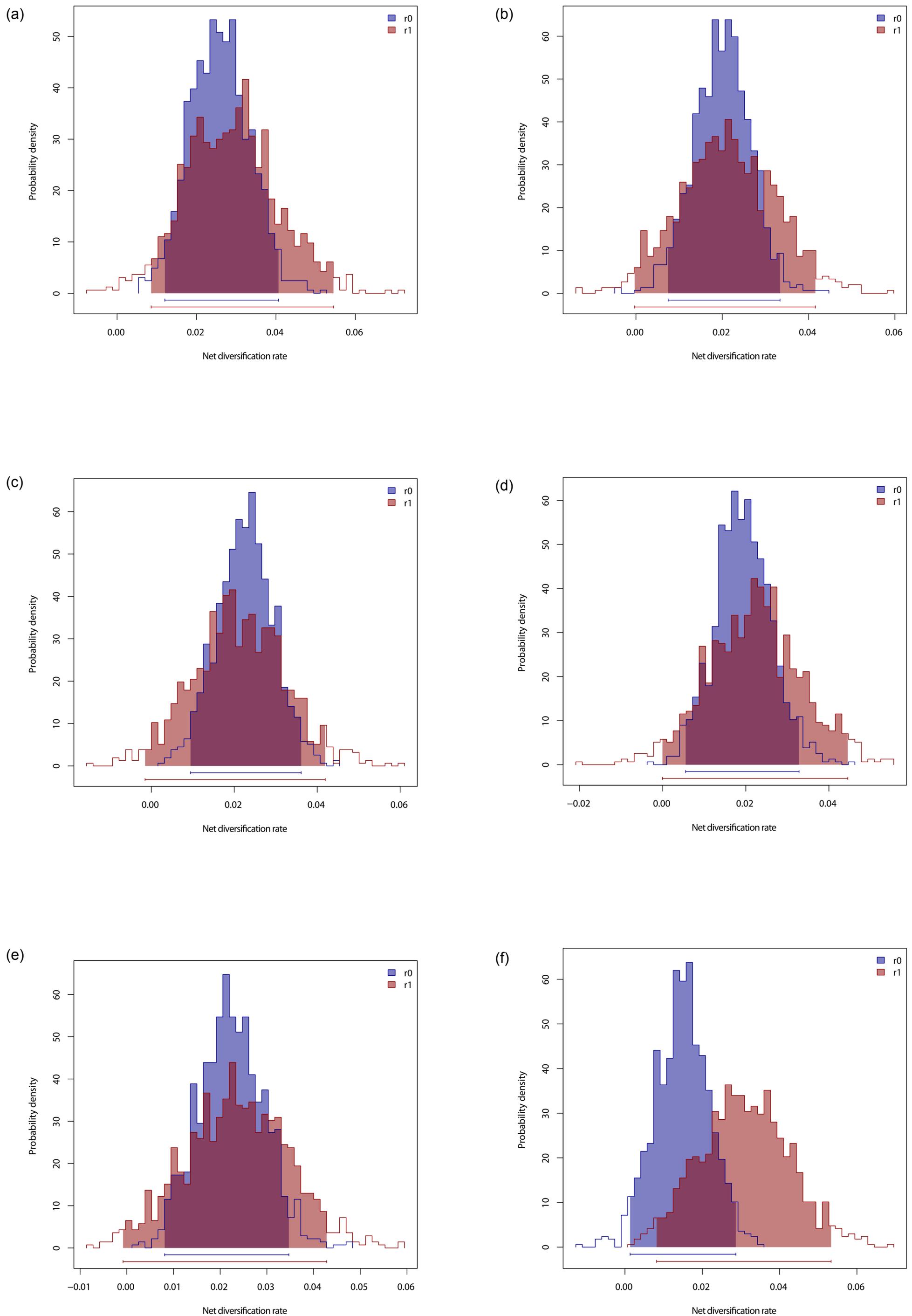
Zygomorphic (1) vs. actinomorphic (0)



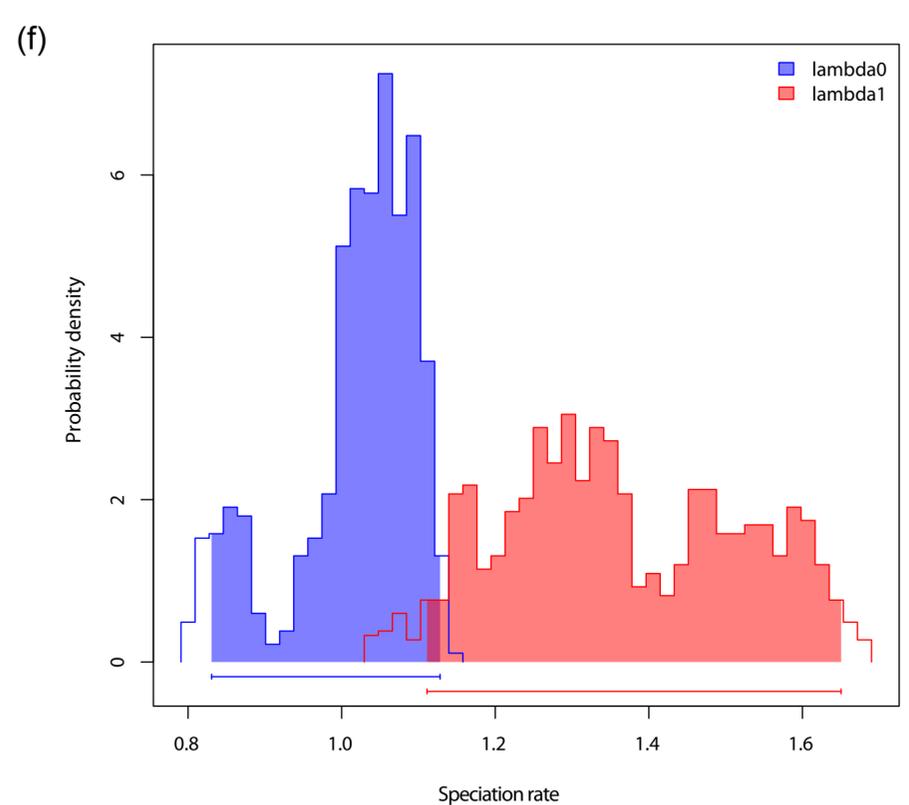
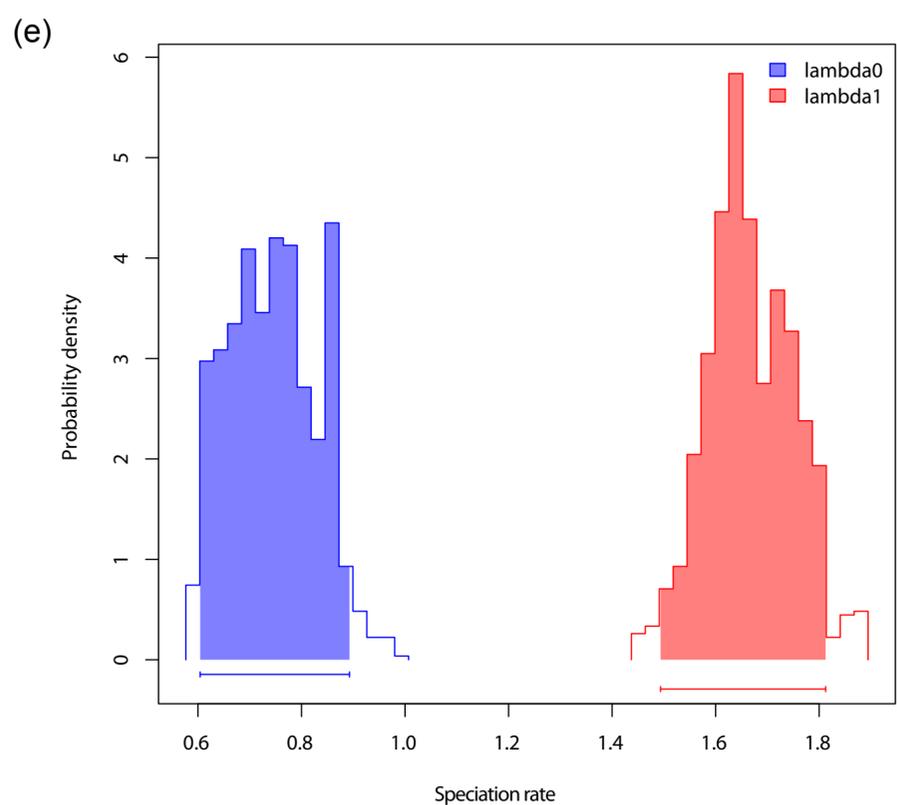
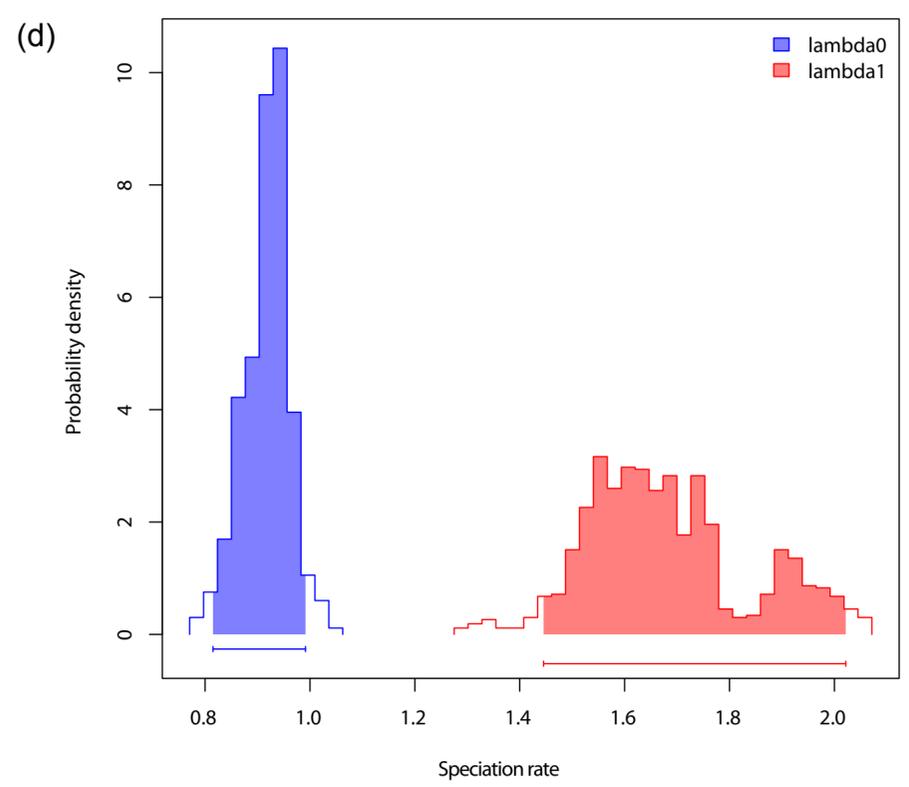
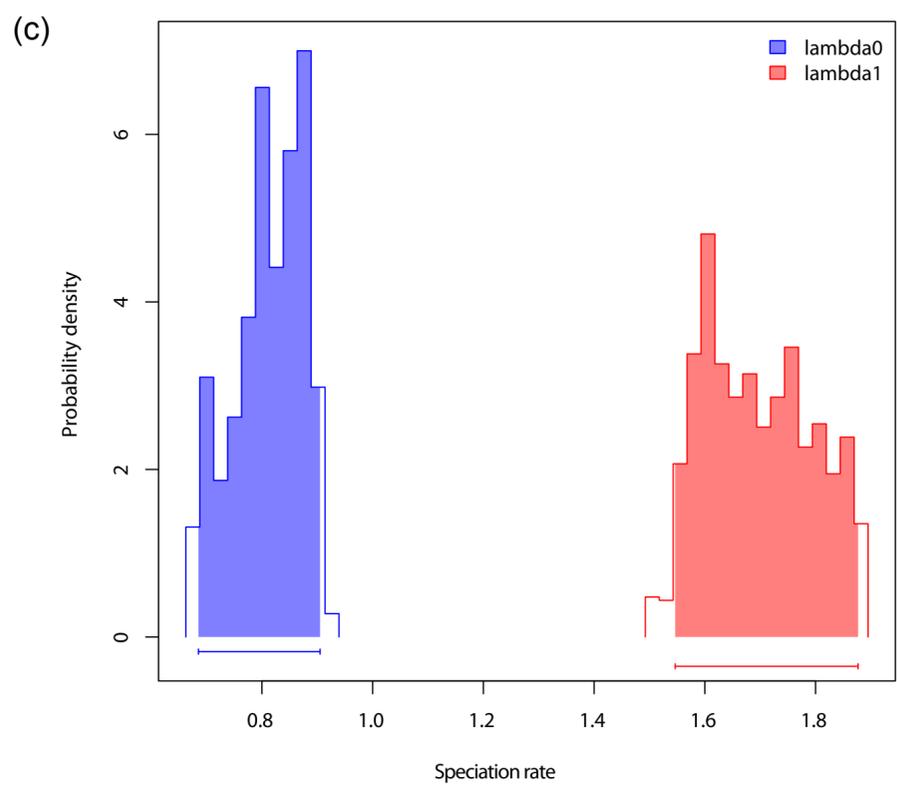
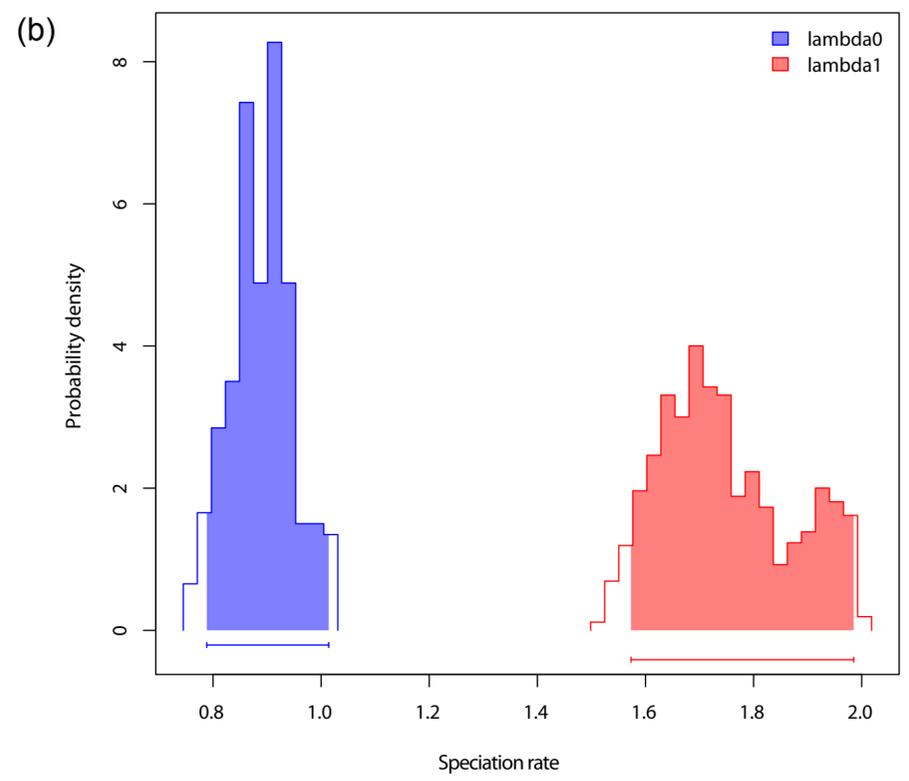
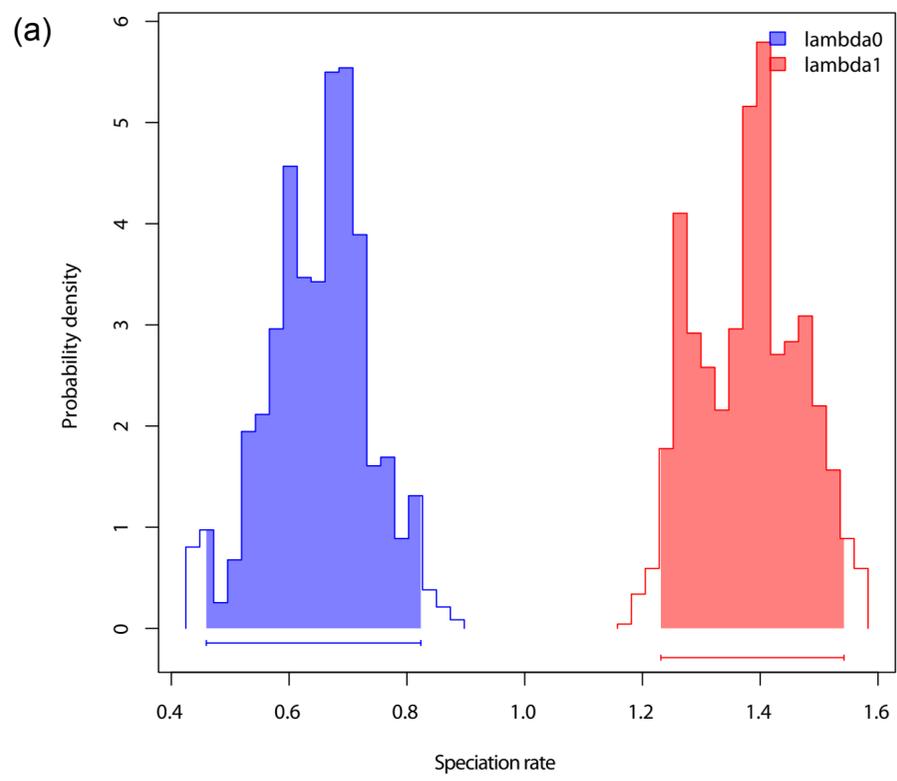
**Figure II.S2.** Parameter estimates obtained for the best BiSSE models selected for each trait of interest when nodes are spread evenly within *Grevillea*. (a,c) Presence in Mediterranean climate regions (red) vs. other regions (blue). (b,d) Floral zygomorphy (red) vs. actinomorphy (blue), assuming *Banksia* is unknown for this trait. (a,b) Net diversification rates. (c,d) Transition rates.



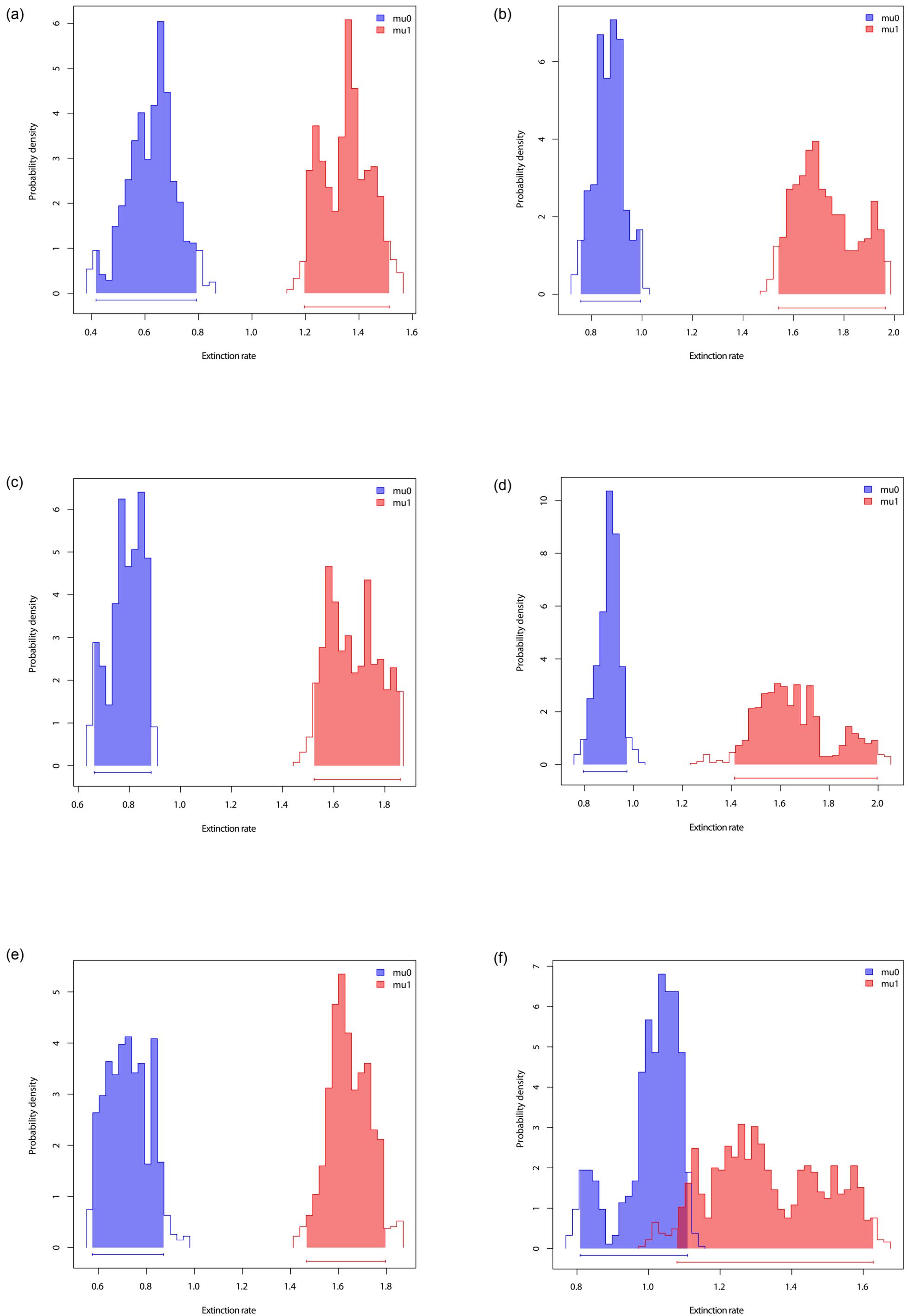
**Figure II.S3.** Maximum likelihood reconstruction of ancestral states for presence in Mediterranean hotspots. Subtending branches are colored according to the highest probability state of the corresponding node. Terminal pie charts represent the actual proportion of species in each state in each terminal taxon. However, these proportions could not be taken into account explicitly in the model used for this reconstruction (see Methods S1), which is instead based on the most common state scored for each polymorphic terminal taxon. The analysis with polymorphic terminal taxa scored as missing data yielded essentially the same character reconstruction (results not shown).



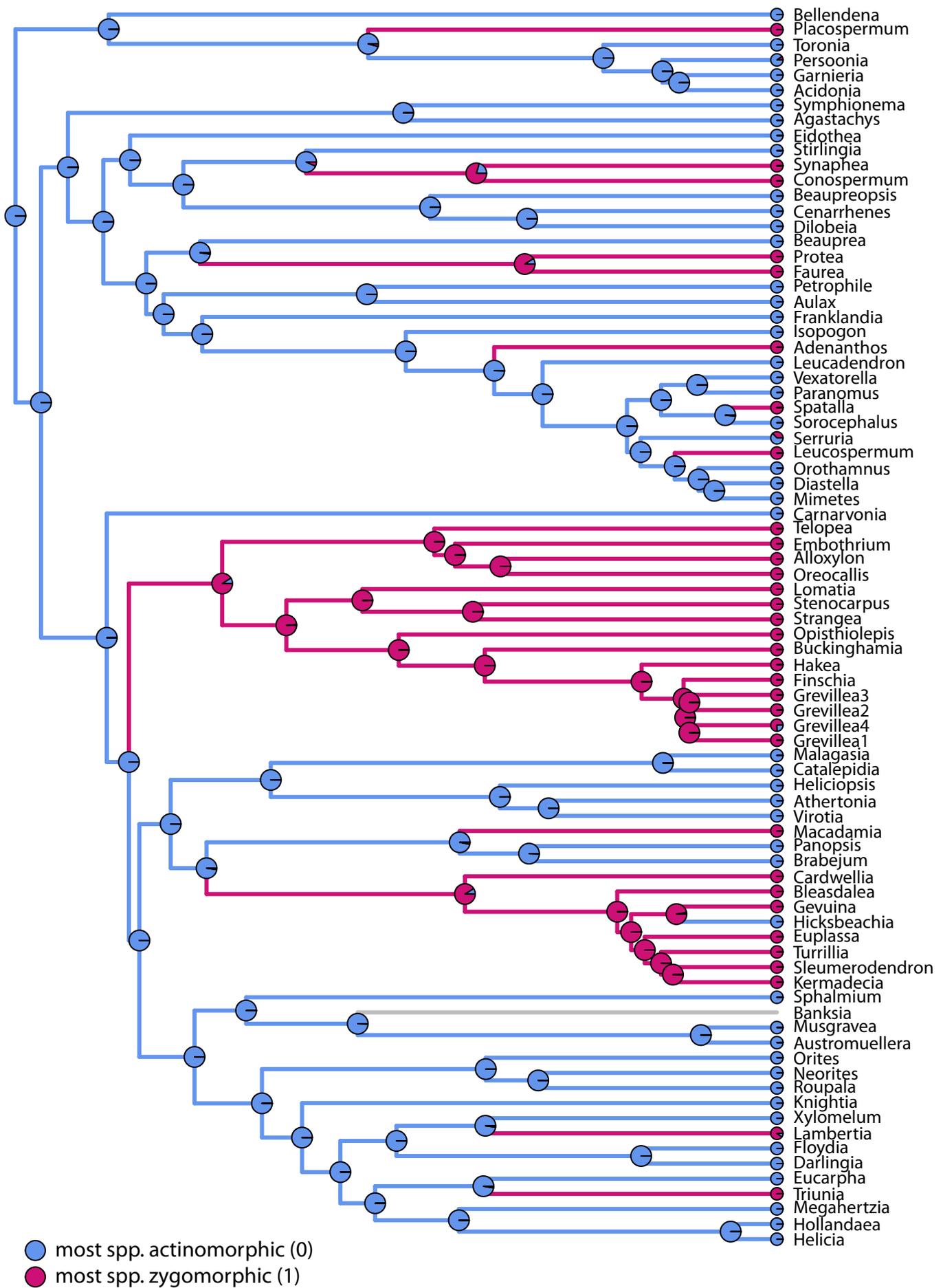
**Figure II.S4.** Sensitivity of BiSSE analyses of floral symmetry to various hypothetical proportions of zygomorphic species in *Banksia* (net diversification rate estimates). (a) Proportion unknown (same as Fig. II.2b). (b) 0% (*Banksia* actinomorphic). (c) 25%. (d) 50%. (e) 75%. (f) 100% (*Banksia* zygomorphic).



**Figure II.S5.** Sensitivity of BiSSE analyses of floral symmetry to various hypothetical proportions of zygomorphic species in *Banksia* (speciation rate estimates). (a) Proportion unknown (same as Fig. II.S1b). (b) 0% (*Banksia* actinomorphic). (c) 25%. (d) 50%. (e) 75%. (f) 100% (*Banksia* zygomorphic).



**Figure II.S6.** Sensitivity of BiSSE analyses of floral symmetry to various hypothetical proportions of zygomorphic species in *Banksia* (extinction rate estimates). (a) Proportion unknown (same as Fig. II.S1d). (b) 0% (*Banksia* actinomorphic). (c) 25%. (d) 50%. (e) 75%. (f) 100% (*Banksia* zygomorphic).



**Figure II.S7.** Maximum likelihood reconstruction of ancestral states for floral symmetry. Subtending branches are colored according to the highest probability state of the corresponding node. Terminal pie charts represent the actual proportion of species in each state in each terminal taxon. However, these proportions could not be taken into account explicitly in the model used for this reconstruction (see Methods S1), which is instead based on the most common state scored for each polymorphic terminal taxon. The analysis with polymorphic terminal taxa scored as missing data yielded essentially the same character reconstruction (results not shown).

**Table II.S1.** Total number of species in each genus of Proteaceae, and number of species in each character state.

<b>Genus</b>	<b>Total</b>	<b>Non-hotspot</b>	<b>Hotspot</b>	<b>Actinomorphic</b>	<b>Zygomorphic</b>
<i>Acidonia</i>	1	0	1	1	0
<i>Adenanthos</i>	31	2	29	0	31
<i>Agastachys</i>	1	1	0	1	0
<i>Alloxylon</i>	4	4	0	0	4
<i>Athertonia</i>	1	1	0	1	0
<i>Aulax</i>	3	0	3	3	0
<i>Austromuellera</i>	2	2	0	2	0
<i>Banksia</i>	169	15	154	?	?
<i>Beauprea</i>	13	13	0	13	0
<i>Beaupreopsis</i>	1	1	0	1	0
<i>Bellendena</i>	1	1	0	1	0
<i>Bleasdalea</i>	2	2	0	0	2
<i>Brabejum</i>	1	0	1	1	0
<i>Buckinghamia</i>	2	2	0	0	2
<i>Cardwellia</i>	1	1	0	0	1
<i>Carnarvon</i>	1	1	0	1	0
<i>Catalepidia</i>	1	1	0	1	0
<i>Cenarrhenes</i>	1	1	0	1	0
<i>Conospermum</i>	53	10	43	0	53
<i>Darlingia</i>	2	2	0	2	0
<i>Diastella</i>	7	0	7	7	0
<i>Dilobeia</i>	2	2	0	2	0
<i>Eidothea</i>	2	2	0	2	0
<i>Embothrium</i>	1	1	0	0	1
<i>Eucarpha</i>	2	2	0	2	0
<i>Euplassa</i>	20	20	0	0	20
<i>Faurea</i>	15	14	1	0	15
<i>Finschia</i>	3	3	0	0	3
<i>Floydia</i>	1	1	0	1	0
<i>Franklandia</i>	2	0	2	2	0
<i>Garnieria</i>	1	1	0	1	0
<i>Gevuina</i>	1	1	0	0	1
<i>Grevillea1</i>	1	0	1	0	1
<i>Grevillea2</i>	90	46	44	0	90
<i>Grevillea3</i>	112	78	34	0	112

<b>Genus</b>	<b>Total</b>	<b>Non-hotspot</b>	<b>Hotspot</b>	<b>Actinomorphic</b>	<b>Zygomorphic</b>
<i>Grevillea</i> <sup>4</sup>	156	56	100	38	118
<i>Hakea</i>	150	55	95	0	150
<i>Helicia</i>	97	97	0	97	0
<i>Heliciopsis</i>	14	14	0	14	0
<i>Hicksbeachia</i>	2	2	0	2	0
<i>Hollandaea</i>	4	4	0	4	0
<i>Isopogon</i>	35	8	27	35	0
<i>Kermadecia</i>	4	4	0	0	4
<i>Knighthia</i>	1	1	0	1	0
<i>Lambertia</i>	10	1	9	1	9
<i>Leucadendron</i>	85	1	84	85	0
<i>Leucospermum</i>	48	3	45	0	48
<i>Lomatia</i>	12	12	0	0	12
<i>Macadamia</i>	9	9	0	0	9
<i>Malagasia</i>	1	1	0	1	0
<i>Megahertzia</i>	1	1	0	1	0
<i>Mimetes</i>	13	0	13	13	0
<i>Musgravea</i>	2	2	0	2	0
<i>Neorites</i>	1	1	0	1	0
<i>Opisthiolepis</i>	1	1	0	0	1
<i>Oreocallis</i>	2	2	0	0	2
<i>Orites</i>	8	8	0	8	0
<i>Orothamnus</i>	1	0	1	1	0
<i>Panopsis</i>	26	26	0	26	0
<i>Paranomus</i>	19	0	19	19	0
<i>Persoonia</i>	100	59	41	90	10
<i>Petrophile</i>	53	6	47	53	0
<i>Placospermum</i>	1	1	0	0	1
<i>Protea</i>	112	43	69	0	112
<i>Roupala</i>	33	33	0	33	0
<i>Serruria</i>	54	0	54	34	20
<i>Sleumerodendron</i>	1	1	0	0	1
<i>Sorocephalus</i>	11	0	11	11	0
<i>Spatalla</i>	20	0	20	0	20
<i>Sphalmium</i>	1	1	0	1	0
<i>Stenocarpus</i>	23	23	0	0	23
<i>Stirlingia</i>	7	0	7	7	0
<i>Strangea</i>	3	1	2	0	3
<i>Symphionema</i>	2	2	0	2	0

<b>Genus</b>	<b>Total</b>	<b>Non-hotspot</b>	<b>Hotspot</b>	<b>Actinomorphic</b>	<b>Zygomorphic</b>
<i>Synaphea</i>	51	0	51	0	51
<i>Telopea</i>	5	5	0	0	5
<i>Toronia</i>	1	1	0	1	0
<i>Triunia</i>	4	4	0	0	4
<i>Turrillia</i>	3	3	0	0	3
<i>Vexatorella</i>	4	0	4	4	0
<i>Virotia</i>	6	6	0	6	0
<i>Xylomelum</i>	6	4	2	6	0

**Table II.S2.** AIC values and delta-AIC (in parentheses) obtained for each diversification model tested in the character-dependent analyses (BiSSE). The lowest AIC score (best model) in each analysis is highlighted in bold.

	Non-hotspot vs hotspot	Floral symmetry ( <i>Banksia</i> unknown)
$\lambda_0 \neq \lambda_1; \mu_0 \neq \mu_1; q_{01} \neq q_{10}$	<b>1538.8</b>	<b>1472.0</b>
$\lambda_0 = \lambda_1; \mu_0 \neq \mu_1; q_{01} \neq q_{10}$	1544.1 (5.3)	1476.2 (4.2)
$\lambda_0 \neq \lambda_1; \mu_0 = \mu_1; q_{01} \neq q_{10}$	1542.6 (3.8)	1475.8 (3.8)
$\lambda_0 \neq \lambda_1; \mu_0 \neq \mu_1; q_{01} = q_{10}$	1548.1 (9.3)	1477.8 (5.8)
$\lambda_0 = \lambda_1; \mu_0 = \mu_1; q_{01} \neq q_{10}$	1564.6 (25.8)	1476.4 (4.4)
$\lambda_0 \neq \lambda_1; \mu_0 = \mu_1; q_{01} = q_{10}$	1564.9 (26.1)	1476.4 (4.9)
$\lambda_0 = \lambda_1; \mu_0 \neq \mu_1; q_{01} = q_{10}$	1565.9 (27.1)	1477.1 (5.1)
$\lambda_0 = \lambda_1; \mu_0 = \mu_1; q_{01} = q_{10}$	1566.5 (27.7)	1479.4 (7.4)

**Table II.S3.** Parameter estimates obtained from the BiSSE analysis testing the impact of occurring in Mediterranean hotspots on diversification rates in Proteaceae when a third of the diversification time passes between each split of *Grevillea* (mean estimates followed with 95% confidence intervals).  $\lambda$  = speciation rate;  $\mu$  = extinction rate;  $r$  = net diversification rate ( $\lambda - \mu$ );  $\varepsilon$  = relative extinction rate ( $\mu / \lambda$ );  $q$  = transition rate.

	$\lambda$	$\mu$	$r$	$\varepsilon$	$q_{01}$	$q_{10}$
non-hotspot (0)	1.39 (1.13 – 1.62)	1.41 (1.15 – 1.65)	-0.02 (-0.05 – 0.00)	1.02 (1.00 – 1.03)	0.001 (0.000 – 0.004)	0.034 (0.022 – 0.048)
hotspot (1)	1.42 (1.13 – 1.68)	1.37 (1.08 – 1.64)	0.05 (0.03 – 0.07)	0.97 (0.95 – 0.98)		

**Table II.S4.** Parameter estimates obtained in the BiSSE analysis of the impact of floral symmetry on diversification rates in Proteaceae when a third of the diversification time passes between each split of *Grevillea* (mean estimates followed with 95% confidence intervals).  $r$  = net diversification rate ( $\lambda - \mu$ );  $\varepsilon$  = relative extinction rate ( $\mu / \lambda$ );  $q$  = transition rate.

	$\lambda$	$\mu$	$r$	$\varepsilon$	$q_{01}$	$q_{10}$
actinomorphic (0)	1.17 (0.98 – 1.36)	1.15 (0.96 – 1.35)	0.02 (0.00 – 0.03)	0.99 (0.97 – 1.00)	0.006 (0.003 – 0.011)	0.001 (0.000 – 0.003)
zygomorphic (1)	1.62 (1.14 – 2.11)	1.60 (1.10 – 2.10)	0.02 (0.00 – 0.04)	0.99 (0.96 – 1.00)		

**Table II.S5.** Results from the split BiSSE analyses on presence in Mediterranean hotspots. Each analysis corresponds to a split tree with two independent BiSSE models, one for the clade descending from the split node ('After'), and one for the rest of the tree ('Before'). The split nodes were chosen to match the nodes identified as major significant rate shifts in the MEDUSA analyses (see Fig. 1).  $\lambda$  = speciation rate;  $\mu$  = extinction rate;  $r$  = net diversification rate ( $\lambda - \mu$ );  $\varepsilon$  = relative extinction rate ( $\mu / \lambda$ );  $q$  = transition rate.

Shift 1

		$\lambda$	$\mu$	$r$	$\varepsilon$	$q_{01}$	$q_{10}$
Before	non-hotspot (0)	0.607	0.622	-0.015	1.02	0.002	0.020
	hotspot (1)	1.593	1.558	0.035	0.98		
After	non-hotspot (0)	0.706	0	0.706	0	0	0.032
	hotspot (1)	0	0	0	0		

Shift 2

		$\lambda$	$\mu$	$r$	$\varepsilon$	$q_{01}$	$q_{10}$
Before	non-hotspot (0)	0.497	0.495	0.002	0.99	0.002	0.012
	hotspot (1)	1.219	1.193	0.026	0.98		
After	non-hotspot (0)	0.629	0.072	0.557	0.11	0.307	0.012
	hotspot (1)	1.715	1.707	0.008	0.99		

Shift 3

		$\lambda$	$\mu$	$r$	$\varepsilon$	$q_{01}$	$q_{10}$
Before	non-hotspot (0)	0.959	0.979	-0.020	1.02	0.000	0.025
	hotspot (1)	1.569	1.529	0.040	0.97		
After	non-hotspot (0)	0.020	0	0.020	0	0.000	0.117
	hotspot (1)	0	0	0	0		

Shift 4

		$\lambda$	$\mu$	$r$	$\varepsilon$	$q_{01}$	$q_{10}$
Before	non-hotspot (0)	0	0.263	-0.263	NA	0.041	0.415
	hotspot (1)	4.582	4.319	0.263	0.94		
After	non-hotspot (0)	0.866	0.879	-0.013	1.02	0.000	0.022
	hotspot (1)	1.454	1.412	0.042	0.97		

Shift 5

		$\lambda$	$\mu$	$r$	$\varepsilon$	$q_{01}$	$q_{10}$
Before	non-hotspot (0)	0.837	0.852	-0.015	1.02	0.000	0.022
	hotspot (1)	1.525	1.488	0.037	0.98		
After	non-hotspot (0)	0	0	0	0	0.216	0.625
	hotspot (1)	0.568	0	0.568	0		

**Table II.S6.** Results from the split BiSSE analyses on floral symmetry. Each analysis corresponds to a split tree with two independent BiSSE models, one for the clade descending from the split node ('After'), and one for the rest of the tree ('Before'). The split nodes were chosen to match the nodes identified as major significant rate shifts in the MEDUSA analyses (see Fig. 1).  $\lambda$  = speciation rate;  $\mu$  = extinction rate;  $r$  = net diversification rate ( $\lambda - \mu$ );  $\varepsilon$  = relative extinction rate ( $\mu / \lambda$ );  $q$  = transition rate.

Shift 1

		$\lambda$	$\mu$	$r$	$\varepsilon$	$q_{01}$	$q_{10}$
Before	actinomorphic(0)	0.541	0.513	0.028	0.95	0.010	0.001
	zygomorphic (1)	1.599	1.577	0.033	0.98		
After	actinomorphic(0)	0.706	0	0.706	0	0.001	0.032
	zygomorphic (1)	0	0	0	0		

Shift 2

		$\lambda$	$\mu$	$r$	$\varepsilon$	$q_{01}$	$q_{10}$
Before	actinomorphic(0)	0.808	0.789	0.019	0.98	0.006	0.001
	zygomorphic (1)	1.521	1.496	0.025	0.98		
After	actinomorphic(0)	0.019	0	0.019	0	0.264	4.368
	zygomorphic (1)	2.274	0	2.274	0		

Shift 3

		$\lambda$	$\mu$	$r$	$\varepsilon$	$q_{01}$	$q_{10}$
Before	actinomorphic(0)	0.931	0.912	0.019	0.98	0.006	0.001
	zygomorphic (1)	1.562	1.538	0.024	0.98		
After	actinomorphic(0)	0.019	0	0.019	0	0.000	0.119
	zygomorphic (1)	0	0	0	0		

Shift 4

		$\lambda$	$\mu$	$r$	$\varepsilon$	$q_{01}$	$q_{10}$
Before	actinomorphic(0)	3.029	3.086	-0.057	1.02	0.043	0.296
	zygomorphic (1)	0	0	0	0		
After	actinomorphic(0)	0.785	0.762	0.023	0.97	0.006	0.001
	zygomorphic (1)	1.526	1.498	0.028	0.98		

Shift 5

		$\lambda$	$\mu$	$r$	$\varepsilon$	$q_{01}$	$q_{10}$
Before	actinomorphic(0)	0.808	0.789	0.019	0.98	0.006	0.001
	zygomorphic (1)	1.514	1.489	0.025	0.98		
After	actinomorphic(0)	0.292	0	0.292	0	0.076	0.412
	zygomorphic (1)	0	0	0	0		

## Methods II.S1

### *Ancestral state reconstruction*

Ancestral states can, in principle, be estimated using the BiSSE model, taking into account the effect of a character on speciation and extinction (Maddison *et al.*, 2007). However, this is not yet implemented for the version handling unresolved terminal clades corresponding to the situation in our study. Therefore, we used a simple maximum likelihood approach to reconstruct ancestral states of both presence in Mediterranean-climate hotspots and floral symmetry in Proteaceae. In doing so, our aim was to help visualizing the distribution of character states on the phylogeny rather than make an inference on exact character states and transitions. Specifically, we used maximum likelihood to optimize the most general (All Rates Different, ARD) Markov model for a binary character, which has two transition rate parameters (Mk2 model). Optimization and character reconstructions were conducted using the rayDISC function of the corHMM package in R (Beaulieu *et al.*, 2013). We handled polymorphic terminal taxa (genera) in two ways: first, we treated them as polymorphic (equivalent to missing data for a binary character); and then we rescored them with their most common state. Using their ancestral state would be more appropriate, but internal phylogenies are lacking for most genera. Results from both reconstructions were essentially identical for each trait and therefore we chose to represent the second reconstruction to maximize graphical appreciation of the character distributions (Figures S5, S6).

**Beaulieu JM, O'Meara BC, Donoghue MJ. 2013.** Identifying hidden rate changes in the evolution of a binary morphological character: the evolution of plant habit in campanulid angiosperms. *Systematic Biology* **62**: 725–737.

Table III.S1: List of sources used to resolve relationships within families in the supertree used for this study.

Clade	Sources
Asteraceae	(Funk et al. 2009)
Boraginaceae	(Cohen 2014)
Borthwickiaceae+Stixaceae+Resedaceae+ Gyrostemonaceae	(Su et al. 2012)
Brassicaceae+Cleomaceae+Capparaceae	(Hall et al. 2002; Warwick et al. 2010; Busch et al. 2012; Patchell et al. 2014; Hohmann et al. 2015)
Bromeliaceae	GRIN Taxonomy for plants ( <a href="http://www.ars-grin.gov/cgi-bin/npgs/html/index.pl?language=en">http://www.ars-grin.gov/cgi-bin/npgs/html/index.pl?language=en</a> )("Germplasm Resources Information Network - (GRIN) [Online Database]" 2015)
Commelinaceae	(Evans et al. 2003; Burns et al. 2011)
Ericaceae	(Kron et al. 2002)
Fabaceae	(Cardoso et al. 2012) GRIN Taxonomy for plants ( <a href="http://www.ars-grin.gov/cgi-bin/npgs/html/index.pl?language=en">http://www.ars-grin.gov/cgi-bin/npgs/html/index.pl?language=en</a> )("Germplasm Resources Information Network - (GRIN) [Online Database]" 2015),
Gentianaceae	(Mansion and Struwe 2004) GRIN Taxonomy for plants ( <a href="http://www.ars-grin.gov/cgi-bin/npgs/html/index.pl?language=en">http://www.ars-grin.gov/cgi-bin/npgs/html/index.pl?language=en</a> )("Germplasm Resources Information Network - (GRIN) [Online Database]" 2015)
Iridaceae	(Goldblatt et al. 2008)
Lecythidaceae	(Tsou and Mori 2007)
Loranthaceae	(Wilson and Calvin 2006; Vidal-Russell and Nickrent 2008)
Proteaceae	(Mast et al. 2008; Sauquet et al. 2009)
Malpighiaceae	(Davis and Anderson 2010)
Plantaginaceae	(Albach et al. 2005)
Sapindaceae	(Buerki et al. 2011)
Saxifragales	(Soltis et al. 2013)
Scrophulariaceae	(Oxelman et al. 2005)
Solanaceae	(Olmstead et al. 2008)
Tecophileaceae	(Buerki et al. 2013)
Violaceae	(Tokuoka 2008)

#### Literature cited

**Albach DC, Meudt HM, Oxelman B. 2005.** Piecing together the “new” Plantaginaceae.

*American Journal of Botany* **92**: 297–315.

**Buerki S, Forest F, Alvarez N, Nylander JAA, Arrigo N, Sanmartín I. 2011.** An evaluation of new parsimony-based versus parametric inference methods in biogeography: a case study using the globally distributed plant family Sapindaceae. *Journal of Biogeography* **38**: 531–550.

**Buerki S, Manning JC, Forest F. 2013.** Spatio-temporal history of the disjunct family Tecophilaeaceae: a tale involving the colonization of three Mediterranean-type ecosystems. *Annals of botany* **111**: 361–373.

**Burns JH, Faden RB, Steppan SJ. 2011.** Phylogenetic studies in the Commelinaceae subfamily Commelinoideae inferred from nuclear ribosomal and chloroplast DNA sequences. *Systematic Botany* **36**: 268–276.

**Busch A, Horn S, Mühlhausen A, Mummenhoff K, Zachgo S. 2012.** Corolla monosymmetry: evolution of a morphological novelty in the Brassicaceae family. *Molecular Biology and Evolution* **29**: 1241–1254.

**Cardoso D, de Queiroz LP, Pennington RT, de Lima HC, Fonty É, Wojciechowsky MF, Lavin M. 2012.** Revisiting the phylogeny of papilionoid legumes: new insights from comprehensively sampled. *American Journal of Botany* **99**: 1–23.

**Cohen JI. 2014.** Cladistics of Boraginaceae: evolutionary relationships, taxonomy, and patterns of character evolution. *Cladistics* **30**: 139–169.

**Davis CC, Anderson WR. 2010.** A complete generic phylogeny of Malpighiaceae inferred from nucleotide sequence data and morphology. *American Journal of Botany* **97**: 2031–2048.

**Evans TM, Sytsma KJ, Faden RB, Givnish TJ. 2003.** Phylogenetic relationships in the Commelinaceae : II . A cladistic analysis of rbcL sequences and morphology. *Systematic Botany* **28**: 270–292.

**Funk VA, Susanna A, Stuessy TF, Robinson H. 2009.** Classification of Compositae. In: Funk VA, In: Susanna A, In: Stuessy TF, In: Bayer RJ, eds. *Systematics, Evolution, and Biogeography of Compositae*. Vienna, Austria: International Association for Plant Taxonomy, 171–189.

**Germplasm Resources Information Network - (GRIN) [Online Database]. 2015.** <http://www.ars-grin.gov/npgs/>.(accessed Jul 2015)

**Goldblatt P, Rodriguez A, Powell MP, Davies TJ, Manning JC, van der Bank M, Savolainen V. 2008.** Iridaceae “Out of Australasia”? Phylogeny, Biogeography, and Divergence Time Based on Plastid DNA Sequences. *Systematic Botany* **33**: 495–508.

**Hall JC, Sytsma KJ, Iltis HH. 2002.** Phylogeny of Capparaceae and Brassicaceae based on chloroplast sequence data. *American Journal of Botany* **89**: 1826–1842.

**Hohmann N, Wolf EM, Lysak MA, Koch MA. 2015.** A time-calibrated road map of Brassicaceae species radiation and evolutionary history. *The Plant Cell* **27**: 2770–2784.

**Kron KA, Judd WS, Stevens PF. 2002.** Phylogenetic classification of Ericaceae: molecular and morphological evidence. *The Botanical Review* **68**: 335–423.

**Mansion G, Struwe L. 2004.** Generic delimitation and phylogenetic relationships within the subtribe Chironiinae (Chironieae: Gentianaceae), with special reference to *Centaurium*: evidence from nrDNA and cpDNA sequences. *Molecular phylogenetics and evolution* **32**: 951–977.

- Mast AR, Willis CL, Jones EH, Downs KM, Weston PH. 2008.** A smaller Macadamia from a more vagile tribe: inference of phylogenetic relationships, divergence times, and diaspore evolution in Macadamia and relatives (tribe Macadamieae; Proteaceae). *American Journal of Botany* **95**: 843–870.
- Olmstead RG, Bohs L, Migid HA, Santiago-Valentin E, Garcia VF, Collier SM. 2008.** A molecular phylogeny of the Solanaceae. *Taxon* **57**: 1159–1181.
- Oxelman B, Kornhall P, Olmstead RG, Bremer B. 2005.** Further disintegration of Scrophulariaceae. *Taxon* **54**: 411.
- Patchell MJ, Roalson EH, Hall JC. 2014.** Resolved phylogeny of Cleomaceae based on all three genomes. *Taxon* **63**: 315–328.
- Sauquet H, Weston PH, Anderson CL, Barker NP, Cantrill DJ, Mast AR, Savolainen V. 2009.** Contrasted patterns of hyperdiversification in Mediterranean hotspots. *Proceedings of the National Academy of Sciences of the United States of America* **106**: 221–225.
- Soltis DE, Mort ME, Latvis M, Mavrodiev E V., O'meara BC, Soltis PS, Burleigh JG, de Casas RR. 2013.** Phylogenetic relationships and character evolution analysis of Saxifragales using a supermatrix approach. *American Journal of Botany* **100**: 916–929.
- Su JX, Wang W, Zhang LB, Chen ZD. 2012.** Phylogenetic placement of two enigmatic genera, *Borthwickia* and *Stixis*, based on molecular and pollen data, and the description of a new family of Brassicales, Borthwickiaceae. *Taxon* **61**: 601–611.
- Tokuoka T. 2008.** Molecular phylogenetic analysis of Violaceae (Malpighiales) based on plastid and nuclear DNA sequences. *Journal of plant research* **121**: 253–260.
- Tsou C-H, Mori SA. 2007.** Floral organogenesis and floral evolution of the Lecythidoideae (Lecythidaceae). *American Journal of Botany* **94**: 716–736.
- Vidal-Russell R, Nickrent DL. 2008.** Evolutionary relationships in the showy mistletoe family (Loranthaceae). *American journal of botany* **95**: 1015–29.
- Warwick SI, Mummenhoff K, Sauder CA, Koch MA, Al-Shehbaz IA. 2010.** Closing the gaps: Phylogenetic relationships in the Brassicaceae based on DNA sequence data of nuclear ribosomal ITS region. *Plant Systematics and Evolution* **285**: 1–24.
- Wilson CA, Calvin CL. 2006.** Character divergences and convergences in canopy-dwelling Loranthaceae. *American journal of botany* **150**: 101–113.

Table III.S2: Summary of family-level states for floral symmetry and transitions reconstructed from this study (Fig. III.2).

Clade	States present	Ancestral state	Origins (min-max)	Reversals (min-max)	Species
<b>Families not assigned to order</b>					
Boraginaceae	actinomorphic, zygomorphic	actinomorphic	6	0	2740
Dasygongonaceae	actinomorphic	actinomorphic	0	0	16
Icacinae	actinomorphic	actinomorphic	0	0	160
Metteniusaceae	actinomorphic	actinomorphic	0	0	49
Oncothecaceae	actinomorphic	actinomorphic	0	0	2
Vahliaceae	actinomorphic	actinomorphic	0	0	8
<b>Acorales</b>	<b>zygomorphic</b>	<b>zygomorphic</b>	<b>0</b>	<b>0</b>	<b>2</b>
Acoraceae	zygomorphic	zygomorphic	0	0	2
<b>Alismatales</b>	<b>actinomorphic, zygomorphic</b>	<b>actinomorphic</b>	<b>1-3</b>	<b>0-2</b>	<b>2844</b>
Alismataceae	actinomorphic	actinomorphic	0	0	88
Aponogetonaceae	zygomorphic	zygomorphic	0	0	50
Araceae	actinomorphic	actinomorphic	0	0	2385
Butomaceae	actinomorphic	actinomorphic	0	0	1
Cymodoceaceae	NA	NA	NA	NA	16
Hydrocharitaceae	actinomorphic	actinomorphic	0	0	116
Juncaginaceae	actinomorphic, zygomorphic	uncertain	0-2	0-1	30
Posidoniaceae	NA	NA	NA	NA	9
Potamogetonaceae	actinomorphic	actinomorphic	0	0	102
Ruppiaceae	NA	NA	NA	NA	1
Scheuchzeriaceae	actinomorphic	actinomorphic	0	0	1
Tofieldiaceae	actinomorphic	actinomorphic	0	0	31
Zosteraceae	NA	NA	NA	NA	14
<b>Amborellales</b>	<b>actinomorphic</b>	<b>actinomorphic</b>	<b>0</b>	<b>0</b>	<b>1</b>
Amborellaceae	actinomorphic	actinomorphic	0	0	1
<b>Apiales</b>	<b>actinomorphic, zygomorphic</b>	<b>actinomorphic</b>	<b>2</b>	<b>1</b>	<b>5294</b>
Apiaceae	actinomorphic, zygomorphic	actinomorphic	1	1	3780
Araliaceae	actinomorphic	actinomorphic	0	0	1275
Griselinaceae	actinomorphic	actinomorphic	0	0	6
Myodocarpaceae	actinomorphic	actinomorphic	0	0	19
Pennantiaceae	actinomorphic	actinomorphic	0	0	4
Pittosporaceae	actinomorphic, zygomorphic	actinomorphic	1	0	200
Toricelliaceae	actinomorphic	actinomorphic	0	0	10
<b>Aquifoliales</b>	<b>actinomorphic</b>	<b>actinomorphic</b>	<b>0</b>	<b>0</b>	<b>550</b>
Aquifoliaceae	actinomorphic	actinomorphic	0	0	405
Cardiopteridaceae	actinomorphic	actinomorphic	0	0	43
Helwingiaceae	actinomorphic	actinomorphic	0	0	3
Phyllonomaceae	actinomorphic	actinomorphic	0	0	4

Clade	States present	Ancestral state	Origins (min-max)	Reversals (min-max)	Species
Stemonuraceae	actinomorphic	actinomorphic	0	0	95
<b>Arecales</b>	<b>actinomorphic</b>	<b>actinomorphic</b>	<b>0</b>	<b>0</b>	<b>2385</b>
Areaceae	actinomorphic	actinomorphic	0	0	2385
<b>Asparagales</b>	<b>actinomorphic, zygomorphic</b>	<b>actinomorphic</b>	<b>11-12</b>	<b>2-3</b>	<b>35020</b>
Amaryllidaceae	actinomorphic, zygomorphic	actinomorphic	6	0	1605
Asparagaceae	actinomorphic	actinomorphic	0	0	2500
Asteliaceae	actinomorphic	actinomorphic	0	0	31
Blandfordiaceae	actinomorphic	actinomorphic	0	0	4
Boryaceae	actinomorphic	actinomorphic	0	0	12
Doryanthaceae	actinomorphic	actinomorphic	0	0	2
Hypoxidaceae	actinomorphic	actinomorphic	0	0	100
Iridaceae	actinomorphic, zygomorphic	actinomorphic	1-2	0-1	2035
Ixioliriaceae	actinomorphic	actinomorphic	0	0	3
Lanariaceae	actinomorphic	actinomorphic	0	0	1
Orchidaceae	actinomorphic, zygomorphic	zygomorphic	0	2	27800
Tecophilaeaceae	actinomorphic, zygomorphic	actinomorphic	1	0	25
Xanthorrhoeaceae	actinomorphic, zygomorphic	actinomorphic	2	0	900
Xeronemataceae	actinomorphic	actinomorphic	0	0	2
<b>Asterales</b>	<b>actinomorphic, zygomorphic</b>	<b>actinomorphic</b>	<b>4-14</b>	<b>5-15</b>	<b>26859</b>
Alseuosmiaceae	actinomorphic	actinomorphic	0	0	10
Argophyllaceae	actinomorphic	actinomorphic	0	0	21
Asteraceae	actinomorphic, zygomorphic	uncertain	0-9	5-14	23600
Calyceraceae	actinomorphic	actinomorphic	0	0	60
Campanulaceae	actinomorphic, zygomorphic	actinomorphic	2	0	2380
Goodeniaceae	actinomorphic, zygomorphic	uncertain	0-2	0-1	430
Menyanthaceae	actinomorphic	actinomorphic	0	0	58
Pentaphragmataceae	actinomorphic	actinomorphic	0	0	30
Phellinaceae	actinomorphic	actinomorphic	0	0	12
Rousseaceae	actinomorphic	actinomorphic	0	0	13
Stylidiaceae	actinomorphic, zygomorphic	actinomorphic	1	0	245
<b>Austrobaileyales</b>	<b>actinomorphic</b>	<b>actinomorphic</b>	<b>0</b>	<b>0</b>	<b>100</b>
Austrobaileyaceae	actinomorphic	actinomorphic	0	0	2
Schisandraceae	actinomorphic	actinomorphic	0	0	92
Trimeniaceae	actinomorphic	actinomorphic	0	0	6
<b>Berberidopsidales</b>	<b>actinomorphic</b>	<b>actinomorphic</b>	<b>0</b>	<b>0</b>	<b>4</b>
Aextoxicaceae	actinomorphic	actinomorphic	0	0	1
Berberidopsidaceae	actinomorphic	actinomorphic	0	0	3
<b>Brassicales</b>	<b>actinomorphic, zygomorphic</b>	<b>actinomorphic</b>	<b>5-9</b>	<b>2-6</b>	<b>4785</b>
Akaniaceae	actinomorphic, zygomorphic	uncertain	0-1	0-1	2

Clade	States present	Ancestral state	Origins (min-max)	Reversals (min-max)	Species
Bataceae	actinomorphic	actinomorphic	0	0	2
Borthwickiaceae	unknown				1
Brassicaceae	zygomorphic	uncertain	0	0	3710
Capparaceae	actinomorphic, zygomorphic	uncertain	0-3	0-3	480
Caricaceae	actinomorphic	actinomorphic	0	0	34
Cleomaceae	actinomorphic, zygomorphic	zygomorphic	0	2	300
Emblingiaceae	zygomorphic	zygomorphic	0	0	1
Gyrostemonaceae	actinomorphic	actinomorphic	0	0	18
Koerberliniaceae	actinomorphic	actinomorphic	0	0	2
Limnanthaceae	actinomorphic	actinomorphic	0	0	8
Moringaceae	actinomorphic, zygomorphic	actinomorphic	1	0	12
Pentadiplandraceae	actinomorphic	actinomorphic	0	0	1
Resedaceae	zygomorphic	zygomorphic	0	0	75
Salvadoraceae	actinomorphic	actinomorphic	0	0	11
Setchellanthaceae	actinomorphic	actinomorphic	0	0	1
Stixaceae	actinomorphic	actinomorphic	0	0	20
Tovariaceae	actinomorphic	actinomorphic	0	0	2
Tropaeolaceae	zygomorphic	zygomorphic	0	0	105
<b>Bruniales</b>	<b>actinomorphic</b>	<b>actinomorphic</b>	<b>0</b>	<b>0</b>	<b>86</b>
Bruniaceae	actinomorphic	actinomorphic	0	0	81
Columelliaceae	actinomorphic	actinomorphic	0	0	5
<b>Buxales</b>	<b>actinomorphic</b>	<b>actinomorphic</b>	<b>0</b>	<b>0</b>	<b>115</b>
Buxaceae	actinomorphic	actinomorphic	0	0	115
<b>Canellales</b>	<b>actinomorphic</b>	<b>actinomorphic</b>	<b>0</b>	<b>0</b>	<b>73</b>
Canellaceae	actinomorphic	actinomorphic	0	0	13
Winteraceae	actinomorphic	actinomorphic	0	0	60
<b>Caryophyllales</b>	<b>actinomorphic, zygomorphic</b>	<b>actinomorphic</b>	<b>5</b>	<b>0</b>	<b>11453</b>
Achatocarpaceae	actinomorphic	actinomorphic	0	0	7
Aizoaceae	actinomorphic	actinomorphic	0	0	2035
Amaranthaceae	actinomorphic	actinomorphic	0	0	2050
Anacampserotaceae	unknown				32
Ancistrocladaceae	actinomorphic	actinomorphic	0	0	12
Asteropeiaceae	actinomorphic	actinomorphic	0	0	8
Barbeuiaceae	actinomorphic	actinomorphic	0	0	1
Basellaceae	actinomorphic	actinomorphic	0	0	19
Cactaceae	actinomorphic, zygomorphic	actinomorphic	2	0	1866
Caryophyllaceae	actinomorphic, zygomorphic	actinomorphic	1	0	2200
Didiereaceae	other (disymmetric)				16
Dioncophyllaceae	actinomorphic	actinomorphic	0	0	3
Droseraceae	actinomorphic	actinomorphic	0	0	115
Drosophyllaceae	actinomorphic	actinomorphic	0	0	1
Frankeniaceae	actinomorphic	actinomorphic	0	0	90

Clade	States present	Ancestral state	Origins (min-max)	Reversals (min-max)	Species
Gisekiaceae	actinomorphic	actinomorphic	0	0	5
Halophytaceae	other (disymmetric)				1
Limeaceae	actinomorphic	actinomorphic	0	0	21
Lophiocarpaceae	actinomorphic	actinomorphic	0	0	6
Microteaceae	unknown				9
Molluginaceae	actinomorphic	actinomorphic	0	0	87
Montiaceae	actinomorphic, zygomorphic	actinomorphic	1	0	225
Nepenthaceae	actinomorphic	actinomorphic	0	0	90
Nyctaginaceae	actinomorphic, zygomorphic	actinomorphic	1	0	395
Physenaceae	actinomorphic	actinomorphic	0	0	2
Phytolaccaceae	actinomorphic	actinomorphic	0	0	32
Plumbaginaceae	actinomorphic	actinomorphic	0	0	836
Polygonaceae	actinomorphic	actinomorphic	0	0	1110
Portulacaceae	actinomorphic	actinomorphic	0	0	40
Rhabdodendraceae	actinomorphic	actinomorphic	0	0	3
Rivinaceae	actinomorphic	actinomorphic	0	0	13
Sarcobataceae	actinomorphic	actinomorphic	0	0	2
Simmondsiaceae	actinomorphic	actinomorphic	0	0	1
Stegnospermataceae	actinomorphic	actinomorphic	0	0	3
Talinaceae	actinomorphic	actinomorphic	0	0	27
Tamaricaceae	actinomorphic	actinomorphic	0	0	90
<b>Celastrales</b>	<b>actinomorphic</b>	<b>actinomorphic</b>	<b>0</b>	<b>0</b>	<b>1402</b>
Celastraceae	actinomorphic	actinomorphic	0	0	1400
Lepidobotryaceae	actinomorphic	actinomorphic	0	0	2
<b>Ceratophyllales</b>	<b>NA</b>	<b>NA</b>	<b>NA</b>	<b>NA</b>	<b>6</b>
Ceratophyllaceae	NA	NA	NA	NA	6
<b>Chloranthales</b>	<b>actinomorphic</b>	<b>actinomorphic</b>	<b>0</b>	<b>0</b>	<b>75</b>
Chloranthaceae	actinomorphic	actinomorphic	0	0	75
<b>Commelinales</b>	<b>actinomorphic, zygomorphic</b>	<b>uncertain</b>	<b>0-6</b>	<b>2-3</b>	<b>816</b>
Commelinaceae	actinomorphic, zygomorphic	actinomorphic	0-5	0-5	652
Haemodoraceae	actinomorphic, zygomorphic	zygomorphic	0	1	116
Hanguanaceae	actinomorphic	actinomorphic	0	0	10
Philydraceae	zygomorphic	zygomorphic	0	0	5
Pontederiaceae	actinomorphic, zygomorphic	zygomorphic	0	1	33
<b>Cornales</b>	<b>actinomorphic</b>	<b>actinomorphic</b>	<b>0</b>	<b>0</b>	<b>564</b>
Cornaceae	actinomorphic	actinomorphic	0	0	85
Curtisiaceae	actinomorphic	actinomorphic	0	0	1
Grubbiaceae	actinomorphic	actinomorphic	0	0	3
Hydrangeaceae	actinomorphic	actinomorphic	0	0	190
Hydrostachyaceae	actinomorphic	actinomorphic	0	0	20
Loasaceae	actinomorphic	actinomorphic	0	0	265

Clade	States present	Ancestral state	Origins (min-max)	Reversals (min-max)	Species
<b>Crossosomatales</b>	<b>actinomorphic, other</b>		<b>0</b>	<b>0</b>	<b>67</b>
Aphloiaceae	unknown				1
Crossosomataceae	actinomorphic	actinomorphic	0	0	12
Geissolomataceae	actinomorphic	actinomorphic	0	0	1
Guamatelaceae	actinomorphic	actinomorphic	0	0	1
Stachyuraceae	actinomorphic	actinomorphic	0	0	5
Staphyleaceae	actinomorphic	actinomorphic	0	0	45
Strasburgeriaceae	actinomorphic	actinomorphic	0	0	2
<b>Cucurbitales</b>	<b>actinomorphic, zygomorphic</b>	<b>actinomorphic</b>	<b>1</b>	<b>0</b>	<b>2635</b>
Anisophylleaceae	actinomorphic	actinomorphic	0	0	34
Apodanthaceae	unknown		0	0	10
Begoniaceae	zygomorphic	zygomorphic	0	0	1601
Coriariaceae	actinomorphic	actinomorphic	0	0	5
Corynocarpaceae	actinomorphic	actinomorphic	0	0	6
Cucurbitaceae	actinomorphic	actinomorphic	0	0	975
Datisceae	actinomorphic	actinomorphic	0	0	2
Tetramelaceae	actinomorphic	actinomorphic	0	0	2
<b>Dilleniales</b>	<b>actinomorphic</b>	<b>actinomorphic</b>	<b>0</b>	<b>0</b>	<b>300</b>
Dilleniales	actinomorphic	actinomorphic	0	0	300
<b>Dioscoreales</b>	<b>actinomorphic, zygomorphic</b>	<b>actinomorphic</b>	<b>1</b>	<b>0</b>	<b>1073</b>
Burmanniaceae	actinomorphic	actinomorphic	0	0	95
Dioscoreaceae	actinomorphic	actinomorphic	0	0	870
Nartheciaceae	actinomorphic	actinomorphic	0	0	41
Taccaceae	actinomorphic	actinomorphic	0	0	12
Thismiaceae	zygomorphic	zygomorphic	0	0	55
<b>Dipsacales</b>	<b>actinomorphic, zygomorphic</b>	<b>actinomorphic</b>	<b>1</b>	<b>3</b>	<b>1090</b>
Adoxaceae	actinomorphic	actinomorphic	0	0	200
Caprifoliaceae	actinomorphic, zygomorphic	zygomorphic	0	3	890
<b>Ericales</b>	<b>actinomorphic, zygomorphic</b>	<b>actinomorphic</b>	<b>9-11</b>	<b>0-2</b>	<b>11636</b>
Actinidiaceae	actinomorphic	actinomorphic	0	0	355
Balsaminaceae	zygomorphic	zygomorphic	0	0	1001
Clethraceae	actinomorphic	actinomorphic	0	0	75
Cyrillaceae	actinomorphic	actinomorphic	0	0	2
Diapensiaceae	actinomorphic	actinomorphic	0	0	18
Ebenaceae	actinomorphic	actinomorphic	0	0	553
Ericaceae	actinomorphic, zygomorphic	actinomorphic	3	0	4010
Fouquieriaceae	actinomorphic, zygomorphic	actinomorphic	1	0	11
Lecythidaceae	actinomorphic, zygomorphic	actinomorphic	1-3	0-2	350
Marcgraviaceae	actinomorphic	actinomorphic	0	0	130
Mitrastemonaceae	actinomorphic	actinomorphic	0	0	2
Pentaphragmaceae	actinomorphic	actinomorphic	0	0	337

Clade	States present	Ancestral state	Origins (min-max)	Reversals (min-max)	Species
Polemoniaceae	actinomorphic, zygomorphic	actinomorphic	2	0	385
Primulaceae	actinomorphic, zygomorphic	actinomorphic	1	0	2590
Roridulaceae	actinomorphic	actinomorphic	0	0	2
Sapotaceae	actinomorphic	actinomorphic	0	0	1100
Sarraceniaceae	actinomorphic	actinomorphic	0	0	32
Sladeniaceae	actinomorphic	actinomorphic	0	0	3
Styracaceae	actinomorphic	actinomorphic	0	0	160
Symplocaceae	actinomorphic	actinomorphic	0	0	320
Tetrameristaceae	actinomorphic	actinomorphic	0	0	5
Theaceae	actinomorphic	actinomorphic	0	0	195
<b>Escalloniales</b>	<b>actinomorphic</b>	<b>actinomorphic</b>	<b>0</b>	<b>0</b>	<b>130</b>
Escalloniaceae	actinomorphic	actinomorphic	0	0	130
<b>Fabales</b>	<b>actinomorphic, zygomorphic</b>	<b>actinomorphic</b>	<b>1-3</b>	<b>2-4</b>	<b>20535</b>
Fabaceae	actinomorphic, zygomorphic	actinomorphic	0-1	2-3	19560
Polygalaceae	zygomorphic	zygomorphic	0	0	965
Quillajaceae	actinomorphic	actinomorphic	0	0	2
Surianaceae	actinomorphic	actinomorphic	0	0	8
<b>Fagales</b>	<b>actinomorphic</b>	<b>actinomorphic</b>	<b>0</b>	<b>0</b>	<b>1053</b>
Betulaceae	unknown				145
Casuarinaceae	unknown				95
Fagaceae	actinomorphic	actinomorphic	0	0	670
Juglandaceae	actinomorphic	actinomorphic	0	0	50
Myricaceae	NA	NA	NA	NA	57
Nothofagaceae	actinomorphic	actinomorphic	0	0	35
Ticodendraceae	NA	NA	NA	NA	1
<b>Garryales</b>	<b>actinomorphic</b>	<b>actinomorphic</b>	<b>0</b>	<b>0</b>	<b>18</b>
Eucommiaceae	NA	NA	NA	NA	1
Garryaceae	actinomorphic	actinomorphic	0	0	17
<b>Gentianales</b>	<b>actinomorphic, zygomorphic</b>	<b>actinomorphic</b>	<b>5-6</b>	<b>0-1</b>	<b>19811</b>
Apocynaceae	actinomorphic	actinomorphic	0	0	4555
Gelsemiaceae	actinomorphic	actinomorphic	0	0	11
Gentianaceae	actinomorphic, zygomorphic	actinomorphic	3-4	0-1	1675
Loganiaceae	actinomorphic	actinomorphic	0	0	420
Rubiaceae	actinomorphic, zygomorphic	actinomorphic	2	0	13150
<b>Geraniales</b>	<b>actinomorphic, zygomorphic</b>	<b>actinomorphic</b>	<b>4</b>	<b>0</b>	<b>831</b>
Geraniaceae	actinomorphic, zygomorphic	actinomorphic	3	0	805
Melianthaceae	zygomorphic	zygomorphic	0	0	8
Vivianiaceae	actinomorphic	actinomorphic	0	0	18
<b>Gunnerales</b>	<b>other (disymmetric)</b>				<b>42</b>
Gunneraceae	other (disymmetric)				40

Clade	States present	Ancestral state	Origins (min-max)	Reversals (min-max)	Species
Myrothamnaceae	NA	NA	NA	NA	2
<b>Huerteales</b>	<b>actinomorphic</b>		<b>0</b>	<b>0</b>	<b>24</b>
Dipentodontaceae	actinomorphic	actinomorphic	0	0	16
Gerrardinaceae	actinomorphic	actinomorphic	0	0	2
Petenaaceae	unknown				1
Tapisciaceae	actinomorphic	actinomorphic	0	0	5
<b>Lamiales</b>	<b>actinomorphic, zygomorphic</b>	<b>uncertain</b>	<b>0-4</b>	<b>10-13</b>	<b>23972</b>
Acanthaceae	zygomorphic	zygomorphic	0	0	4000
Bignoniaceae	zygomorphic	zygomorphic	0	0	800
Byblidaceae	actinomorphic	actinomorphic	0	0	6
Calceolariaceae	zygomorphic	zygomorphic	0	0	260
Carlemanniaceae	zygomorphic	zygomorphic	0	0	5
Gesneriaceae	actinomorphic, zygomorphic	zygomorphic	0	2	3460
Lamiaceae	actinomorphic, zygomorphic	zygomorphic	0	3	7173
Lentibulariaceae	zygomorphic	zygomorphic	0	0	330
Linderniaceae	zygomorphic	zygomorphic	0	0	255
Martyniaceae	zygomorphic	zygomorphic	0	0	16
Mazaceae	zygomorphic	zygomorphic	0	0	33
Oleaceae	actinomorphic	actinomorphic	0	0	615
Orobanchaceae	zygomorphic	zygomorphic	0	0	2060
Paulowniaceae	zygomorphic	zygomorphic	0	0	6
Pedaliaceae	zygomorphic	zygomorphic	0	0	70
Phrymaceae	zygomorphic	zygomorphic	0	0	188
Plantaginaceae	actinomorphic, zygomorphic	zygomorphic	0	1	1900
Plocospermataceae	zygomorphic	zygomorphic	0	0	1
Schlegeliaceae	zygomorphic	zygomorphic	0	0	28
Scrophulariaceae	actinomorphic, zygomorphic	zygomorphic	0-1	1-2	1800
Stilbaceae	zygomorphic	zygomorphic	0	0	39
Tetrachondraceae	actinomorphic	actinomorphic	0	0	3
Thomandersiaceae	zygomorphic	zygomorphic	0	0	6
Verbenaceae	actinomorphic, zygomorphic	zygomorphic	0	1	918
<b>Laurales</b>	<b>actinomorphic, zygomorphic</b>	<b>actinomorphic</b>	<b>1</b>	<b>0</b>	<b>2858</b>
Atherospermataceae	actinomorphic	actinomorphic	0	0	16
Calycanthaceae	actinomorphic	actinomorphic	0	0	11
Gomortegaceae	actinomorphic	actinomorphic	0	0	1
Hernandiaceae	actinomorphic	actinomorphic	0	0	55
Lauraceae	actinomorphic	actinomorphic	0	0	2500
Monimiaceae	actinomorphic	actinomorphic	0	0	200
Siparunaceae	actinomorphic, zygomorphic	actinomorphic	1	0	75
<b>Liliales</b>	<b>actinomorphic, zygomorphic</b>	<b>actinomorphic</b>	<b>3</b>	<b>0</b>	<b>1448</b>

Clade	States present	Ancestral state	Origins (min-max)	Reversals (min-max)	Species
Alstroemeriaceae	actinomorphic, zygomorphic	actinomorphic	1	0	170
Campynemataceae	actinomorphic	actinomorphic	0	0	4
Colchicaceae	actinomorphic	actinomorphic	0	0	245
Corsiaceae	zygomorphic	zygomorphic	0	0	30
Liliaceae	actinomorphic	actinomorphic	0	0	610
Melanthiaceae	actinomorphic, zygomorphic	actinomorphic	1	0	170
Petermanniaceae	actinomorphic	actinomorphic	0	0	1
Philesiaceae	actinomorphic	actinomorphic	0	0	2
Rhipogonaceae	actinomorphic	actinomorphic	0	0	6
Smilacaceae	actinomorphic	actinomorphic	0	0	210
<b>Magnoliales</b>	<b>actinomorphic</b>	<b>actinomorphic</b>	<b>0</b>	<b>0</b>	<b>2929</b>
Annonaceae	actinomorphic	actinomorphic	0	0	2400
Degeneriaceae	actinomorphic	actinomorphic	0	0	2
Eupomatiaceae	NA	NA	NA	NA	3
Himantandraceae	actinomorphic	actinomorphic	0	0	2
Magnoliaceae	actinomorphic	actinomorphic	0	0	227
Myristicaceae	actinomorphic	actinomorphic	0	0	475
<b>Malpighiales</b>	<b>actinomorphic, zygomorphic</b>	<b>actinomorphic</b>	<b>6-9</b>	<b>4-7</b>	<b>17852</b>
Achariaceae	actinomorphic	actinomorphic			145
Balanopaceae	NA	NA	NA	NA	9
Bonnetiaceae	actinomorphic	actinomorphic	0	0	35
Calophyllaceae	actinomorphic	actinomorphic	0	0	460
Caryocaraceae	actinomorphic	actinomorphic	0	0	27
Centroplacaceae	actinomorphic	actinomorphic	0	0	6
Chrysobalanaceae	actinomorphic, zygomorphic	actinomorphic	1	0	460
Clusiaceae	actinomorphic	actinomorphic	0	0	595
Ctenolophonaceae	actinomorphic	actinomorphic	0	0	3
Dichapetalaceae	actinomorphic, zygomorphic	uncertain	0-1	0-1	165
Elatinaceae	actinomorphic	actinomorphic	0	0	35
Erythroxylaceae	actinomorphic	actinomorphic	0	0	240
Euphorbiaceae	actinomorphic	actinomorphic	0	0	6745
Euphroniaceae	actinomorphic	actinomorphic	0	0	1
Goupiaceae	actinomorphic	actinomorphic	0	0	2
Humiriaceae	actinomorphic	actinomorphic	0	0	50
Hypericaceae	actinomorphic	actinomorphic	0	0	560
Irvingiaceae	actinomorphic	actinomorphic	0	0	10
Ixonanthaceae	actinomorphic	actinomorphic	0	0	21
Lacistemataceae	unknown				14
Linaceae	actinomorphic	actinomorphic	0	0	300
Lophopyxidaceae	actinomorphic	actinomorphic	0	0	1
Malpighiaceae	actinomorphic, zygomorphic	zygomorphic	0	4	1250
Ochnaceae	actinomorphic	actinomorphic	0	0	495

Clade	States present	Ancestral state	Origins (min-max)	Reversals (min-max)	Species
Pandaceae	actinomorphic	actinomorphic	0	0	15
Passifloraceae	actinomorphic, zygomorphic	actinomorphic	1	0	975
Peraceae	unknown				135
Phyllanthaceae	actinomorphic	actinomorphic	0	0	2330
Picrodendraceae	actinomorphic	actinomorphic	0	0	96
Podostemaceae	actinomorphic, zygomorphic	actinomorphic	1	0	270
Putranjivaceae	actinomorphic	actinomorphic	0	0	210
Rafflesiaceae	actinomorphic	actinomorphic	0	0	20
Rhizophoraceae	actinomorphic	actinomorphic	0	0	149
Salicaceae	actinomorphic	actinomorphic	0	0	1010
Trigoniaceae	zygomorphic	zygomorphic	0	0	28
Violaceae	actinomorphic, zygomorphic	uncertain	0-3	0-2	985
<b>Malvales</b>	<b>actinomorphic</b>	<b>actinomorphic</b>	<b>0</b>	<b>0</b>	<b>6093</b>
Bixaceae	actinomorphic	actinomorphic	0	0	21
Cistaceae	actinomorphic	actinomorphic	0	0	175
Cytinaceae	actinomorphic	actinomorphic	0	0	10
Dipterocarpaceae	actinomorphic	actinomorphic	0	0	680
Malvaceae	actinomorphic	actinomorphic	0	0	4225
Muntingiaceae	actinomorphic	actinomorphic	0	0	3
Neuradaceae	actinomorphic	actinomorphic	0	0	10
Sarcolaenaceae	actinomorphic	actinomorphic	0	0	60
Sphaerosepalaceae	actinomorphic	actinomorphic	0	0	18
Thymelaeaceae	actinomorphic	actinomorphic	0	0	891
<b>Myrtales</b>	<b>actinomorphic, zygomorphic</b>	<b>actinomorphic</b>	<b>6</b>	<b>0</b>	<b>11732</b>
Alzateaceae	actinomorphic	actinomorphic	0	0	2
Combretaceae	actinomorphic	actinomorphic	0	0	500
Crypteroniaceae	actinomorphic	actinomorphic	0	0	10
Lythraceae	actinomorphic, zygomorphic	actinomorphic	1	0	620
Melastomataceae	actinomorphic, zygomorphic	actinomorphic	1	0	5105
Myrtaceae	actinomorphic	actinomorphic	0	0	4620
Onagraceae	actinomorphic, zygomorphic	actinomorphic	3	0	656
Penaecaceae	actinomorphic	actinomorphic	0	0	29
Vochysiaceae	zygomorphic	zygomorphic	0	0	190
<b>Nymphaeales</b>	<b>actinomorphic</b>	<b>actinomorphic</b>	<b>0</b>	<b>0</b>	<b>74</b>
Cabombaceae	actinomorphic	actinomorphic	0	0	6
Hydatellaceae	NA	NA	NA	NA	10
Nymphaeaceae	actinomorphic	actinomorphic	0	0	58
<b>Oxalidales</b>	<b>actinomorphic</b>	<b>actinomorphic</b>	<b>0</b>	<b>0</b>	<b>1894</b>
Brunelliaceae	actinomorphic	actinomorphic	0	0	55
Cephalotaceae	actinomorphic	actinomorphic	0	0	1
Connaraceae	actinomorphic	actinomorphic	0	0	180

Clade	States present	Ancestral state	Origins (min-max)	Reversals (min-max)	Species
Cunoniaceae	actinomorphic	actinomorphic	0	0	280
Elaeocarpaceae	actinomorphic	actinomorphic	0	0	605
Huaceae	actinomorphic	actinomorphic	0	0	3
Oxalidaceae	actinomorphic	actinomorphic	0	0	770
<b>Pandanales</b>	<b>actinomorphic</b>	<b>actinomorphic</b>	<b>0</b>	<b>0</b>	<b>1427</b>
Cyclanthaceae	actinomorphic	actinomorphic	0	0	225
Pandanaceae	NA	NA	NA	NA	885
Stemonaceae	actinomorphic	actinomorphic	0	0	27
Triuridaceae	actinomorphic	actinomorphic	0	0	50
Velloziaceae	actinomorphic	actinomorphic	0	0	240
<b>Paracryphiales</b>	<b>actinomorphic</b>	<b>actinomorphic</b>	<b>0</b>	<b>0</b>	<b>36</b>
Paracryphiaceae	actinomorphic	actinomorphic	0	0	36
<b>Petrosaviales</b>	<b>actinomorphic</b>	<b>actinomorphic</b>	<b>0</b>	<b>0</b>	<b>4</b>
Petrosaviaceae	actinomorphic	actinomorphic	0	0	4
<b>Picramniales</b>	<b>actinomorphic</b>	<b>actinomorphic</b>	<b>0</b>	<b>0</b>	<b>49</b>
Picramniaceae	actinomorphic	actinomorphic	0	0	49
<b>Piperales</b>	<b>actinomorphic, zygomorphic</b>	<b>actinomorphic</b>	<b>1</b>	<b>0</b>	<b>4108</b>
Aristolochiaceae	actinomorphic, zygomorphic	actinomorphic	1	0	480
Hydnoraceae	actinomorphic	actinomorphic	0	0	7
Piperaceae	NA	NA	NA	NA	3615
Saururaceae	NA	NA	NA	NA	6
<b>Poales</b>	<b>actinomorphic, zygomorphic</b>	<b>actinomorphic</b>	<b>5</b>	<b>0</b>	<b>18637</b>
Anarthriaceae	actinomorphic	actinomorphic	0	0	11
Bromeliaceae	actinomorphic, zygomorphic	actinomorphic	3	0	3350
Centrolepidaceae	NA	NA	NA	NA	35
Cyperaceae	unknown				5680
Ecdeiocoleaceae	actinomorphic	actinomorphic	0	0	3
Eriocaulaceae	actinomorphic, zygomorphic	actinomorphic	1	0	1160
Flagellariaceae	actinomorphic	actinomorphic	0	0	4
Joinvilleaceae	actinomorphic	actinomorphic	0	0	2
Juncaceae	actinomorphic	actinomorphic	0	0	430
Mayacaceae	actinomorphic	actinomorphic	0	0	4
Poaceae	unknown				11337
Rapateaceae	actinomorphic	actinomorphic	0	0	94
Restionaceae	unknown				535
Thurniaceae	actinomorphic	actinomorphic	0	0	4
Typhaceae	unknown				25
Xyridaceae	actinomorphic, zygomorphic	actinomorphic	1	0	260
<b>Proteales</b>	<b>actinomorphic, zygomorphic, other (asymmetric)</b>	<b>actinomorphic</b>	<b>13-14</b>	<b>3-4</b>	<b>1767</b>
Nelumbonaceae	actinomorphic	actinomorphic	0	0	1

Clade	States present	Ancestral state	Origins (min-max)	Reversals (min-max)	Species
Platanaceae	other (asymmetric)				10
Proteaceae	actinomorphic, zygomorphic	actinomorphic	13-14	3-4	1756
<b>Ranunculales</b>	<b>actinomorphic, zygomorphic</b>	<b>actinomorphic</b>	<b>3</b>	<b>0</b>	<b>4537</b>
Berberidaceae	actinomorphic	actinomorphic	0	0	701
Circaeasteraceae	actinomorphic	actinomorphic			2
Eupteleaceae	NA	NA	NA	NA	2
Lardizabalaceae	actinomorphic	actinomorphic	0	0	40
Menispermaceae	actinomorphic, zygomorphic	actinomorphic	1	0	442
Papaveraceae	actinomorphic, zygomorphic	actinomorphic	1	0	825
Ranunculaceae	actinomorphic, zygomorphic	actinomorphic	1	0	2525
<b>Rosales</b>	<b>actinomorphic, zygomorphic</b>	<b>actinomorphic</b>	<b>1</b>	<b>0</b>	<b>7448</b>
Barbeyaceae	actinomorphic	actinomorphic	0	0	1
Cannabaceae	zygomorphic	zygomorphic	0	0	170
Dirachmaceae	actinomorphic	actinomorphic	0	0	2
Elaeagnaceae	actinomorphic	actinomorphic	0	0	45
Moraceae	actinomorphic	actinomorphic	0	0	1125
Rhamnaceae	actinomorphic	actinomorphic	0	0	925
Rosaceae	actinomorphic	actinomorphic	0	0	2520
Ulmaceae	unknown				35
Urticaceae	actinomorphic	actinomorphic	0	0	2625
<b>Sabiales</b>	<b>actinomorphic, zygomorphic</b>	<b>actinomorphic</b>	<b>1</b>	<b>0</b>	<b>100</b>
Sabiaceae	actinomorphic, zygomorphic	actinomorphic	1	0	100
<b>Santalales</b>	<b>actinomorphic, zygomorphic</b>	<b>actinomorphic</b>	<b>9</b>	<b>0</b>	<b>2260</b>
Aptandraceae	actinomorphic	actinomorphic	0	0	34
Balanophoraceae	actinomorphic, zygomorphic	actinomorphic	1	0	42
Coulaceae	actinomorphic	actinomorphic	0	0	3
Erythrolalaceae	actinomorphic	actinomorphic	0	0	40
Loranthaceae	actinomorphic, zygomorphic	actinomorphic	7	0	950
Misodendraceae	actinomorphic	actinomorphic	0	0	8
Octoknemaceae	actinomorphic	actinomorphic	0	0	14
Olacaceae	actinomorphic	actinomorphic	0	0	57
Opiliaceae	actinomorphic	actinomorphic	0	0	36
Santalaceae	actinomorphic	actinomorphic	0	0	990
Schoepfiaceae	actinomorphic, zygomorphic	actinomorphic	1	0	55
Strombosiaceae	unknown				18
Ximeniaceae	actinomorphic	actinomorphic	0	0	13
<b>Sapindales</b>	<b>actinomorphic, zygomorphic</b>	<b>actinomorphic</b>	<b>4</b>	<b>1</b>	<b>6172</b>
Anacardiaceae	actinomorphic	actinomorphic	0	0	873
Biebersteiniaceae	actinomorphic	actinomorphic	0	0	5

Clade	States present	Ancestral state	Origins (min-max)	Reversals (min-max)	Species
Burseraceae	actinomorphic	actinomorphic	0	0	755
Kirkiaceae	actinomorphic	actinomorphic	0	0	8
Meliaceae	actinomorphic	actinomorphic	0	0	705
Nitrariaceae	actinomorphic	actinomorphic	0	0	16
Rutaceae	actinomorphic, zygomorphic	actinomorphic	1	0	2070
Sapindaceae	actinomorphic, zygomorphic	actinomorphic	3	1	1630
Simaroubaceae	actinomorphic	actinomorphic	0	0	110
<b>Saxifragales</b>	<b>actinomorphic, zygomorphic</b>	<b>actinomorphic</b>	<b>5</b>	<b>0</b>	<b>2474</b>
Altingiaceae	NA	NA	NA	NA	13
Aphanopetalaceae	actinomorphic	actinomorphic	0	0	2
Cercidiphyllaceae	NA	NA	NA	NA	2
Crassulaceae	actinomorphic	actinomorphic	0	0	1400
Cynomoriaceae	unknown				2
Daphniphyllaceae	actinomorphic	actinomorphic	0	0	10
Grossulariaceae	actinomorphic	actinomorphic	0	0	150
Haloragaceae	actinomorphic	actinomorphic	0	0	145
Hamamelidaceae	actinomorphic, zygomorphic	actinomorphic	1	0	82
Iteaceae	actinomorphic	actinomorphic	0	0	21
Paeoniaceae	actinomorphic	actinomorphic	0	0	33
Penthoraceae	actinomorphic	actinomorphic	0	0	2
Peridiscaceae	actinomorphic	actinomorphic	0	0	11
Saxifragaceae	actinomorphic, zygomorphic	actinomorphic	4	0	600
Tetracarpaeaceae	actinomorphic	actinomorphic	0	0	1
<b>Solanales</b>	<b>actinomorphic, zygomorphic</b>	<b>actinomorphic</b>	<b>5-9</b>	<b>0-4</b>	<b>4104</b>
Convolvulaceae	actinomorphic	actinomorphic	0	0	1625
Hydroleaceae	actinomorphic	actinomorphic	0	0	12
Montiniaceae	actinomorphic	actinomorphic	0	0	5
Solanaceae	actinomorphic, zygomorphic	zygomorphic	4-9	0-4	2460
Sphenocleaceae	actinomorphic	actinomorphic	0	0	2
<b>Trochodendrales</b>	<b>actinomorphic</b>	<b>actinomorphic</b>	<b>0</b>	<b>0</b>	<b>2</b>
Trochodendraceae	actinomorphic	actinomorphic	0	0	2
<b>Vitales</b>	<b>actinomorphic</b>	<b>actinomorphic</b>	<b>0</b>	<b>0</b>	<b>850</b>
Vitaceae	actinomorphic	actinomorphic	0	0	850
<b>Zingiberales</b>	<b>zygomorphic, other</b>	<b>zygomorphic</b>	<b>0</b>	<b>0</b>	<b>1917</b>
Cannaceae	other (asymmetric)				10
Costaceae	zygomorphic	zygomorphic	0	0	110
Heliconiaceae	zygomorphic	zygomorphic	0	0	100
Lowiaceae	zygomorphic	zygomorphic	0	0	20
Marantaceae	other (asymmetric)				550
Musaceae	zygomorphic	zygomorphic	0	0	41
Strelitziaceae	zygomorphic	zygomorphic	0	0	7

Clade	States present	Ancestral state	Origins (min-max)	Reversals (min-max)	Species
Zingiberaceae	zygomorphic	zygomorphic	0	0	1075
<b>Zygophyllales</b>	<b>actinomorphic, zygomorphic</b>	<b>actinomorphic</b>	<b>2</b>	<b>0</b>	<b>303</b>
Krameriaceae	zygomorphic	zygomorphic	0	0	18
Zygophyllaceae	actinomorphic, zygomorphic	actinomorphic	1	0	285

Appendix IV.2: Parsimony, Mk1 and Mk2 for 15 key nodes

**Symmetry**

no star= 0.51-0.6 probability    \*= 61-89% probability    \*\*= 90-100% probability  
 full = full tree; part = partitioned tree; high rate= high rate equilibrium

**Difference between models**

REScoreD DATA	Parsimony	Maximum Likelihood (Mk1 model)		Maximum Likelihood (Mk2 model)	
	Both "full" and "part"	Mk1 Likelihood (full)	Mk1 Likelihood (part)	Mk2 Likelihood (full)	Mk2 Likelihood (part)
Angiospermae	actinomorphic	actinomorphic**	actinomorphic**	actinomorphic*	actinomorphic**
Mesangiospermae	actinomorphic	actinomorphic**	?	actinomorphic*	?
Magnoliidae	actinomorphic	actinomorphic**	actinomorphic**	actinomorphic*	actinomorphic**
Monocotyledoneae	actinomorphic, zygomorphic	actinomorphic**	equivocal (high rate)	actinomorphic*	actinomorphic (high rate)
Commelinidae	actinomorphic	<b>actinomorphic**</b>	equivocal (high rate)	<b>zygomorphic*</b>	actinomorphic (high rate)
Eudicotyledoneae	actinomorphic	actinomorphic**	equivocal (high rate)	actinomorphic*	actinomorphic*
Pentapetalae	actinomorphic	actinomorphic**	?	actinomorphic*	?
Superrosidae	actinomorphic	actinomorphic**	actinomorphic**	actinomorphic*	actinomorphic**
Rosidae	actinomorphic	actinomorphic**	actinomorphic**	actinomorphic*	actinomorphic**
Malvidae	actinomorphic	actinomorphic**	actinomorphic**	actinomorphic*	actinomorphic**
Fabidae	actinomorphic	actinomorphic**	actinomorphic**	actinomorphic**	actinomorphic**
Superasteridae	actinomorphic	actinomorphic**	actinomorphic**	actinomorphic*	actinomorphic*
Asteridae	actinomorphic	actinomorphic**	actinomorphic**	actinomorphic*	actinomorphic*
Campanulidae	actinomorphic	actinomorphic**	actinomorphic**	actinomorphic*	actinomorphic*
Lamiidae	actinomorphic	actinomorphic**	actinomorphic**	actinomorphic*	actinomorphic*

ORIGINAL DATA	Parsimony	Maximum Likelihood (Mk1 model)		Maximum Likelihood (Mk2 model)	
	Both "full" and "part"	Mk1 Likelihood (full)	Mk1 Likelihood (part)	Mk2 Likelihood (full)	Mk2 Likelihood (part)
Angiospermae	actinomorphic	actinomorphic**	actinomorphic**	actinomorphic*	actinomorphic**
Mesangiospermae	actinomorphic	actinomorphic**	?	actinomorphic*	?
Magnoliidae	actinomorphic	actinomorphic**	actinomorphic**	actinomorphic*	actinomorphic**
Monocotyledoneae	actinomorphic, zygomorphic	actinomorphic**	equivocal (high rate)	actinomorphic*	actinomorphic (high rate)
Commelinidae	actinomorphic	<b>actinomorphic**</b>	equivocal (high rate)	<b>zygomorphic*</b>	actinomorphic (high rate)
Eudicotyledoneae	actinomorphic	actinomorphic**	actinomorphic*	actinomorphic*	actinomorphic*
Pentapetalae	actinomorphic	actinomorphic**	?	actinomorphic**	?
Superrosidae	actinomorphic	actinomorphic**	actinomorphic**	actinomorphic**	actinomorphic**
Rosidae	actinomorphic	actinomorphic**	actinomorphic**	actinomorphic**	actinomorphic**
Malvidae	actinomorphic	actinomorphic**	actinomorphic**	actinomorphic*	actinomorphic**
Fabidae	actinomorphic	actinomorphic**	actinomorphic**	actinomorphic**	actinomorphic**
Superasteridae	actinomorphic	actinomorphic**	actinomorphic**	actinomorphic**	actinomorphic**
Asteridae	actinomorphic	actinomorphic**	actinomorphic**	actinomorphic**	actinomorphic**
Campanulidae	actinomorphic	actinomorphic**	actinomorphic**	actinomorphic*	actinomorphic*
Lamiidae	actinomorphic	actinomorphic**	actinomorphic**	actinomorphic*	actinomorphic*

Appendix IV.2: Parsimony, Mk1 and Mk2 for 15 key nodes

**Fusion** no star= 0.51-0.6 probability \* = 61-89% probability \*\* = 90-100% probability  
 full = full tree; part = partitioned tree; high rate= high rate equilibrium

**Difference between models**

RESCORED DATA	Parsimony	Maximum Likelihood (Mk1 model)		Maximum Likelihood (Mk2 model)	
	Both "full" and "part"	Mk1 Likelihood (full)	Mk1 Likelihood (part)	Mk2 Likelihood (full)	Mk2 Likelihood (part)
Angiospermae	free	free**	equivocal (high rate)	free**	free (high rate)
Mesangiospermae	free	free**	?	free**	?
Magnoliidae	free	free**	equivocal (high rate)	free**	equivocal (high rate)
Monocotyledoneae	free	free**	free	free**	free*
Commelinidae	free	free**	free	free**	free*
Eudicotyledoneae	free	free**	free**	free**	free*
Pentapetalae	free, fused	free**	?	free**	?
Superrosidae	fused	free**	equivocal (high rate)	free**	equivocal (high rate)
Rosidae	fused	free**	equivocal (high rate)	free**	equivocal (high rate)
Malvidae	fused	free*	equivocal (high rate)	free*	equivocal (high rate)
Fabidae	free	free**	equivocal (high rate)	free**	equivocal (high rate)
Superasteridae	free, fused	<b>free*</b>	<b>fused**</b>	<b>free*</b>	<b>fused*</b>
Asteridae	fused	fused*	fused**	fused	fused*
Campanulidae	fused	fused**	fused**	fused**	fused**
Lamiidae	fused	fused**	fused**	fused**	fused**

ORIGINAL DATA	Parsimony	Maximum Likelihood (Mk1 model)		Maximum Likelihood (Mk2 model)	
	Both "full" and "part"	Mk1 Likelihood (full)	Mk1 Likelihood (part)	Mk2 Likelihood (full)	Mk2 Likelihood (part)
Angiospermae	free	free**	free**	free**	free (high rate)
Mesangiospermae	free	free**	?	free**	?
Magnoliidae	free	free**	free**	free**	free (high rate)
Monocotyledoneae	free	free**	free*	free**	free*
Comelinidae	free	free**	free**	free**	free*
Eudicotyledoneae	free	free**	free**	free**	free**
Pentapetalae	free	free**	?	free**	?
Superrosidae	free	free**	free**	free**	free*
Rosidae	free	free**	free**	free**	free*
Malvidae	free	free**	free**	free**	free*
Fabidae	free	free**	free**	free**	free*
Superasteridae	free, fused	<b>free**</b>	<b>fused*</b>	free*	<b>free**</b>
Asteridae	free, fused	<b>free*</b>	<b>fused*</b>	free*	<b>free**</b>
Campanulidae	fused	fused*	fused**	<b>fused**</b>	<b>free**</b>
Lamiidae	fused	fused*	fused**	<b>fused**</b>	<b>free**</b>

Appendix IV.2: Parsimony, Mk1 and Mk2 for 15 key nodes

**Phylogeny**

no star= 0.51-0.6 probability    \* = 61-89% probability    \*\* = 90-100% probability  
 full = full tree; part = partitioned tree; high rate= high rate equilibrium

**Difference between models**

RESCORED DATA	Parsimony	Maximum Likelihood (Mk1 model)		Maximum Likelihood (Mk2 model)	
	Both "full" and "part"	Mk1 Likelihood (full)	Mk1 Likelihood (part)	Mk2 Likelihood (full)	Mk2 Likelihood (part)
Angiospermae	whorled, spiral	<b>whorled**</b>	whorled**	<b>spiral**</b>	whorled**
Mesangiospermae	whorled	<b>whorled**</b>	?	<b>spiral**</b>	?
Magnoliidae	whorled	<b>whorled**</b>	whorled**	<b>spiral**</b>	whorled**
Monocotyledoneae	whorled	<b>whorled**</b>	whorled**	<b>spiral*</b>	whorled**
Commelinidae	whorled	<b>whorled**</b>	whorled**	whorled**	whorled**
Eudicotyledoneae	whorled	<b>whorled**</b>	whorled**	<b>spiral**</b>	whorled*
Pentapetalae	whorled	<b>whorled**</b>	?	whorled**	?
Superrosidae	whorled	<b>whorled**</b>	whorled**	whorled**	whorled**
Rosidae	whorled	<b>whorled**</b>	whorled**	whorled**	whorled**
Malvidae	whorled	<b>whorled**</b>	whorled**	whorled**	whorled**
Fabidae	whorled	<b>whorled**</b>	whorled**	whorled**	whorled**
Superasteridae	whorled	<b>whorled**</b>	whorled**	whorled**	whorled**
Asteridae	whorled	<b>whorled**</b>	whorled**	whorled**	whorled**
Campanulidae	whorled	<b>whorled**</b>	whorled**	whorled**	whorled**
Lamiidae	whorled	<b>whorled**</b>	whorled**	whorled**	whorled**

ORIGINAL DATA	Parsimony	Maximum Likelihood (Mk1 model)		Maximum Likelihood (Mk2 model)	
	Both "full" and "part"	Mk1 Likelihood (full)	Mk1 Likelihood (part)	Mk2 Likelihood (full)	Mk2 Likelihood (part)
Angiospermae	whorled, spiral	<b>whorled**</b>	whorled**	<b>spiral**</b>	whorled**
Mesangiospermae	whorled	<b>whorled**</b>	?	<b>spiral**</b>	?
Magnoliidae	whorled	<b>whorled**</b>	whorled**	<b>spiral**</b>	whorled**
Monocotyledoneae	whorled	<b>whorled**</b>	whorled**	<b>spiral*</b>	whorled**
Commelinidae	whorled	<b>whorled**</b>	whorled**	whorled**	whorled**
Eudicotyledoneae	whorled	<b>whorled**</b>	whorled**	<b>spiral**</b>	whorled*
Pentapetalae	whorled	<b>whorled**</b>	?	whorled**	?
Superrosidae	whorled	<b>whorled**</b>	whorled**	whorled**	whorled**
Rosidae	whorled	<b>whorled**</b>	whorled**	whorled**	whorled**
Malvidae	whorled	<b>whorled**</b>	whorled**	whorled**	whorled**
Fabidae	whorled	<b>whorled**</b>	whorled**	whorled**	whorled**
Superasteridae	whorled	<b>whorled**</b>	whorled**	whorled**	whorled**
Asteridae	whorled	<b>whorled**</b>	whorled**	whorled**	whorled**
Campanulidae	whorled	<b>whorled**</b>	whorled**	whorled**	whorled**
Lamiidae	whorled	<b>whorled**</b>	whorled**	whorled**	whorled**

Appendix IV.2: Parsimony, Mk1 and Mk2 for 15 key nodes

**Pentamery**

no star= 0.51-0.6 probability    \*= 61-89% probability    \*\*= 90-100% probability  
 full = full tree; part = partitioned tree; high rate= high rate equilibrium

**Difference between models**

RESCORED DATA	Parsimony	Maximum Likelihood (Mk1 model)		Maximum Likelihood (Mk2 model)	
	Both "full" and "part"	Mk1 Likelihood (full)	Mk1 Likelihood (part)	Mk2 Likelihood (full)	Mk2 Likelihood (part)
Angiospermae	non-penta	non-penta**	non-penta**	non-penta**	non-penta*
Mesangiospermae	non-penta	non-penta**	?	non-penta**	?
Magnoliidae	non-penta	non-penta**	non-penta**	non-penta**	non-penta*
Monocotyledoneae	non-penta	non-penta**	non-penta**	non-penta**	non-penta**
Commelinidae	non-penta	non-penta**	non-penta**	non-penta**	non-penta**
Eudicotyledoneae	non-penta	non-penta**	non-penta**	non-penta**	non-penta**
Pentapetalae	pentamerous	pentamerous**	?	pentamerous**	?
Superrosidae	pentamerous	pentamerous**	pentamerous**	pentamerous**	pentamerous
Rosidae	pentamerous	pentamerous**	pentamerous**	pentamerous**	pentamerous
Malvidae	pentamerous	pentamerous**	pentamerous**	pentamerous**	pentamerous
Fabidae	pentamerous	pentamerous**	pentamerous**	pentamerous**	pentamerous
Superasteridae	pentamerous	pentamerous**	pentamerous**	pentamerous**	pentamerous*
Asteridae	pentamerous	pentamerous**	pentamerous**	pentamerous**	pentamerous*
Campanulidae	pentamerous	pentamerous**	pentamerous**	pentamerous**	pentamerous*
Lamiidae	pentamerous	pentamerous**	pentamerous**	pentamerous**	pentamerous*

ORIGINAL DATA	Parsimony	Maximum Likelihood (Mk1 model)		Maximum Likelihood (Mk2 model)	
	Both "full" and "part"	Mk1 Likelihood (full)	Mk1 Likelihood (part)	Mk2 Likelihood (full)	Mk2 Likelihood (part)
Angiospermae	non-penta	non-penta**	non-penta**	non-penta**	non-penta**
Mesangiospermae	non-penta	non-penta**	?	non-penta**	?
Magnoliidae	non-penta	non-penta**	non-penta**	non-penta**	non-penta**
Monocotyledoneae	non-penta	non-penta**	non-penta**	non-penta**	non-penta**
Commelinidae	non-penta	non-penta**	non-penta**	non-penta**	non-penta**
Eudicotyledoneae	non-penta	non-penta**	non-penta**	non-penta**	non-penta*
Pentapetalae	non-penta, pentamerous	pentamerous*	?	pentamerous**	?
Superrosidae	pentamerous	pentamerous**	pentamerous**	pentamerous**	non-penta
Rosidae	pentamerous	pentamerous**	pentamerous**	pentamerous**	non-penta
Malvidae	pentamerous	pentamerous**	pentamerous**	pentamerous**	non-penta
Fabidae	pentamerous	pentamerous**	pentamerous**	pentamerous**	non-penta
Superasteridae	non-penta, pentamerous	pentamerous*	pentamerous*	pentamerous*	pentamerous
Asteridae	pentamerous	pentamerous**	pentamerous**	pentamerous**	pentamerous
Campanulidae	pentamerous	pentamerous**	pentamerous**	pentamerous**	pentamerous*
Lamiidae	pentamerous	pentamerous**	pentamerous**	pentamerous**	pentamerous

Appendix IV.2: Parsimony, Mk1 and Mk2 for 15 key nodes

**Differentiation**

no star= 0.51-0.6 probability

\*= 61-89% probability

\*\*= 90-100% probability

full = full tree; part = partitioned tree; high rate= high rate equilibrium

**Difference between models**

REScoreD DATA	Parsimony	Maximum Likelihood (Mk1 model)		Maximum Likelihood (Mk2 model)	
	Both "full" and "part"	Mk1 Likelihood (full)	Mk1 Likelihood (part)	Mk2 Likelihood (full)	Mk2 Likelihood (part)
Angiospermae	undifferentiated	<b>differentiated*</b>	differentiated	<b>undifferentiated**</b>	<b>differentiated</b>
Mesangiospermae	undifferentiated	<b>differentiated**</b>	?	<b>undifferentiated**</b>	?
Magnoliidae	undifferentiated	<b>differentiated*</b>	differentiated	<b>undifferentiated**</b>	<b>differentiated</b>
Monocotyledoneae	undifferentiated	<b>differentiated**</b>	<b>undifferentiated</b>	<b>undifferentiated**</b>	undifferentiated
Commelinidae	undifferentiated, differentiated	<b>differentiated**</b>	differentiated*	<b>undifferentiated**</b>	<b>differentiated</b>
Eudicotyledoneae	differentiated	<b>differentiated**</b>	differentiated**	<b>undifferentiated*</b>	<b>differentiated**</b>
Pentapetalae	differentiated	differentiated**	?	differentiated**	?
Superrosidae	differentiated	differentiated**	differentiated**	differentiated**	differentiated**
Rosidae	differentiated	differentiated**	differentiated**	differentiated**	differentiated**
Malvidae	differentiated	differentiated**	differentiated**	differentiated**	differentiated**
Fabidae	differentiated	differentiated**	differentiated**	differentiated**	differentiated**
Superasteridae	differentiated	differentiated**	differentiated**	differentiated**	differentiated*
Asteridae	differentiated	differentiated**	differentiated**	differentiated**	differentiated**
Campanulidae	differentiated	differentiated**	differentiated**	differentiated**	differentiated**
Lamiidae	differentiated	differentiated**	differentiated**	differentiated**	differentiated**

ORIGINAL DATA	Parsimony	Maximum Likelihood (Mk1 model)		Maximum Likelihood (Mk2 model)	
	Both "full" and "part"	Mk1 Likelihood (full)	Mk1 Likelihood (part)	Mk2 Likelihood (full)	Mk2 Likelihood (part)
Angiospermae	undifferentiated	<b>differentiated*</b>	<b>undifferentiated</b>	<b>undifferentiated**</b>	undifferentiated
Mesangiospermae	undifferentiated	<b>differentiated*</b>	?	<b>undifferentiated**</b>	?
Magnoliidae	undifferentiated	<b>differentiated*</b>	equivocal	<b>undifferentiated**</b>	undifferentiated
Monocotyledoneae	undifferentiated	<b>differentiated*</b>	<b>undifferentiated</b>	<b>undifferentiated**</b>	undifferentiated
Comelinidae	undifferentiated, differentiated	<b>differentiated*</b>	differentiated	<b>undifferentiated**</b>	<b>differentiated</b>
Eudicotyledoneae	differentiated	<b>differentiated**</b>	differentiated**	<b>undifferentiated*</b>	<b>differentiated**</b>
Pentapetalae	differentiated	differentiated**	?	differentiated**	?
Superrosidae	differentiated	differentiated**	differentiated**	differentiated**	differentiated**
Rosidae	differentiated	differentiated**	differentiated**	differentiated**	differentiated**
Malvidae	differentiated	differentiated**	differentiated**	differentiated**	differentiated**
Fabidae	differentiated	differentiated**	differentiated**	differentiated**	differentiated**
Superasteridae	differentiated	differentiated**	differentiated**	differentiated**	differentiated*
Asteridae	differentiated	differentiated**	differentiated**	differentiated**	differentiated**
Campanulidae	differentiated	differentiated**	differentiated**	differentiated**	differentiated**
Lamiidae	differentiated	differentiated**	differentiated**	differentiated**	differentiated**

Appendix IV.3: Parsimony, Mk1 and Mk2 for the 54 order nodes

**Symmetry** no star= 0.51-0.6 probability \* = 61-89% probability \*\* = 90-100% probability  
 full = full tree; part = partitioned tree; high rate= high rate equilibrium

**Difference between models**

REScoreD DATA	Parsimony	Maximum Likelihood (Mk1 model)		Maximum Likelihood (Mk2 model)	
	Both "full" and "part"	Mk1 Likelihood (full)	Mk1 Likelihood (part)	Mk2 Likelihood (full)	Mk2 Likelihood (part)
Alismatales	actinomorphic	actinomorphic**	equivocal (high rate)	actinomorphic*	actinomorphic (high rate)
Apiales	actinomorphic	actinomorphic**	actinomorphic**	actinomorphic*	actinomorphic*
Aquifoliales	actinomorphic	actinomorphic**	actinomorphic**	actinomorphic*	actinomorphic*
Arecales	actinomorphic	actinomorphic**	equivocal (high rate)	actinomorphic*	actinomorphic (high rate)
Asparagales	actinomorphic	<b>actinomorphic**</b>	equivocal (high rate)	<b>zygomorphic</b>	actinomorphic (high rate)
Asterales	actinomorphic	actinomorphic**	actinomorphic**	actinomorphic	actinomorphic
Austrobaileyales	actinomorphic	actinomorphic**	actinomorphic**	actinomorphic*	actinomorphic**
Berberidopsidales	actinomorphic	actinomorphic**	actinomorphic**	actinomorphic**	actinomorphic**
Boraginales	actinomorphic	<b>actinomorphic**</b>	actinomorphic**	<b>zygomorphic</b>	actinomorphic*
Brassicales	actinomorphic	actinomorphic**	actinomorphic**	actinomorphic*	actinomorphic
Bruniales	actinomorphic	actinomorphic**	actinomorphic**	actinomorphic*	actinomorphic*
Buxales	actinomorphic	actinomorphic**	equivocal (high rate)	actinomorphic*	actinomorphic*
Canellales	actinomorphic	actinomorphic**	actinomorphic**	actinomorphic*	actinomorphic**
Caryophyllales	actinomorphic	actinomorphic**	actinomorphic**	actinomorphic**	actinomorphic**
Celastrales	actinomorphic	actinomorphic**	actinomorphic**	actinomorphic*	actinomorphic**
Commelinales	actinomorphic, zygomorphic	zygomorphic*	equivocal (high rate)	zygomorphic**	actinomorphic (high rate)
Cornales	actinomorphic	actinomorphic**	actinomorphic**	actinomorphic*	actinomorphic*
Crossosomatales	actinomorphic	actinomorphic**	actinomorphic**	actinomorphic**	actinomorphic**
Cucurbitales	actinomorphic	actinomorphic**	actinomorphic**	actinomorphic**	actinomorphic**
Dioscoreales	actinomorphic	actinomorphic**	equivocal (high rate)	actinomorphic*	actinomorphic (high rate)
Dipsacales	actinomorphic	actinomorphic*	actinomorphic*	equivocal	actinomorphic
Ericales	actinomorphic	actinomorphic**	actinomorphic**	actinomorphic**	actinomorphic*
Escalloniales	actinomorphic	actinomorphic**	actinomorphic**	actinomorphic*	actinomorphic*
Fabales	actinomorphic	actinomorphic*	actinomorphic*	actinomorphic*	actinomorphic
Fagales	actinomorphic	actinomorphic**	actinomorphic**	actinomorphic**	actinomorphic**
Garryales	actinomorphic	actinomorphic**	actinomorphic**	actinomorphic**	actinomorphic**
Gentianales	actinomorphic	actinomorphic**	actinomorphic**	actinomorphic*	actinomorphic*
Geraniales	actinomorphic	actinomorphic**	actinomorphic**	actinomorphic*	actinomorphic*
Hurteales	actinomorphic	actinomorphic**	actinomorphic**	actinomorphic**	actinomorphic**
Icacinales	actinomorphic	actinomorphic**	actinomorphic**	actinomorphic*	actinomorphic*
Lamiales	actinomorphic, zygomorphic	<b>actinomorphic*</b>	<b>actinomorphic*</b>	<b>zygomorphic*</b>	<b>zygomorphic*</b>
Laurales	actinomorphic	actinomorphic**	actinomorphic**	actinomorphic*	actinomorphic**
Liliales	actinomorphic	actinomorphic**	equivocal (high rate)	actinomorphic	actinomorphic (high rate)
Magnoliales	actinomorphic	actinomorphic**	actinomorphic**	actinomorphic*	actinomorphic**
Malpighiales	actinomorphic	actinomorphic**	actinomorphic**	actinomorphic**	actinomorphic**
Malvales	actinomorphic	actinomorphic**	actinomorphic**	actinomorphic*	actinomorphic**
Myrtales	actinomorphic	actinomorphic**	actinomorphic**	actinomorphic*	actinomorphic*
Nymphaeales	actinomorphic	actinomorphic**	actinomorphic**	actinomorphic*	actinomorphic**
Oxalidales	actinomorphic	actinomorphic**	actinomorphic**	actinomorphic**	actinomorphic**
Pandanales	actinomorphic	actinomorphic**	equivocal (high rate)	actinomorphic*	actinomorphic (high rate)
Paracryphiales	actinomorphic	actinomorphic**	actinomorphic**	actinomorphic**	actinomorphic**
Petrosaviales	actinomorphic	actinomorphic**	equivocal (high rate)	actinomorphic*	actinomorphic (high rate)
Piperales	actinomorphic	actinomorphic**	actinomorphic**	actinomorphic*	actinomorphic**
Poales	actinomorphic	<b>actinomorphic**</b>	equivocal (high rate)	<b>zygomorphic*</b>	actinomorphic (high rate)
Proteales	actinomorphic	actinomorphic**	equivocal (high rate)	actinomorphic*	actinomorphic*
Ranunculales	actinomorphic	actinomorphic**	equivocal (high rate)	actinomorphic*	actinomorphic*
Rosales	actinomorphic	actinomorphic**	actinomorphic**	actinomorphic**	actinomorphic**
Santalales	actinomorphic	actinomorphic**	actinomorphic**	actinomorphic*	actinomorphic*
Sapindales	actinomorphic	actinomorphic**	actinomorphic**	actinomorphic**	actinomorphic**
Saxifragales	actinomorphic	actinomorphic**	actinomorphic**	actinomorphic**	actinomorphic**
Solanales	actinomorphic	<b>actinomorphic**</b>	actinomorphic**	<b>zygomorphic*</b>	actinomorphic
Vitales	actinomorphic	actinomorphic**	actinomorphic**	actinomorphic*	actinomorphic**
Zingiberales	zygomorphic	zygomorphic**	equivocal (high rate)	zygomorphic**	actinomorphic (high rate)
Zygophyllales	actinomorphic	actinomorphic**	actinomorphic**	actinomorphic**	actinomorphic**

ORIGINAL DATA	Parsimony	Maximum Likelihood (Mk1 model)		Maximum Likelihood (Mk2 model)	
	Both "full" and "part"	Mk1 Likelihood (full)	Mk1 Likelihood (part)	Mk2 Likelihood (full)	Mk2 Likelihood (part)
Alismatales	actinomorphic	actinomorphic**	equivocal (high rate)	actinomorphic*	actinomorphic (high rate)
Apiales	actinomorphic	actinomorphic**	actinomorphic**	actinomorphic*	actinomorphic*
Aquifoliales	actinomorphic	actinomorphic**	actinomorphic**	actinomorphic*	actinomorphic*
Arecales	actinomorphic	actinomorphic**	equivocal (high rate)	actinomorphic*	actinomorphic (high rate)
Asparagales	actinomorphic	<b>actinomorphic**</b>	equivocal (high rate)	<b>zygomorphic</b>	actinomorphic (high rate)
Asterales	actinomorphic	actinomorphic**	actinomorphic**	actinomorphic*	actinomorphic*
Austrobaileyales	actinomorphic	actinomorphic**	actinomorphic**	actinomorphic*	actinomorphic**
Berberidopsidales	actinomorphic	actinomorphic**	actinomorphic**	actinomorphic**	actinomorphic**
Boraginales	actinomorphic	<b>actinomorphic**</b>	actinomorphic**	<b>zygomorphic</b>	actinomorphic
Brassicales	actinomorphic	actinomorphic**	actinomorphic**	actinomorphic*	actinomorphic
Bruniales	actinomorphic	actinomorphic**	actinomorphic**	actinomorphic*	actinomorphic*
Buxales	actinomorphic	actinomorphic**	actinomorphic	actinomorphic*	actinomorphic*
Canellales	actinomorphic	actinomorphic**	actinomorphic**	actinomorphic*	actinomorphic**
Caryophyllales	actinomorphic	actinomorphic**	actinomorphic**	actinomorphic**	actinomorphic**
Celastrales	actinomorphic	actinomorphic**	actinomorphic**	actinomorphic**	actinomorphic**

Commelinales	actinomorphic, zygomorphic	zygomorphic	equivocal (high rate)	zygomorphic**	actinomorphic (high rate)
Cornales	actinomorphic	actinomorphic**	actinomorphic**	actinomorphic**	actinomorphic*
Crossosomatales	actinomorphic	actinomorphic**	actinomorphic**	actinomorphic**	actinomorphic**
Cucurbitales	actinomorphic	actinomorphic**	actinomorphic**	actinomorphic**	actinomorphic**
Dioscoreales	actinomorphic	actinomorphic**	equivocal (high rate)	actinomorphic*	actinomorphic (high rate)
Dipsacales	actinomorphic	actinomorphic*	actinomorphic*	actinomorphic	actinomorphic*
Ericales	actinomorphic	actinomorphic**	actinomorphic**	actinomorphic**	actinomorphic**
Escalloniales	actinomorphic	actinomorphic**	actinomorphic**	actinomorphic*	actinomorphic*
Fabales	actinomorphic	actinomorphic*	actinomorphic*	actinomorphic*	actinomorphic
Fagales	actinomorphic	actinomorphic**	actinomorphic**	actinomorphic**	actinomorphic**
Garryales	actinomorphic	actinomorphic**	actinomorphic**	actinomorphic**	actinomorphic**
Gentianales	actinomorphic	actinomorphic**	actinomorphic**	actinomorphic*	actinomorphic*
Geraniales	actinomorphic	actinomorphic**	actinomorphic**	actinomorphic*	actinomorphic*
Hurteales	actinomorphic	actinomorphic**	actinomorphic**	actinomorphic**	actinomorphic**
Icacinales	actinomorphic	actinomorphic**	actinomorphic**	actinomorphic*	actinomorphic*
Lamiales	actinomorphic, zygomorphic	<b>actinomorphic*</b>	<b>actinomorphic*</b>	<b>zygomorphic*</b>	<b>zygomorphic*</b>
Laurales	actinomorphic	actinomorphic**	actinomorphic**	actinomorphic**	actinomorphic**
Liliales	actinomorphic	actinomorphic**	equivocal (high rate)	actinomorphic	actinomorphic (high rate)
Magnoliales	actinomorphic	actinomorphic**	actinomorphic**	actinomorphic**	actinomorphic**
Malpighiales	actinomorphic	actinomorphic**	actinomorphic**	actinomorphic**	actinomorphic**
Malvales	actinomorphic	actinomorphic**	actinomorphic**	actinomorphic**	actinomorphic**
Myrtales	actinomorphic	actinomorphic**	actinomorphic**	actinomorphic*	actinomorphic**
Nymphaeales	actinomorphic	actinomorphic**	actinomorphic**	actinomorphic*	actinomorphic**
Oxalidales	actinomorphic	actinomorphic**	actinomorphic**	actinomorphic**	actinomorphic**
Pandanales	actinomorphic	actinomorphic**	equivocal (high rate)	actinomorphic*	actinomorphic (high rate)
Paracryphiales	actinomorphic	actinomorphic**	actinomorphic**	actinomorphic*	actinomorphic*
Petrosaviales	actinomorphic	actinomorphic**	equivocal (high rate)	actinomorphic*	actinomorphic (high rate)
Piperales	actinomorphic	actinomorphic**	actinomorphic**	actinomorphic*	actinomorphic**
Poales	actinomorphic	<b>actinomorphic**</b>	equivocal (high rate)	<b>zygomorphic*</b>	actinomorphic (high rate)
Proteales	actinomorphic	actinomorphic**	actinomorphic	actinomorphic*	actinomorphic*
Ranunculales	actinomorphic	actinomorphic**	actinomorphic	actinomorphic*	actinomorphic*
Rosales	actinomorphic	actinomorphic**	actinomorphic**	actinomorphic**	actinomorphic**
Santalales	actinomorphic	actinomorphic**	actinomorphic**	actinomorphic**	actinomorphic*
Sapindales	actinomorphic	actinomorphic**	actinomorphic**	actinomorphic**	actinomorphic**
Saxifragales	actinomorphic	actinomorphic**	actinomorphic**	actinomorphic**	actinomorphic**
Solanales	actinomorphic	<b>actinomorphic**</b>	actinomorphic**	<b>zygomorphic</b>	actinomorphic
Vitales	actinomorphic	actinomorphic**	actinomorphic**	actinomorphic*	actinomorphic**
Zingiberales	zygomorphic	zygomorphic**	equivocal (high rate)	zygomorphic**	actinomorphic (high rate)
Zygophyllales	actinomorphic	actinomorphic*	actinomorphic*	actinomorphic*	actinomorphic*

## Appendix IV.3: Parsimony, Mk1 and Mk2 for the 54 order nodes

**Fusion** no star= 0.51-0.6 probability \* = 61-89% probability \*\* = 90-100% probability  
full = full tree; part = partitioned tree; high rate = high rate equilibrium

**Difference between models**

RESCORED DATA	Parsimony	Maximum Likelihood (Mk1 model)		Maximum Likelihood (Mk2 model)	
	Both "full" and "part"	Mk1 Likelihood (full)	Mk1 Likelihood (part)	Mk2 Likelihood (full)	Mk2 Likelihood (part)
Alismatales	free	free**	free*	free**	free*
Apiales	free, fused	fused**	fused**	fused**	fused*
Aquifoliales	fused	free**	fused**	fused*	fused*
Arecales	free	free*	free*	free*	free*
Asparagales	free	free**	free*	free**	free*
Asterales	fused	fused**	fused**	fused**	fused**
Austrobaileyales	free	free**	equivocal (high rate)	free**	equivocal (high rate)
Berberidopsidales	free	free**	free**	free**	free**
Boraginales	fused	fused**	fused**	fused**	fused**
Brassicales	free, fused	free*	equivocal (high rate)	free*	equivocal (high rate)
Bruniales	free, fused	fused*	fused	fused	fused*
Buxales	free	free*	free*	free*	free*
Canellales	free, fused	free**		free**	equivocal (high rate)
Caryophyllales	fused	free*	fused*	free*	fused
Celastrales	free	free**	equivocal (high rate)	free**	equivocal (high rate)
Commelinales	free, fused	free*	free*	free*	free*
Cornales	fused	fused	fused**	fused	fused*
Crossosomatales	fused	free*	equivocal (high rate)	free*	equivocal (high rate)
Cucurbitales	free	free*	equivocal (high rate)	free*	equivocal (high rate)
Dioscoreales	free	free*	free	free*	free
Dipsacales	fused	fused**	fused**	fused**	fused**
Ericales	fused	fused**	fused**	fused**	fused**
Escalloniales	fused	fused**	fused**	fused**	fused**
Fabales	free	free*	equivocal (high rate)	free*	equivocal (high rate)
Fagales	free, fused	free**	equivocal (high rate)	free**	equivocal (high rate)
Garryales	free, fused	free*	free*	free*	free*
Gentianales	fused	fused**	fused**	fused**	fused**
Geraniales	fused	free**	equivocal (high rate)	free**	equivocal (high rate)
Huerteales	free, fused	free*	equivocal (high rate)	free*	equivocal (high rate)
Icacinales	fused	fused**	fused**	fused**	fused**
Lamiales	fused	fused**	fused**	fused**	fused**
Laurales	free	free**	equivocal (high rate)	free**	equivocal (high rate)
Liliales	free	free**	free*	free**	free*
Magnoliales	free	free**	equivocal (high rate)	free**	equivocal (high rate)
Malpighiales	free	free**	equivocal (high rate)	free**	equivocal (high rate)
Malvales	free, fused	fused*	equivocal (high rate)	fused*	equivocal (high rate)
Myrtales	fused	free*	equivocal (high rate)	free	equivocal (high rate)
Nymphaeales	free	free*	equivocal (high rate)	free	equivocal (high rate)
Oxalidales	free	free*	equivocal (high rate)	free**	equivocal (high rate)
Pandanales	free	free*	free*	free*	free*
Paracryphiales	free	free*	free*	free*	free*
Petrosaviales	free	free*	free*	free*	free*
Piperales	free, fused	free*	equivocal (high rate)	free*	equivocal (high rate)
Poales	free, fused	free**	free*	free**	free
Proteales	free	free**	free**	free**	free*
Ranunculales	free	free**	free**	free**	free*
Rosales	free	free*	equivocal (high rate)	free**	equivocal (high rate)
Santalales	fused	fused*	fused**	fused*	fused**
Sapindales	free, fused	free*	equivocal (high rate)	free*	equivocal (high rate)
Saxifragales	fused	free*	equivocal (high rate)	free*	equivocal (high rate)
Solanales	fused	fused**	fused**	fused**	fused**
Vitales	fused	fused*	equivocal (high rate)	fused**	equivocal (high rate)
Zingiberales	fused	fused**	fused*	fused**	fused*
Zygophyllales	free	free**	equivocal (high rate)	free**	equivocal (high rate)

ORIGINAL DATA	Parsimony	Maximum Likelihood (Mk1 model)		Maximum Likelihood (Mk2 model)	
	Both "full" and "part"	Mk1 Likelihood (full)	Mk1 Likelihood (part)	Mk2 Likelihood (full)	Mk2 Likelihood (part)
Alismatales	free	free**	free**	free**	free*
Apiales	free	free	free	fused	free**
Aquifoliales	fused	fused*	fused**	fused**	free**
Arecales	free	free**	free*	free**	free*
Asparagales	free	free**	free**	free**	free*
Asterales	fused	fused**	fused**	fused**	fused
Austrobaileyales	free	free**	free**	free**	free (high rate)
Berberidopsidales	free	free**	free**	free**	free**
Boraginales	fused	fused**	fused**	fused**	fused**
Brassicales	free	free**	free**	free**	free*
Bruniales	free, fused	fused*	fused*	fused*	free**
Buxales	free	free**	free**	free**	free**
Canellales	free, fused	free**	free**	free**	free (high rate)
Caryophyllales	free, fused	free**	fused*	free*	free**

Celastrales	free	free**	free**	free**	free*
Commelinales	free	free**	free*	free**	free*
Cornales	free	free*	free	free*	free**
Crossosomatales	free, fused	free*	free*	free*	free*
Cucurbitales	free	free*	free*	free*	free
Dioscoreales	free	free*	free*	free*	free
Dipsacales	fused	fused**	fused**	fused*	fused
Ericales	free, fused	fused*	<b>fused**</b>	<b>fused*</b>	<b>free**</b>
Escalloniales	fused	fused**	fused**	fused**	fused**
Fabales	free	free**	free*	free*	free*
Fagales	free	free**	free**	free**	free*
Garryales	free, fused	free*	free*	free*	free**
Gentianales	fused	fused**	fused**	fused**	fused**
Geraniales	free	free**	free**	free**	free*
Hurteales	free	free**	free*	free**	free
Icacinales	fused	fused*	fused**	fused**	fused
Lamiales	fused	fused**	fused**	fused**	fused**
Laurales	free	free**	free**	free**	free (high rate)
Liliales	free	free**	free**	free**	free*
Magnoliales	free	free**	free**	free**	free (high rate)
Malpighiales	free	free**	free**	free**	free*
Malvales	free, fused	fused*	<b>fused</b>	<b>fused*</b>	<b>free</b>
Myrtales	free, fused	free**	free*	free*	free*
Nymphaeales	free	free**	free**	free**	free (high rate)
Oxalidales	free	free**	free**	free**	free*
Pandanales	free	free*	free*	free*	free*
Paracryphiales	free	free*	free*	free*	free**
Petrosaviales	free	free*	free*	free*	free*
Piperales	free, fused	free**	free**	free*	free (high rate)
Poales	free, fused	free**	free**	free**	free*
Proteales	free	free**	free**	free**	free**
Ranunculales	free	free**	free**	free**	free**
Rosales	free	free**	free**	free**	free*
Santalales	fused	fused	<b>fused**</b>	<b>fused</b>	<b>free**</b>
Sapindales	free	free**	free**	free**	free
Saxifragales	free	free**	free*	free*	free
Solanales	fused	fused**	fused**	fused**	fused**
Vitales	free, fused	fused	<b>fused</b>	equivocal	<b>free</b>
Zingiberales	free, fused	fused	fused	fused	fused
Zygophyllales	free	free**	free**	free**	free**

Appendix IV.3: Parsimony, Mk1 and Mk2 for the 54 order nodes

**Phylogeny**

no star= 0.51-0.6 probability    \* = 61-89% probability    \*\* = 90-100% probability  
 full = full tree; part = partitioned tree; high rate= high rate equilibrium

**Difference between models**

RESCORED DATA	Parsimony	Maximum Likelihood (Mk1 model)		Maximum Likelihood (Mk2 model)	
	Both "full" and "part"	Mk1 Likelihood (full)	Mk1 Likelihood (part)	Mk2 Likelihood (full)	Mk2 Likelihood (part)
Alismatales	whorled	whorled**	whorled**	whorled*	whorled**
Apiales	whorled	whorled**	whorled**	whorled**	whorled**
Aquifoliales	whorled	whorled**	whorled**	whorled**	whorled**
Arecales	whorled	whorled**	whorled**	whorled**	whorled**
Asparagales	whorled	whorled**	whorled**	whorled**	whorled**
Asterales	whorled	whorled**	whorled**	whorled**	whorled**
Austrobaileyales	spiral	<b>spiral**</b>	<b>whorled</b>	<b>spiral**</b>	<b>whorled</b>
Berberidopsidales	whorled	whorled**	whorled**	whorled*	whorled*
Boraginales	whorled	whorled**	whorled**	whorled**	whorled**
Brassicales	whorled	whorled**	whorled**	whorled**	whorled**
Bruniales	whorled	whorled**	whorled**	whorled**	whorled**
Buxales	whorled	<b>whorled**</b>	whorled*	<b>spiral**</b>	<b>whorled*</b>
Canellales	whorled	<b>whorled**</b>	whorled**	<b>spiral**</b>	<b>whorled**</b>
Caryophyllales	whorled	whorled**	whorled**	whorled**	whorled**
Celastrales	whorled	whorled**	whorled**	whorled**	whorled**
Commelinales	whorled	whorled**	whorled**	whorled**	whorled**
Cornales	whorled	whorled**	whorled**	whorled**	whorled**
Crossosomatales	whorled	whorled**	whorled**	whorled**	whorled**
Cucurbitales	whorled	whorled**	whorled**	whorled**	whorled**
Dioscoreales	whorled	whorled**	whorled**	whorled**	whorled**
Dipsacales	whorled	whorled**	whorled**	whorled**	whorled**
Ericales	whorled	whorled**	whorled**	whorled**	whorled**
Escalloniales	whorled	whorled**	whorled**	whorled**	whorled**
Fabales	whorled	whorled**	whorled**	whorled**	whorled**
Fagales	whorled	whorled**	whorled**	whorled**	whorled**
Garryales	whorled	whorled**	whorled**	whorled**	whorled**
Gentianales	whorled	whorled**	whorled**	whorled**	whorled**
Geraniales	whorled	whorled**	whorled**	whorled**	whorled**
Huerteales	whorled	whorled**	whorled**	whorled**	whorled**
Icacinales	whorled	whorled**	whorled**	whorled**	whorled**
Lamiales	whorled	whorled**	whorled**	whorled**	whorled**
Laurales	whorled	<b>whorled**</b>	whorled**	<b>spiral**</b>	<b>whorled**</b>
Liliales	whorled	whorled**	whorled**	whorled**	whorled**
Magnoliales	whorled	<b>whorled**</b>	whorled**	<b>spiral**</b>	<b>whorled**</b>
Malpighiales	whorled	whorled**	whorled**	whorled**	whorled**
Malvales	whorled	whorled**	whorled**	whorled**	whorled**
Myrtales	whorled	whorled**	whorled**	whorled**	whorled**
Nymphaeales	whorled, spiral	<b>whorled**</b>	whorled**	<b>spiral**</b>	<b>whorled**</b>
Oxalidales	whorled	whorled**	whorled**	whorled**	whorled**
Pandanales	whorled	whorled**	whorled**	whorled**	whorled**
Paracryphiales	whorled	whorled**	whorled**	whorled**	whorled**
Petrosaviales	whorled	whorled**	whorled**	whorled**	whorled**
Piperales	whorled	whorled**	whorled**	whorled	whorled**
Poales	whorled	whorled**	whorled**	whorled**	whorled*
Proteales	whorled	<b>whorled**</b>	whorled**	<b>spiral**</b>	<b>whorled*</b>
Ranunculales	whorled	<b>whorled**</b>	whorled**	<b>spiral**</b>	<b>whorled*</b>
Rosales	whorled	whorled**	whorled**	whorled**	whorled**
Santalales	whorled	whorled**	whorled**	whorled**	whorled**
Sapindales	whorled	whorled**	whorled**	whorled**	whorled**
Saxifragales	whorled	whorled**	whorled**	whorled**	whorled**
Solanales	whorled	whorled**	whorled**	whorled**	whorled**
Vitales	whorled	whorled**	whorled**	whorled**	whorled**
Zingiberales	whorled	whorled**	whorled**	whorled**	whorled**
Zygophyllales	whorled	whorled**	whorled**	whorled**	whorled**

ORIGINAL DATA	Parsimony	Maximum Likelihood (Mk1 model)		Maximum Likelihood (Mk2 model)	
	Both "full" and "part"	Mk1 Likelihood (full)	Mk1 Likelihood (part)	Mk2 Likelihood (full)	Mk2 Likelihood (part)
Alismatales	whorled	whorled**	whorled**	whorled*	whorled**
Apiales	whorled	whorled**	whorled**	whorled**	whorled**
Aquifoliales	whorled	whorled**	whorled**	whorled**	whorled**
Arecales	whorled	whorled**	whorled**	whorled**	whorled**
Asparagales	whorled	whorled**	whorled**	whorled**	whorled**
Asterales	whorled	whorled**	whorled**	whorled**	whorled**
Austrobaileyales	spiral	<b>spiral**</b>	<b>whorled</b>	<b>spiral**</b>	<b>whorled</b>
Berberidopsidales	whorled	whorled**	whorled**	whorled*	whorled*
Boraginales	whorled	whorled**	whorled**	whorled**	whorled**
Brassicales	whorled	whorled**	whorled**	whorled**	whorled**
Bruniales	whorled	whorled**	whorled**	whorled**	whorled**
Buxales	whorled	whorled**	whorled**	whorled*	whorled*
Canellales	whorled	<b>whorled**</b>	whorled**	<b>spiral**</b>	<b>whorled**</b>
Caryophyllales	whorled	whorled**	whorled**	whorled**	whorled**

Celastrales	whorled	whorled**	whorled**	whorled**	whorled**
Commelinales	whorled	whorled**	whorled**	whorled**	whorled**
Cornales	whorled	whorled**	whorled**	whorled**	whorled**
Crossosomatales	whorled	whorled**	whorled**	whorled**	whorled**
Cucurbitales	whorled	whorled**	whorled**	whorled**	whorled**
Dioscoreales	whorled	whorled**	whorled**	whorled**	whorled**
Dipsacales	whorled	whorled**	whorled**	whorled**	whorled**
Ericales	whorled	whorled**	whorled**	whorled**	whorled**
Escalloniales	whorled	whorled**	whorled**	whorled**	whorled**
Fabales	whorled	whorled**	whorled**	whorled**	whorled**
Fagales	whorled	whorled**	whorled**	whorled**	whorled**
Garryales	whorled	whorled**	whorled**	whorled**	whorled**
Gentianales	whorled	whorled**	whorled**	whorled**	whorled**
Geraniales	whorled	whorled**	whorled**	whorled**	whorled**
Hurteales	whorled	whorled**	whorled**	whorled**	whorled**
Icacinales	whorled	whorled**	whorled**	whorled**	whorled**
Lamiales	whorled	whorled**	whorled**	whorled**	whorled**
Laurales	whorled	<b>whorled**</b>	whorled**	<b>spiral**</b>	<b>whorled**</b>
Liliales	whorled	whorled**	whorled**	whorled**	whorled**
Magnoliales	whorled	<b>whorled**</b>	whorled**	<b>spiral**</b>	<b>whorled**</b>
Malpighiales	whorled	whorled**	whorled**	whorled**	whorled**
Malvales	whorled	whorled**	whorled**	whorled**	whorled**
Myrtales	whorled	whorled**	whorled**	whorled**	whorled**
Nymphaeales	whorled, spiral	<b>whorled**</b>	whorled**	<b>spiral*</b>	<b>whorled**</b>
Oxalidales	whorled	whorled**	whorled**	whorled**	whorled**
Pandanales	whorled	whorled**	whorled**	whorled**	whorled**
Paracryphiales	whorled	whorled**	whorled**	whorled**	whorled**
Petrosaviales	whorled	whorled**	whorled**	whorled**	whorled**
Piperales	whorled	whorled**	whorled**	whorled	whorled**
Poales	whorled	whorled**	whorled**	whorled**	whorled*
Proteales	whorled	<b>whorled**</b>	whorled**	<b>spiral**</b>	<b>whorled*</b>
Ranunculales	whorled	<b>whorled**</b>	whorled**	<b>spiral**</b>	<b>whorled*</b>
Rosales	whorled	whorled**	whorled**	whorled**	whorled**
Santalales	whorled	whorled**	whorled**	whorled**	whorled**
Sapindales	whorled	whorled**	whorled**	whorled**	whorled**
Saxifragales	whorled	whorled**	whorled**	whorled**	whorled**
Solanales	whorled	whorled**	whorled**	whorled**	whorled**
Vitales	whorled	whorled**	whorled**	whorled**	whorled**
Zingiberales	whorled	whorled**	whorled**	whorled**	whorled**
Zygophyllales	whorled	whorled**	whorled**	whorled**	whorled**

Appendix IV.3: Parsimony, Mk1 and Mk2 for the 54 order nodes

**Pentamery**

no star= 0.51-0.6 probability \* = 61-89% probability \*\* = 90-100% probability  
 full = full tree; part = partitioned tree; high rate= high rate equilibrium

**Difference between models**

RESCORED DATA	Parsimony	Maximum Likelihood (Mk1 model)		Maximum Likelihood (Mk2 model)	
	Both "full" and "part"	Mk1 Likelihood (full)	Mk1 Likelihood (part)	Mk2 Likelihood (full)	Mk2 Likelihood (part)
Alismatales	non-penta	non-penta**	non-penta**	non-penta**	non-penta**
Apiales	pentamerous	pentamerous**	pentamerous**	pentamerous**	pentamerous*
Aquifoliales	pentamerous	pentamerous**	pentamerous**	pentamerous**	pentamerous*
Arecales	non-penta	non-penta**	non-penta**	non-penta**	non-penta**
Asparagales	non-penta	non-penta**	non-penta**	non-penta**	non-penta**
Asterales	pentamerous	pentamerous**	pentamerous**	pentamerous**	pentamerous*
Austrobaileyales	?	?	?	?	?
Berberidopsidales	pentamerous	?	?	?	?
Boraginales	pentamerous	pentamerous**	pentamerous**	pentamerous**	pentamerous*
Brassicales	pentamerous	pentamerous**	pentamerous**	pentamerous**	pentamerous
Bruniales	pentamerous	pentamerous**	pentamerous**	pentamerous**	pentamerous*
Buxales	non-penta	non-penta**	non-penta**	non-penta*	non-penta*
Canellales	non-penta	non-penta**	non-penta**	non-penta**	non-penta*
Caryophyllales	pentamerous	pentamerous**	pentamerous**	pentamerous**	pentamerous*
Celastrales	pentamerous	pentamerous**	pentamerous**	pentamerous**	pentamerous*
Commelinales	non-penta	non-penta**	non-penta**	non-penta**	non-penta**
Cornales	pentamerous	pentamerous**	pentamerous**	pentamerous**	pentamerous*
Crossosomatales	pentamerous	pentamerous**	pentamerous*	pentamerous**	pentamerous*
Cucurbitales	pentamerous	pentamerous**	pentamerous*	pentamerous**	pentamerous*
Dioscoreales	non-penta	non-penta**	non-penta**	non-penta**	non-penta**
Dipsacales	pentamerous	pentamerous**	pentamerous**	pentamerous**	pentamerous*
Ericales	pentamerous	pentamerous**	pentamerous**	pentamerous**	pentamerous*
Escalloniales	pentamerous	pentamerous**	pentamerous**	pentamerous**	pentamerous*
Fabales	pentamerous	pentamerous**	pentamerous**	pentamerous**	pentamerous*
Fagales	non-penta	pentamerous	pentamerous*	pentamerous	pentamerous
Garryales	non-penta, pentamerous	non-penta*	non-penta*	non-penta*	non-penta*
Gentianales	pentamerous	pentamerous**	pentamerous**	pentamerous**	pentamerous*
Geraniales	pentamerous	pentamerous**	pentamerous**	pentamerous**	pentamerous*
Huerteales	pentamerous	pentamerous**	pentamerous*	pentamerous**	pentamerous*
Icacinales	pentamerous	pentamerous*	pentamerous*	pentamerous**	pentamerous*
Lamiales	pentamerous	pentamerous**	pentamerous**	pentamerous**	pentamerous*
Laurales	non-penta	non-penta**	non-penta**	non-penta**	non-penta*
Liliales	non-penta	non-penta**	non-penta**	non-penta**	non-penta**
Magnoliales	non-penta	non-penta**	non-penta**	non-penta**	non-penta*
Malpighiales	pentamerous	pentamerous**	pentamerous**	pentamerous**	pentamerous*
Malvales	pentamerous	pentamerous**	pentamerous**	pentamerous**	pentamerous*
Myrtales	pentamerous	pentamerous**	pentamerous*	pentamerous**	pentamerous
Nymphaeales	non-penta	non-penta**	non-penta**	non-penta**	non-penta*
Oxalidales	pentamerous	pentamerous**	pentamerous**	pentamerous**	pentamerous*
Pandanales	non-penta	non-penta**	non-penta**	non-penta**	non-penta**
Paracryphiales	pentamerous	pentamerous*	pentamerous*	pentamerous*	pentamerous*
Petrosaviales	non-penta	non-penta**	non-penta**	non-penta*	non-penta*
Piperales	non-penta	non-penta**	non-penta**	non-penta**	non-penta**
Poales	non-penta	non-penta**	non-penta**	non-penta**	non-penta**
Proteales	non-penta, pentamerous	non-penta*	non-penta**	non-penta*	non-penta*
Ranunculales	non-penta	non-penta**	non-penta**	non-penta**	non-penta*
Rosales	pentamerous	pentamerous**	pentamerous*	pentamerous**	pentamerous
Santalales	pentamerous	pentamerous*	pentamerous*	pentamerous**	pentamerous*
Sapindales	pentamerous	pentamerous**	pentamerous**	pentamerous**	pentamerous*
Saxifragales	pentamerous	pentamerous**	pentamerous**	pentamerous**	pentamerous*
Solanales	pentamerous	pentamerous**	pentamerous**	pentamerous**	pentamerous*
Vitales	pentamerous	pentamerous*	pentamerous*	pentamerous*	pentamerous*
Zingiberales	non-penta	non-penta**	non-penta**	non-penta**	non-penta**
Zygophyllales	pentamerous	pentamerous**	pentamerous*	pentamerous**	pentamerous*

ORIGINAL DATA	Parsimony	Maximum Likelihood (Mk1 model)		Maximum Likelihood (Mk2 model)	
	Both "full" and "part"	Mk1 Likelihood (full)	Mk1 Likelihood (part)	Mk2 Likelihood (full)	Mk2 Likelihood (part)
Alismatales	non-penta	non-penta**	non-penta**	non-penta**	non-penta**
Apiales	pentamerous	pentamerous**	pentamerous**	pentamerous**	pentamerous*
Aquifoliales	pentamerous	pentamerous**	pentamerous**	pentamerous**	pentamerous*
Arecales	non-penta	non-penta**	non-penta**	non-penta**	non-penta**
Asparagales	non-penta	non-penta**	non-penta**	non-penta**	non-penta**
Asterales	pentamerous	pentamerous**	pentamerous**	pentamerous**	pentamerous*
Austrobaileyales	?	?	?	?	?
Berberidopsidales	non-penta, pentamerous	?	?	?	?
Boraginales	pentamerous	pentamerous**	pentamerous**	pentamerous**	pentamerous*
Brassicales	pentamerous	pentamerous**	pentamerous*	pentamerous**	non-penta
Bruniales	pentamerous	pentamerous**	pentamerous**	pentamerous**	pentamerous*
Buxales	non-penta	non-penta*	non-penta**	non-penta*	non-penta**
Canellales	non-penta	non-penta**	non-penta**	non-penta**	non-penta**
Caryophyllales	pentamerous	pentamerous**	pentamerous**	pentamerous**	pentamerous*

Celastrales	pentamerous	pentamerous**	pentamerous*	pentamerous**	pentamerous
Commelinales	non-penta	non-penta**	non-penta**	non-penta**	non-penta**
Cornales	pentamerous	pentamerous*	pentamerous*	pentamerous**	pentamerous
Crossosomatales	pentamerous	pentamerous**	pentamerous*	pentamerous**	pentamerous
Cucurbitales	non-penta	<b>non-penta</b>	<b>pentamerous</b>	<b>pentamerous**</b>	<b>non-penta</b>
Dioscoreales	non-penta	non-penta**	non-penta**	non-penta**	non-penta**
Dipsacales	pentamerous	pentamerous**	pentamerous**	pentamerous**	pentamerous*
Ericales	pentamerous	pentamerous**	pentamerous**	pentamerous**	pentamerous*
Escalloniales	pentamerous	pentamerous**	pentamerous**	pentamerous**	pentamerous*
Fabales	pentamerous	pentamerous**	pentamerous**	pentamerous**	pentamerous*
Fagales	non-penta	<b>non-penta*</b>	<b>pentamerous</b>	<b>pentamerous</b>	<b>non-penta*</b>
Garryales	non-penta, pentamerous	non-penta*	non-penta*	non-penta*	non-penta*
Gentianales	pentamerous	pentamerous**	pentamerous*	pentamerous**	pentamerous
Geraniales	pentamerous	pentamerous**	<b>pentamerous**</b>	<b>pentamerous**</b>	<b>non-penta</b>
Hurteales	pentamerous	pentamerous**	pentamerous*	pentamerous**	pentamerous*
Icacinales	pentamerous	pentamerous*	pentamerous*	pentamerous**	pentamerous*
Lamiales	pentamerous	pentamerous**	pentamerous**	pentamerous**	pentamerous*
Laurales	non-penta	non-penta**	non-penta**	non-penta**	non-penta**
Liliales	non-penta	non-penta**	non-penta**	non-penta**	non-penta**
Magnoliales	non-penta	non-penta**	non-penta**	non-penta**	non-penta**
Malpighiales	pentamerous	pentamerous**	pentamerous**	pentamerous**	pentamerous*
Malvales	pentamerous	pentamerous**	pentamerous*	pentamerous**	pentamerous
Myrtales	pentamerous	pentamerous**	<b>pentamerous*</b>	<b>pentamerous**</b>	<b>non-penta</b>
Nymphaeales	non-penta	non-penta**	non-penta**	non-penta**	non-penta**
Oxalidales	pentamerous	pentamerous**	<b>pentamerous**</b>	<b>pentamerous**</b>	<b>equivocal</b>
Pandanales	non-penta	non-penta**	non-penta**	non-penta**	non-penta**
Paracryphiales	pentamerous	pentamerous*	pentamerous*	pentamerous**	pentamerous*
Petrosaviales	non-penta	non-penta**	non-penta**	non-penta*	non-penta*
Piperales	non-penta	non-penta**	non-penta**	non-penta**	non-penta**
Poales	non-penta	non-penta**	non-penta**	non-penta**	non-penta**
Proteales	non-penta	non-penta**	non-penta**	non-penta*	non-penta*
Ranunculales	non-penta	non-penta**	non-penta**	non-penta**	non-penta*
Rosales	non-penta	pentamerous*	<b>pentamerous*</b>	<b>pentamerous**</b>	<b>non-penta*</b>
Santalales	non-penta, pentamerous	<b>non-penta*</b>	<b>pentamerous</b>	<b>pentamerous**</b>	pentamerous
Sapindales	pentamerous	pentamerous**	<b>pentamerous*</b>	<b>pentamerous**</b>	<b>non-penta*</b>
Saxifragales	pentamerous	pentamerous*	<b>pentamerous*</b>	<b>pentamerous**</b>	<b>non-penta</b>
Solanales	pentamerous	pentamerous**	pentamerous**	pentamerous**	pentamerous*
Vitales	pentamerous	pentamerous*	pentamerous	pentamerous*	pentamerous
Zingiberales	non-penta	non-penta**	non-penta**	non-penta**	non-penta**
Zygophyllales	pentamerous	pentamerous**	pentamerous*	pentamerous**	pentamerous*

Appendix IV.3: Parsimony, Mk1 and Mk2 for the 54 order nodes.

**Differentiation** no star= 0.51-0.6 probability \* = 61-89% probability \*\* = 90-100% probability  
 full = full tree; part = partitioned tree; high rate= high rate equilibrium

**Difference between models**

REScoreD DATA	Parsimony	Maximum Likelihood (Mk1 model)		Maximum Likelihood (Mk2 model)	
	Both "full" and "part"	Mk1 Likelihood (full)	Mk1 Likelihood (part)	Mk2 Likelihood (full)	Mk2 Likelihood (part)
Alismatales	undifferentiated	<b>differentiated*</b>	<b>undifferentiated</b>	<b>undifferentiated**</b>	undifferentiated
Apiales	differentiated	differentiated**	differentiated**	differentiated**	differentiated**
Aquifoliales	differentiated	differentiated**	differentiated**	differentiated**	differentiated**
Arecales	undifferentiated, differentiated	<b>differentiated*</b>	differentiated	<b>undifferentiated*</b>	<b>differentiated</b>
Asparagales	undifferentiated, differentiated	<b>differentiated*</b>	equivocal	<b>undifferentiated**</b>	undifferentiated
Asterales	differentiated	differentiated**	differentiated**	differentiated**	differentiated**
Austrobaileyales	undifferentiated, differentiated	<b>differentiated*</b>	<b>equivocal</b>	<b>undifferentiated**</b>	<b>equivocal</b>
Berberidopsidales	differentiated	differentiated*	differentiated*	differentiated*	differentiated
Boraginales	differentiated	differentiated**	differentiated**	differentiated**	differentiated**
Brassicales	differentiated	differentiated**	differentiated**	differentiated**	differentiated**
Bruniales	differentiated	differentiated**	differentiated**	differentiated**	differentiated**
Buxales	differentiated	differentiated**	differentiated**	differentiated*	differentiated**
Canellales	differentiated	<b>differentiated**</b>	differentiated	<b>undifferentiated**</b>	<b>differentiated</b>
Caryophyllales	differentiated	differentiated**	<b>differentiated**</b>	<b>differentiated*</b>	<b>undifferentiated</b>
Celastrales	differentiated	differentiated**	differentiated**	differentiated**	differentiated**
Commelinales	differentiated	differentiated**	differentiated**	differentiated	differentiated*
Cornales	differentiated	differentiated**	differentiated**	differentiated**	differentiated**
Crossosomatales	differentiated	differentiated**	differentiated**	differentiated**	differentiated**
Cucurbitales	differentiated	<b>differentiated**</b>	<b>differentiated**</b>	<b>undifferentiated*</b>	<b>undifferentiated</b>
Dioscoreales	undifferentiated	undifferentiated	undifferentiated	undifferentiated**	undifferentiated
Dipsacales	differentiated	differentiated**	differentiated**	differentiated**	differentiated**
Ericales	differentiated	differentiated**	differentiated**	differentiated**	differentiated**
Escalloniales	differentiated	differentiated**	differentiated**	differentiated**	differentiated**
Fabales	differentiated	differentiated**	differentiated**	differentiated*	differentiated*
Fagales	undifferentiated	undifferentiated*	undifferentiated*	undifferentiated**	undifferentiated*
Garryales	differentiated	differentiated**	differentiated**	differentiated*	differentiated*
Gentianales	differentiated	differentiated**	differentiated**	differentiated**	differentiated**
Geraniales	differentiated	differentiated**	differentiated**	differentiated**	differentiated**
Hurteales	differentiated	<b>differentiated*</b>	differentiated*	<b>equivocal</b>	differentiated
Icacinales	differentiated	differentiated**	differentiated**	differentiated**	differentiated**
Lamiales	differentiated	differentiated**	differentiated**	differentiated**	differentiated**
Laurales	undifferentiated	<b>differentiated*</b>	differentiated	<b>undifferentiated**</b>	<b>equivocal</b>
Liliales	undifferentiated, differentiated	<b>differentiated*</b>	differentiated*	<b>undifferentiated**</b>	<b>differentiated</b>
Magnoliales	undifferentiated	<b>differentiated**</b>	differentiated*	<b>undifferentiated*</b>	<b>differentiated</b>
Malpighiales	differentiated	differentiated**	differentiated**	differentiated**	differentiated**
Malvales	differentiated	differentiated**	differentiated**	differentiated**	differentiated**
Myrtales	differentiated	differentiated**	differentiated**	differentiated**	differentiated**
Nymphaeales	undifferentiated	<b>differentiated*</b>	differentiated	<b>undifferentiated**</b>	<b>differentiated</b>
Oxalidales	differentiated	differentiated**	differentiated**	differentiated**	differentiated**
Pandanales	undifferentiated	undifferentiated**	undifferentiated*	undifferentiated**	undifferentiated*
Paracryphiales	differentiated	differentiated*	differentiated**	differentiated*	differentiated*
Petrosaviales	differentiated	<b>differentiated*</b>	differentiated	<b>undifferentiated</b>	<b>differentiated</b>
Piperales	undifferentiated	<b>differentiated</b>	undifferentiated	<b>undifferentiated**</b>	undifferentiated
Poales	differentiated	<b>differentiated**</b>	differentiated*	<b>undifferentiated**</b>	<b>differentiated*</b>
Proteales	differentiated	<b>differentiated**</b>	differentiated**	<b>undifferentiated**</b>	<b>differentiated**</b>
Ranunculales	differentiated	<b>differentiated**</b>	differentiated**	<b>undifferentiated*</b>	<b>differentiated**</b>
Rosales	differentiated	<b>differentiated*</b>	<b>differentiated*</b>	<b>undifferentiated*</b>	<b>undifferentiated</b>
Santalales	differentiated	differentiated**	differentiated**	differentiated**	differentiated*
Sapindales	differentiated	differentiated**	differentiated**	differentiated**	differentiated**
Saxifragales	differentiated	differentiated**	differentiated**	differentiated**	differentiated**
Solanales	differentiated	differentiated**	differentiated**	differentiated**	differentiated**
Vitales	differentiated	differentiated**	differentiated**	differentiated**	differentiated**
Zingiberales	differentiated	differentiated**	differentiated*	differentiated	differentiated*
Zygophyllales	differentiated	differentiated**	differentiated**	differentiated**	differentiated**

ORIGINAL DATA	Parsimony	Maximum Likelihood (Mk1 model)		Maximum Likelihood (Mk2 model)	
	Both "full" and "part"	Mk1 Likelihood (full)	Mk1 Likelihood (part)	Mk2 Likelihood (full)	Mk2 Likelihood (part)
Alismatales	undifferentiated	<b>differentiated*</b>	<b>undifferentiated</b>	<b>undifferentiated**</b>	undifferentiated
Apiales	differentiated	differentiated**	differentiated**	differentiated**	differentiated**
Aquifoliales	differentiated	differentiated**	differentiated**	differentiated**	differentiated**
Arecales	undifferentiated, differentiated	<b>differentiated*</b>	differentiated	<b>undifferentiated*</b>	<b>differentiated</b>
Asparagales	undifferentiated, differentiated	<b>differentiated*</b>	<b>undifferentiated</b>	<b>undifferentiated**</b>	undifferentiated
Asterales	differentiated	differentiated**	differentiated**	differentiated**	differentiated**
Austrobaileyales	undifferentiated	<b>differentiated*</b>	<b>undifferentiated</b>	<b>undifferentiated**</b>	undifferentiated
Berberidopsidales	differentiated	differentiated*	differentiated*	differentiated*	differentiated
Boraginales	differentiated	differentiated**	differentiated**	differentiated**	differentiated**
Brassicales	differentiated	differentiated**	differentiated**	differentiated**	differentiated**
Bruniales	differentiated	differentiated**	differentiated**	differentiated**	differentiated**
Buxales	differentiated	differentiated**	differentiated**	differentiated*	differentiated**
Canellales	differentiated	<b>differentiated*</b>	differentiated	<b>undifferentiated**</b>	<b>differentiated</b>
Caryophyllales	differentiated	differentiated**	differentiated**	differentiated*	differentiated
Celastrales	differentiated	differentiated**	differentiated**	differentiated**	differentiated**

Commelinales	differentiated	differentiated**	differentiated**	differentiated*	differentiated*
Cornales	differentiated	differentiated**	differentiated**	differentiated**	differentiated**
Crossosomatales	differentiated	differentiated**	differentiated**	differentiated**	differentiated**
Cucurbitales	differentiated	<b>differentiated**</b>	<b>differentiated**</b>	<b>undifferentiated</b>	<b>undifferentiated</b>
Dioscoreales	undifferentiated	undifferentiated	undifferentiated	undifferentiated**	undifferentiated
Dipsacales	differentiated	differentiated**	differentiated**	differentiated**	differentiated**
Ericales	differentiated	differentiated**	differentiated**	differentiated**	differentiated**
Escalloniales	differentiated	differentiated**	differentiated**	differentiated**	differentiated**
Fabales	differentiated	differentiated**	differentiated**	differentiated**	differentiated**
Fagales	undifferentiated	undifferentiated*	undifferentiated*	undifferentiated**	undifferentiated*
Garryales	differentiated	differentiated**	differentiated**	differentiated*	differentiated*
Gentianales	differentiated	differentiated**	differentiated**	differentiated**	differentiated**
Geraniales	differentiated	differentiated**	differentiated**	differentiated**	differentiated**
Hurteales	differentiated	<b>differentiated*</b>	differentiated*	<b>equivocal</b>	differentiated
Icacinales	differentiated	differentiated**	differentiated**	differentiated**	differentiated**
Lamiales	differentiated	differentiated**	differentiated**	differentiated**	differentiated**
Laurales	undifferentiated	<b>differentiated*</b>	<b>undifferentiated</b>	<b>undifferentiated**</b>	undifferentiated
Liliales	undifferentiated, differentiated	<b>differentiated*</b>	differentiated	<b>undifferentiated**</b>	<b>differentiated</b>
Magnoliales	undifferentiated	<b>differentiated*</b>	differentiated	<b>undifferentiated*</b>	<b>undifferentiated*</b>
Malpighiales	differentiated	differentiated**	differentiated**	differentiated**	differentiated**
Malvales	differentiated	differentiated**	differentiated**	differentiated**	differentiated**
Myrtales	differentiated	differentiated**	differentiated**	differentiated**	differentiated**
Nymphaeales	undifferentiated	<b>differentiated*</b>	<b>equivocal</b>	<b>undifferentiated**</b>	<b>equivocal</b>
Oxalidales	differentiated	differentiated**	differentiated**	differentiated**	differentiated**
Pandanales	undifferentiated	undifferentiated**	undifferentiated*	undifferentiated**	undifferentiated*
Paracryphiales	differentiated	differentiated*	differentiated**	differentiated*	differentiated*
Petrosaviales	differentiated	<b>differentiated*</b>	differentiated	<b>undifferentiated*</b>	<b>differentiated</b>
Piperales	undifferentiated	undifferentiated	undifferentiated	undifferentiated**	undifferentiated
Poales	differentiated	<b>differentiated*</b>	differentiated*	<b>undifferentiated**</b>	<b>differentiated</b>
Proteales	differentiated	<b>differentiated**</b>	differentiated**	<b>undifferentiated</b>	<b>differentiated**</b>
Ranunculales	differentiated	<b>differentiated**</b>	differentiated**	<b>undifferentiated*</b>	<b>differentiated**</b>
Rosales	differentiated	<b>differentiated*</b>	<b>differentiated*</b>	<b>undifferentiated*</b>	<b>undifferentiated*</b>
Santalales	differentiated	differentiated**	differentiated**	differentiated**	differentiated*
Sapindales	differentiated	differentiated**	differentiated**	differentiated**	differentiated**
Saxifragales	differentiated	differentiated**	differentiated**	differentiated**	differentiated**
Solanales	differentiated	differentiated**	differentiated**	differentiated**	differentiated**
Vitales	differentiated	differentiated**	differentiated**	differentiated**	differentiated**
Zingiberales	differentiated	differentiated**	differentiated*	differentiated*	differentiated*
Zygophyllales	differentiated	differentiated**	differentiated**	differentiated**	differentiated**

Appendix IV.4: Parsimony and Mk1 with imposed rates for 15 key nodes

**Symmetry**                      **Confirmed state change**                      *Suspected state change*  
 q= transition rate                      high rate= high transition rate equilibrium

RESCORED DATA	Parsimony	State for q=0.0001	State probability	State for q=0.001	State probability	State for q=0.01	State probability	State for q=0.1	State probability
Angiospermae	actinomorphic	actinomorphic		1 actinomorphic		1 actinomorphic	0.98	equivocal (high rate)	0.5
Mesangiospermae	actinomorphic	actinomorphic		1 actinomorphic		1 actinomorphic		1 equivocal (high rate)	0.5
Magnoliidae	actinomorphic	actinomorphic		1 actinomorphic		1 actinomorphic		1 equivocal (high rate)	0.5
Monocotyledoneae	actinomorphic	actinomorphic		1 actinomorphic		1 actinomorphic		1 equivocal (high rate)	0.5
Commelinidae	actinomorphic	actinomorphic		1 actinomorphic		1 actinomorphic	0.98	equivocal (high rate)	0.5
Eudicotyledoneae	actinomorphic	actinomorphic		1 actinomorphic		1 actinomorphic		1 equivocal (high rate)	0.5
Pentapetalae	actinomorphic	actinomorphic		1 actinomorphic		1 actinomorphic		1 equivocal (high rate)	0.5
Superrosidae	actinomorphic	actinomorphic		1 actinomorphic		1 actinomorphic		1 equivocal (high rate)	0.5
Rosidae	actinomorphic	actinomorphic		1 actinomorphic		1 actinomorphic		1 equivocal (high rate)	0.5
Malvidae	actinomorphic	actinomorphic		1 actinomorphic		1 actinomorphic		1 equivocal (high rate)	0.5
Fabidae	actinomorphic	actinomorphic		1 actinomorphic		1 actinomorphic		1 equivocal (high rate)	0.5
Superasteridae	actinomorphic	actinomorphic		1 actinomorphic		1 actinomorphic		1 equivocal (high rate)	0.5
Asteridae	actinomorphic	actinomorphic		1 actinomorphic		1 actinomorphic		1 equivocal (high rate)	0.5
Campanulidae	actinomorphic	actinomorphic		1 actinomorphic		1 actinomorphic		1 equivocal (high rate)	0.5
Lamiidae	actinomorphic	actinomorphic		1 actinomorphic		1 actinomorphic		1 equivocal (high rate)	0.5

ORIGINAL DATA	Parsimony	State for q=0.0001	State probability	State for q=0.001	State probability	State for q=0.01	State probability	State for q=0.1	State probability
Angiospermae	actinomorphic	actinomorphic		1 actinomorphic		1 actinomorphic	0.98	equivocal (high rate)	0.5
Mesangiospermae	actinomorphic	actinomorphic		1 actinomorphic		1 actinomorphic		1 equivocal (high rate)	0.5
Magnoliidae	actinomorphic	actinomorphic		1 actinomorphic		1 actinomorphic		1 equivocal (high rate)	0.5
Monocotyledoneae	actinomorphic	actinomorphic		1 actinomorphic		1 actinomorphic		1 equivocal (high rate)	0.5
Commelinidae	actinomorphic	actinomorphic		1 actinomorphic		1 actinomorphic	0.98	equivocal (high rate)	0.5
Eudicotyledoneae	actinomorphic	actinomorphic		1 actinomorphic		1 actinomorphic		1 equivocal (high rate)	0.5
Pentapetalae	actinomorphic	actinomorphic		1 actinomorphic		1 actinomorphic		1 equivocal (high rate)	0.5
Superrosidae	actinomorphic	actinomorphic		1 actinomorphic		1 actinomorphic		1 equivocal (high rate)	0.5
Rosidae	actinomorphic	actinomorphic		1 actinomorphic		1 actinomorphic		1 equivocal (high rate)	0.5
Malvidae	actinomorphic	actinomorphic		1 actinomorphic		1 actinomorphic		1 equivocal (high rate)	0.5
Fabidae	actinomorphic	actinomorphic		1 actinomorphic		1 actinomorphic		1 equivocal (high rate)	0.5
Superasteridae	actinomorphic	actinomorphic		1 actinomorphic		1 actinomorphic		1 equivocal (high rate)	0.5
Asteridae	actinomorphic	actinomorphic		1 actinomorphic		1 actinomorphic		1 equivocal (high rate)	0.5
Campanulidae	actinomorphic	actinomorphic		1 actinomorphic		1 actinomorphic		1 equivocal (high rate)	0.5
Lamiidae	actinomorphic	actinomorphic		1 actinomorphic		1 actinomorphic		1 equivocal (high rate)	0.5

Appendix IV.4: Parsimony and Mk1 with imposed rates for 15 key nodes

**Fusion**                      **Confirmed state change**                      *Suspected state change*  
 q= transition rate                      high rate= high transition rate equilibrium

RESCORED DATA	Parsimony	State for q=0.0001	State probability	State for q=0.001	State probability	State for q=0.01	State probability	State for q=0.1	State probability
Angiospermae	free	free		1 free		1 free	0.98	equivocal (high rate)	0.5
Mesangiospermae	free	free		1 free		1 free		1 equivocal (high rate)	0.5
Magnoliidae	free	free		1 free		1 free	0.99	equivocal (high rate)	0.5
Monocotyledoneae	free	free		1 free		1 free	0.99	equivocal (high rate)	0.5
Commelinidae	free	free		1 free		1 free	0.95	equivocal (high rate)	0.5
Eudicotyledoneae	free	free		1 free		1 free	0.99	equivocal (high rate)	0.5
Pentapetalae	free, fused	free	0.73	free	0.84	free	0.92	equivocal (high rate)	0.5
Superrosidae	fused	free	0.73	free	0.84	free	0.92	equivocal (high rate)	0.5
Rosidae	fused	free	0.73	free	0.85	free	0.93	equivocal (high rate)	0.5
Malvidae	fused	free	0.86	free	0.97	free	0.79	equivocal (high rate)	0.5
Fabidae	free	free		1 free		1 free	0.97	equivocal (high rate)	0.5
Superasteridae	free, fused	<b>fused</b>	<b>0.86</b>	<b>fused</b>	<b>0.77</b>	<b>free</b>	<b>0.7</b>	equivocal (high rate)	0.5
Asteridae	fused	fused		1 fused	0.95	fused	0.65	equivocal (high rate)	0.5
Campanulidae	fused	fused		1 fused		1 fused	0.97	equivocal (high rate)	0.5
Lamiidae	fused	fused		1 fused		1 fused	0.95	equivocal (high rate)	0.5

ORIGINAL DATA	Parsimony	State for q=0.0001	State probability	State for q=0.001	State probability	State for q=0.01	State probability	State for q=0.1	State probability
Angiospermae	free	free		1 free		1 free	0.98	equivocal (high rate)	0.5
Mesangiospermae	free	free		1 free		1 free		1 equivocal (high rate)	0.5
Magnoliidae	free	free		1 free		1 free		1 equivocal (high rate)	0.5
Monocotyledoneae	free	free		1 free		1 free		1 equivocal (high rate)	0.5
Commelinidae	free	free		1 free		1 free	0.98	equivocal (high rate)	0.5
Eudicotyledoneae	free	free		1 free		1 free		1 equivocal (high rate)	0.5
Pentapetalae	free	free		1 free		1 free		1 equivocal (high rate)	0.5
Superrosidae	free	free		1 free		1 free		1 equivocal (high rate)	0.5
Rosidae	free	free		1 free		1 free		1 equivocal (high rate)	0.5
Malvidae	free	free		1 free		1 free	0.97	equivocal (high rate)	0.5
Fabidae	free	free		1 free		1 free		1 equivocal (high rate)	0.5
Superasteridae	free, fused	free	0.98	free	0.98	free	0.93	equivocal (high rate)	0.5
Asteridae	free, fused	free	0.97	free	0.96	free	0.75	equivocal (high rate)	0.5
Campanulidae	fused	fused		0 fused		0 fused	0.77	equivocal (high rate)	0.5
Lamiidae	fused	fused		0 fused		0 fused	0.76	equivocal (high rate)	0.5

Appendix IV.4: Parsimony and Mk1 with imposed rates for 15 key nodes

**Phyllotaxy**      **Confirmed state change**      *Suspected state change*  
 q= transition rate      high rate= high transition rate equilibrium

RESCORED DATA	Parsimony	State for q=0.0001	State probability	State for q=0.001	State probability	State for q=0.01	State probability	State for q=0.1	State probability
Angiospermae	whorled, spiral	whorled	0.98	whorled	0.98	whorled	0.96	equivocal (high rate)	0.5
Mesangiospermae	whorled	whorled	1	whorled	1	whorled	1	equivocal (high rate)	0.5
Magnoliidae	whorled	whorled	1	whorled	1	whorled	1	equivocal (high rate)	0.5
Monocotyledoneae	whorled	whorled	1	whorled	1	whorled	1	equivocal (high rate)	0.5
Commelinidae	whorled	whorled	1	whorled	1	whorled	1	equivocal (high rate)	0.5
Eudicotyledoneae	whorled	whorled	1	whorled	1	whorled	1	equivocal (high rate)	0.5
Pentapetalae	whorled	whorled	1	whorled	1	whorled	1	equivocal (high rate)	0.5
Superrosidae	whorled	whorled	1	whorled	1	whorled	1	equivocal (high rate)	0.5
Rosidae	whorled	whorled	1	whorled	1	whorled	1	equivocal (high rate)	0.5
Malvidae	whorled	whorled	1	whorled	1	whorled	1	equivocal (high rate)	0.5
Fabidae	whorled	whorled	1	whorled	1	whorled	1	equivocal (high rate)	0.5
Superasteridae	whorled	whorled	1	whorled	1	whorled	1	equivocal (high rate)	0.5
Asteridae	whorled	whorled	1	whorled	1	whorled	1	equivocal (high rate)	0.5
Campanulidae	whorled	whorled	1	whorled	1	whorled	1	equivocal (high rate)	0.5
Lamiidae	whorled	whorled	1	whorled	1	whorled	1	equivocal (high rate)	0.5

ORIGINAL DATA	Parsimony	State for q=0.0001	State probability	State for q=0.001	State probability	State for q=0.01	State probability	State for q=0.1	State probability
Angiospermae	whorled, spiral	whorled	0.98	whorled	0.98	whorled	0.96	equivocal (high rate)	0.5
Mesangiospermae	whorled	whorled	1	whorled	1	whorled	1	equivocal (high rate)	0.5
Magnoliidae	whorled	whorled	1	whorled	1	whorled	1	equivocal (high rate)	0.5
Monocotyledoneae	whorled	whorled	1	whorled	1	whorled	1	equivocal (high rate)	0.5
Commelinidae	whorled	whorled	1	whorled	1	whorled	1	equivocal (high rate)	0.5
Eudicotyledoneae	whorled	whorled	1	whorled	1	whorled	1	equivocal (high rate)	0.5
Pentapetalae	whorled	whorled	1	whorled	1	whorled	1	equivocal (high rate)	0.5
Superrosidae	whorled	whorled	1	whorled	1	whorled	1	equivocal (high rate)	0.5
Rosidae	whorled	whorled	1	whorled	1	whorled	1	equivocal (high rate)	0.5
Malvidae	whorled	whorled	1	whorled	1	whorled	1	equivocal (high rate)	0.5
Fabidae	whorled	whorled	1	whorled	1	whorled	1	equivocal (high rate)	0.5
Superasteridae	whorled	whorled	1	whorled	1	whorled	1	equivocal (high rate)	0.5
Asteridae	whorled	whorled	1	whorled	1	whorled	1	equivocal (high rate)	0.5
Campanulidae	whorled	whorled	1	whorled	1	whorled	1	equivocal (high rate)	0.5
Lamiidae	whorled	whorled	1	whorled	1	whorled	1	equivocal (high rate)	0.5

Appendix IV.4: Parsimony and Mk1 with imposed rates for 15 key nodes

**Pentamery**      **Confirmed state change**      *Suspected state change*  
 q= transition rate      high rate= high transition rate equilibrium

RESCORED DATA	Parsimony	State for q=0.0001	State probability	State for q=0.001	State probability	State for q=0.01	State probability	State for q=0.1	State probability
Angiospermae	non-penta	non-penta		1 non-penta		1 non-penta	0.98	equivocal (high rate)	0.5
Mesangiospermae	non-penta	non-penta		1 non-penta		1 non-penta	0.99	equivocal (high rate)	0.5
Magnoliidae	non-penta	non-penta		1 non-penta		1 non-penta	0.99	equivocal (high rate)	0.5
Monocotyledoneae	non-penta	non-penta		1 non-penta		1 non-penta	0.99	equivocal (high rate)	0.5
Commelinidae	non-penta	non-penta		1 non-penta		1 non-penta		1 equivocal (high rate)	0.5
Eudicotyledoneae	non-penta	non-penta		1 non-penta		1 non-penta	0.94	equivocal (high rate)	0.5
Pentapetalae	pentamerous	pentamerous		1 pentamerous		1 pentamerous	0.98	equivocal (high rate)	0.5
Superrosidae	pentamerous	pentamerous		1 pentamerous		1 pentamerous	0.99	equivocal (high rate)	0.5
Rosidae	pentamerous	pentamerous		1 pentamerous		1 pentamerous	0.99	equivocal (high rate)	0.5
Malvidae	pentamerous	pentamerous		1 pentamerous		1 pentamerous		1 equivocal (high rate)	0.5
Fabidae	pentamerous	pentamerous		1 pentamerous		1 pentamerous		1 equivocal (high rate)	0.5
Superasteridae	pentamerous	pentamerous		1 pentamerous		1 pentamerous	0.99	equivocal (high rate)	0.5
Asteridae	pentamerous	pentamerous		1 pentamerous		1 pentamerous		1 equivocal (high rate)	0.5
Campanulidae	pentamerous	pentamerous		1 pentamerous		1 pentamerous		1 equivocal (high rate)	0.5
Lamiidae	pentamerous	pentamerous		1 pentamerous		1 pentamerous	0.98	equivocal (high rate)	0.5

ORIGINAL DATA	Parsimony	State for q=0.0001	State probability	State for q=0.001	State probability	State for q=0.01	State probability	State for q=0.1	State probability
Angiospermae	non-penta	non-penta		1 non-penta		1 non-penta	0.98	equivocal (high rate)	0.5
Mesangiospermae	non-penta	non-penta		1 non-penta		1 non-penta		1 equivocal (high rate)	0.5
Magnoliidae	non-penta	non-penta		1 non-penta		1 non-penta		1 equivocal (high rate)	0.5
Monocotyledoneae	non-penta	non-penta		1 non-penta		1 non-penta		1 equivocal (high rate)	0.5
Commelinidae	non-penta	non-penta		1 non-penta		1 non-penta		1 equivocal (high rate)	0.5
Eudicotyledoneae	non-penta	non-penta		1 non-penta		1 non-penta	0.96	equivocal (high rate)	0.5
Pentapetalae	non-penta, pentamerous	pentamerous	0.92	pentamerous	0.92	pentamerous	0.86	equivocal (high rate)	0.5
Superrosidae	pentamerous	pentamerous		1 pentamerous	0.99	pentamerous	0.88	equivocal (high rate)	0.5
Rosidae	pentamerous	pentamerous		1 pentamerous		1 pentamerous	0.9	equivocal (high rate)	0.5
Malvidae	pentamerous	pentamerous		1 pentamerous		1 pentamerous	0.95	equivocal (high rate)	0.5
Fabidae	pentamerous	pentamerous		1 pentamerous		1 pentamerous	0.96	equivocal (high rate)	0.5
Superasteridae	non-penta, pentamerous	pentamerous	0.92	pentamerous	0.92	pentamerous	0.87	equivocal (high rate)	0.5
Asteridae	pentamerous	pentamerous		1 pentamerous		1 pentamerous	0.98	equivocal (high rate)	0.5
Campanulidae	pentamerous	pentamerous		1 pentamerous		1 pentamerous	0.99	equivocal (high rate)	0.5
Lamiidae	pentamerous	pentamerous		1 pentamerous		1 pentamerous	0.98	equivocal (high rate)	0.5

Appendix IV.4: Parsimony and Mk1 with imposed rates for 15 key nodes

**Differentiation**                      **Confirmed state change**                      *Suspected state change*  
 q= transition rate                      high rate= high transition rate equilibrium

RESCORED DATA	Parsimony	State for q=0.0001	State probability	State for q=0.001	State probability	State for q=0.01	State probability	State for q=0.1	State probability
Angiospermae	undifferentiated	<b>undifferentiated</b>	<b>0.96</b>	<b>undifferentiated</b>	<b>0.57</b>	<b>differentiated</b>	<b>0.91</b>	equivocal (high rate)	0.5
Mesangiospermae	undifferentiated	<b>undifferentiated</b>	<b>0.95</b>	<b>undifferentiated</b>	<b>0.53</b>	<b>differentiated</b>	<b>0.94</b>	equivocal (high rate)	0.5
Magnoliidae	undifferentiated	<b>undifferentiated</b>	<b>0.95</b>	<b>undifferentiated</b>	<b>0.54</b>	<b>differentiated</b>	<b>0.93</b>	equivocal (high rate)	0.5
Monocotyledoneae	undifferentiated	<b>undifferentiated</b>	<b>0.98</b>	<b>undifferentiated</b>	<b>0.66</b>	<b>differentiated</b>	<b>0.91</b>	equivocal (high rate)	0.5
Commelinidae	undifferentiated, differentiated	<i>differentiated</i>	<i>0.74</i>	<i>differentiated</i>	<i>0.81</i>	<i>differentiated</i>	<i>0.87</i>	equivocal (high rate)	0.5
Eudicotyledoneae	differentiated	differentiated		1 differentiated		1 differentiated	0.98	equivocal (high rate)	0.5
Pentapetalae	differentiated	differentiated		1 differentiated		1 differentiated		1 equivocal (high rate)	0.5
Superrosidae	differentiated	differentiated		1 differentiated		1 differentiated		1 equivocal (high rate)	0.5
Rosidae	differentiated	differentiated		1 differentiated		1 differentiated		1 equivocal (high rate)	0.5
Malvidae	differentiated	differentiated		1 differentiated		1 differentiated		1 equivocal (high rate)	0.5
Fabidae	differentiated	differentiated		1 differentiated		1 differentiated		1 equivocal (high rate)	0.5
Superasteridae	differentiated	differentiated		1 differentiated		1 differentiated		1 equivocal (high rate)	0.5
Asteridae	differentiated	differentiated		1 differentiated		1 differentiated		1 equivocal (high rate)	0.5
Campanulidae	differentiated	differentiated		1 differentiated		1 differentiated		1 equivocal (high rate)	0.5
Lamiidae	differentiated	differentiated		1 differentiated		1 differentiated		1 equivocal (high rate)	0.5

ORIGINAL DATA	Parsimony	State for q=0.0001	State probability	State for q=0.001	State probability	State for q=0.01	State probability	State for q=0.1	State probability
Angiospermae	undifferentiated	<b>undifferentiated</b>	<b>0.97</b>	<b>undifferentiated</b>	<b>0.67</b>	<b>differentiated</b>	<b>0.89</b>	equivocal (high rate)	0.5
Mesangiospermae	undifferentiated	<b>undifferentiated</b>	<b>0.96</b>	<b>undifferentiated</b>	<b>0.64</b>	<b>differentiated</b>	<b>0.92</b>	equivocal (high rate)	0.5
Magnoliidae	undifferentiated	<b>undifferentiated</b>	<b>0.95</b>	<b>undifferentiated</b>	<b>0.64</b>	<b>differentiated</b>	<b>0.91</b>	equivocal (high rate)	0.5
Monocotyledoneae	undifferentiated	<b>undifferentiated</b>	<b>0.99</b>	<b>undifferentiated</b>	<b>0.75</b>	<b>differentiated</b>	<b>0.88</b>	equivocal (high rate)	0.5
Commelinidae	undifferentiated, differentiated	<i>differentiated</i>	<i>0.74</i>	<i>differentiated</i>	<i>0.76</i>	<i>differentiated</i>	<i>0.82</i>	equivocal (high rate)	0.5
Eudicotyledoneae	differentiated	differentiated		1 differentiated		1 differentiated	0.98	equivocal (high rate)	0.5
Pentapetalae	differentiated	differentiated		1 differentiated		1 differentiated		1 equivocal (high rate)	0.5
Superrosidae	differentiated	differentiated		1 differentiated		1 differentiated		1 equivocal (high rate)	0.5
Rosidae	differentiated	differentiated		1 differentiated		1 differentiated		1 equivocal (high rate)	0.5
Malvidae	differentiated	differentiated		1 differentiated		1 differentiated		1 equivocal (high rate)	0.5
Fabidae	differentiated	differentiated		1 differentiated		1 differentiated		1 equivocal (high rate)	0.5
Superasteridae	differentiated	differentiated		1 differentiated		1 differentiated		1 equivocal (high rate)	0.5
Asteridae	differentiated	differentiated		1 differentiated		1 differentiated		1 equivocal (high rate)	0.5
Campanulidae	differentiated	differentiated		1 differentiated		1 differentiated		1 equivocal (high rate)	0.5
Lamiidae	differentiated	differentiated		1 differentiated		1 differentiated		1 equivocal (high rate)	0.5

Appendix IV.5: Parsimony and Mk1 with imposed rates for the 54 order nodes

**Symmetry**                      **Confirmed state change**                      *Suspected state change*  
 q= transition rate                      high rate= high transition rate equilibrium

RESCORED DATA	Parsimony	State for q=0.0001	State probability	State for q=0.001	State probability	State for q=0.01	State probability	State for q=0.1	State probability
Alismatales	actinomorphic	actinomorphic		1 actinomorphic		1 actinomorphic	0.99	equivocal (high rate)	0.5
Apiales	actinomorphic	actinomorphic		1 actinomorphic		1 actinomorphic	0.99	equivocal (high rate)	0.5
Aquifoliales	actinomorphic	actinomorphic		1 actinomorphic		1 actinomorphic	0.99	equivocal (high rate)	0.5
Arecales	actinomorphic	actinomorphic		1 actinomorphic		1 actinomorphic	0.94	equivocal (high rate)	0.5
Asparagales	actinomorphic	actinomorphic		1 actinomorphic		1 actinomorphic	0.98	equivocal (high rate)	0.5
Asterales	actinomorphic	actinomorphic		1 actinomorphic		1 actinomorphic	0.98	equivocal (high rate)	0.5
Austrobaileyales	actinomorphic	actinomorphic		1 actinomorphic		1 actinomorphic	0.91	equivocal (high rate)	0.5
Berberidopsidales	actinomorphic	actinomorphic		1 actinomorphic		1 actinomorphic	0.95	equivocal (high rate)	0.5
Boraginales	actinomorphic	actinomorphic		1 actinomorphic		1 actinomorphic	0.96	equivocal (high rate)	0.5
Brassicales	actinomorphic	actinomorphic		1 actinomorphic		1 actinomorphic	0.97	equivocal (high rate)	0.5
Bruniales	actinomorphic	actinomorphic		1 actinomorphic		1 actinomorphic	0.94	equivocal (high rate)	0.5
Buxales	actinomorphic	actinomorphic		1 actinomorphic		1 actinomorphic	0.84	equivocal (high rate)	0.5
Caneliales	actinomorphic	actinomorphic		1 actinomorphic		1 actinomorphic	0.97	equivocal (high rate)	0.5
Caryophyllales	actinomorphic	actinomorphic		1 actinomorphic		1 actinomorphic		1 equivocal (high rate)	0.5
Celastrales	actinomorphic	actinomorphic		1 actinomorphic		1 actinomorphic	0.95	equivocal (high rate)	0.5
Commelinales	actinomorphic, zygomorphic	zygomorphic	0.89	zygomorphic	0.85	zygomorphic	0.61	equivocal (high rate)	0.5
Cornales	actinomorphic	actinomorphic		1 actinomorphic		1 actinomorphic	0.99	equivocal (high rate)	0.5
Crossosomatales	actinomorphic	actinomorphic		1 actinomorphic		1 actinomorphic	0.96	equivocal (high rate)	0.5
Cucurbitales	actinomorphic	actinomorphic		1 actinomorphic		1 actinomorphic	0.98	equivocal (high rate)	0.5
Dioscoreales	actinomorphic	actinomorphic		1 actinomorphic		1 actinomorphic	0.98	equivocal (high rate)	0.5
Dipsacales	actinomorphic	actinomorphic		1 actinomorphic	0.97	actinomorphic	0.78	equivocal (high rate)	0.5
Ericales	actinomorphic	actinomorphic		1 actinomorphic		1 actinomorphic		1 equivocal (high rate)	0.5
Escalloniales	actinomorphic	actinomorphic		1 actinomorphic		1 actinomorphic	0.97	equivocal (high rate)	0.5
Fabales	actinomorphic	actinomorphic	0.99	actinomorphic	0.93	actinomorphic	0.8	equivocal (high rate)	0.5
Fagales	actinomorphic	actinomorphic		1 actinomorphic		1 actinomorphic	0.99	equivocal (high rate)	0.5
Garryales	actinomorphic	actinomorphic		1 actinomorphic		1 actinomorphic	0.99	actinomorphic (high rate)	0.51
Gentianales	actinomorphic	actinomorphic		1 actinomorphic		1 actinomorphic	0.98	equivocal (high rate)	0.5
Geraniales	actinomorphic	actinomorphic		1 actinomorphic		1 actinomorphic	0.95	equivocal (high rate)	0.5
Huerteales	actinomorphic	actinomorphic		1 actinomorphic		1 actinomorphic	0.97	equivocal (high rate)	0.5
Icacinales	actinomorphic	actinomorphic		1 actinomorphic		1 actinomorphic	0.93	equivocal (high rate)	0.5
Lamiales	actinomorphic, zygomorphic	actinomorphic	0.73	actinomorphic	0.73	actinomorphic	0.72	equivocal (high rate)	0.5
Laurales	actinomorphic	actinomorphic		1 actinomorphic		1 actinomorphic	0.99	equivocal (high rate)	0.5
Liliales	actinomorphic	actinomorphic		1 actinomorphic		1 actinomorphic	0.95	equivocal (high rate)	0.5
Magnoliales	actinomorphic	actinomorphic		1 actinomorphic		1 actinomorphic	0.98	equivocal (high rate)	0.5
Malpighiales	actinomorphic	actinomorphic		1 actinomorphic		1 actinomorphic		1 equivocal (high rate)	0.5
Malvales	actinomorphic	actinomorphic		1 actinomorphic		1 actinomorphic	0.99	equivocal (high rate)	0.5
Myrtales	actinomorphic	actinomorphic		1 actinomorphic		1 actinomorphic	0.99	equivocal (high rate)	0.5
Nymphaeales	actinomorphic	actinomorphic		1 actinomorphic		1 actinomorphic	0.9	equivocal (high rate)	0.5
Oxalidales	actinomorphic	actinomorphic		1 actinomorphic		1 actinomorphic	0.99	equivocal (high rate)	0.5
Pandanales	actinomorphic	actinomorphic		1 actinomorphic		1 actinomorphic	0.98	equivocal (high rate)	0.5
Paracryphiales	actinomorphic	actinomorphic		1 actinomorphic		1 actinomorphic	0.94	equivocal (high rate)	0.5
Petrosaviales	actinomorphic	actinomorphic		1 actinomorphic		1 actinomorphic	0.87	equivocal (high rate)	0.5
Piperales	actinomorphic	actinomorphic		1 actinomorphic		1 actinomorphic	0.95	equivocal (high rate)	0.5
Poales	actinomorphic	actinomorphic		1 actinomorphic		1 actinomorphic	0.96	equivocal (high rate)	0.5
Proteales	actinomorphic	actinomorphic		1 actinomorphic		1 actinomorphic	0.99	equivocal (high rate)	0.5
Ranunculales	actinomorphic	actinomorphic		1 actinomorphic		1 actinomorphic		1 equivocal (high rate)	0.5
Rosales	actinomorphic	actinomorphic		1 actinomorphic		1 actinomorphic	0.99	equivocal (high rate)	0.5

Santalales	actinomorphic	actinomorphic	1	actinomorphic	1	actinomorphic	0.99	equivocal (high rate)	0.5
Sapindales	actinomorphic	actinomorphic	1	actinomorphic	1	actinomorphic	0.99	equivocal (high rate)	0.5
Saxifragales	actinomorphic	actinomorphic	1	actinomorphic	1	actinomorphic	1	equivocal (high rate)	0.5
Solanales	actinomorphic	actinomorphic	1	actinomorphic	1	actinomorphic	0.95	equivocal (high rate)	0.5
Vitales	actinomorphic	actinomorphic	1	actinomorphic	1	actinomorphic	1	equivocal (high rate)	0.5
Zingiberales	zygomorphic	zygomorphic	1	zygomorphic	1	zygomorphic	0.96	equivocal (high rate)	0.5
Zygophyllales	actinomorphic	actinomorphic	1	actinomorphic	0.96	actinomorphic	0.73	equivocal (high rate)	0.5

ORIGINAL DATA	Parsimony	State for q=0.0001	State probability	State for q=0.001	State probability	State for q=0.01	State probability	State for q=0.1	State probability
Alismatales	actinomorphic	actinomorphic	1	actinomorphic	1	actinomorphic	0.99	equivocal (high rate)	0.5
Apiales	actinomorphic	actinomorphic	1	actinomorphic	1	actinomorphic	0.99	equivocal (high rate)	0.5
Aquifoliales	actinomorphic	actinomorphic	1	actinomorphic	1	actinomorphic	0.99	equivocal (high rate)	0.5
Arecales	actinomorphic	actinomorphic	1	actinomorphic	1	actinomorphic	0.94	equivocal (high rate)	0.5
Asparagales	actinomorphic	actinomorphic	1	actinomorphic	1	actinomorphic	0.99	equivocal (high rate)	0.5
Asterales	actinomorphic	actinomorphic	1	actinomorphic	1	actinomorphic	0.99	equivocal (high rate)	0.5
Austrobaileyales	actinomorphic	actinomorphic	1	actinomorphic	1	actinomorphic	0.91	equivocal (high rate)	0.5
Berberidopsidales	actinomorphic	actinomorphic	1	actinomorphic	1	actinomorphic	0.95	equivocal (high rate)	0.5
Boraginales	actinomorphic	actinomorphic	1	actinomorphic	1	actinomorphic	0.96	equivocal (high rate)	0.5
Brassicales	actinomorphic	actinomorphic	1	actinomorphic	1	actinomorphic	0.97	equivocal (high rate)	0.5
Bruniales	actinomorphic	actinomorphic	1	actinomorphic	1	actinomorphic	0.94	equivocal (high rate)	0.5
Buxales	actinomorphic	<b>actinomorphic</b>	<b>1</b>	<b>actinomorphic</b>	<b>1</b>	<b>actinomorphic</b>	<b>0.84</b>	equivocal (high rate)	0.5
Canellales	actinomorphic	actinomorphic	1	actinomorphic	1	actinomorphic	0.97	equivocal (high rate)	0.5
Caryophyllales	actinomorphic	actinomorphic	1	actinomorphic	1	actinomorphic	1	equivocal (high rate)	0.5
Celastrales	actinomorphic	actinomorphic	1	actinomorphic	1	actinomorphic	0.95	equivocal (high rate)	0.5
Commelinales	actinomorphic, zygomorphic	zygomorphic	0.52	equivocal	0.5	actinomorphic	0.51	equivocal (high rate)	0.5
Cornales	actinomorphic	actinomorphic	1	actinomorphic	1	actinomorphic	0.99	equivocal (high rate)	0.5
Crossosomatales	actinomorphic	actinomorphic	1	actinomorphic	1	actinomorphic	0.96	equivocal (high rate)	0.5
Cucurbitales	actinomorphic	actinomorphic	1	actinomorphic	1	actinomorphic	0.98	equivocal (high rate)	0.5
Dioscoreales	actinomorphic	actinomorphic	1	actinomorphic	1	actinomorphic	0.93	equivocal (high rate)	0.5
Dipsacales	actinomorphic	<b>actinomorphic</b>	<b>1</b>	<b>actinomorphic</b>	<b>0.97</b>	<b>actinomorphic</b>	<b>0.78</b>	equivocal (high rate)	0.5
Ericales	actinomorphic	actinomorphic	1	actinomorphic	1	actinomorphic	1	equivocal (high rate)	0.5
Escalloniales	actinomorphic	actinomorphic	1	actinomorphic	1	actinomorphic	0.97	equivocal (high rate)	0.5
Fabales	actinomorphic	<b>actinomorphic</b>	<b>0.99</b>	<b>actinomorphic</b>	<b>0.93</b>	<b>actinomorphic</b>	<b>0.8</b>	equivocal (high rate)	0.5
Fagales	actinomorphic	actinomorphic	1	actinomorphic	1	actinomorphic	0.99	equivocal (high rate)	0.5
Garryales	actinomorphic	actinomorphic	1	actinomorphic	1	actinomorphic	0.99	actinomorphic (high rate)	0.51
Gentianales	actinomorphic	actinomorphic	1	actinomorphic	1	actinomorphic	0.98	equivocal (high rate)	0.5
Geraniales	actinomorphic	actinomorphic	1	actinomorphic	1	actinomorphic	0.95	equivocal (high rate)	0.5
Huerteales	actinomorphic	actinomorphic	1	actinomorphic	1	actinomorphic	0.97	equivocal (high rate)	0.5
Icacinales	actinomorphic	actinomorphic	1	actinomorphic	1	actinomorphic	0.93	equivocal (high rate)	0.5
Lamiales	actinomorphic, zygomorphic	actinomorphic	1	actinomorphic	1	actinomorphic	1	equivocal (high rate)	0.5
Laurales	actinomorphic	actinomorphic	1	actinomorphic	1	actinomorphic	0.99	equivocal (high rate)	0.5
Liliales	actinomorphic	actinomorphic	1	actinomorphic	1	actinomorphic	0.95	equivocal (high rate)	0.5
Magnoliales	actinomorphic	actinomorphic	1	actinomorphic	1	actinomorphic	0.98	equivocal (high rate)	0.5
Malpighiales	actinomorphic	actinomorphic	1	actinomorphic	1	actinomorphic	1	equivocal (high rate)	0.5
Malvales	actinomorphic	actinomorphic	1	actinomorphic	1	actinomorphic	0.99	equivocal (high rate)	0.5
Myrtales	actinomorphic	actinomorphic	1	actinomorphic	1	actinomorphic	0.99	equivocal (high rate)	0.5
Nymphaeales	actinomorphic	actinomorphic	1	actinomorphic	1	actinomorphic	0.9	equivocal (high rate)	0.5
Oxalidales	actinomorphic	actinomorphic	1	actinomorphic	1	actinomorphic	0.99	equivocal (high rate)	0.5
Pandanales	actinomorphic	actinomorphic	1	actinomorphic	1	actinomorphic	0.98	equivocal (high rate)	0.5
Paracryphiales	actinomorphic	actinomorphic	1	actinomorphic	1	actinomorphic	0.94	equivocal (high rate)	0.5
Petrosaviales	actinomorphic	<b>actinomorphic</b>	<b>1</b>	<b>actinomorphic</b>	<b>1</b>	<b>actinomorphic</b>	<b>0.87</b>	equivocal (high rate)	0.5
Piperiales	actinomorphic	actinomorphic	1	actinomorphic	1	actinomorphic	0.95	equivocal (high rate)	0.5

Poales	actinomorphic	actinomorphic	1	actinomorphic	1	actinomorphic	0.96	equivocal (high rate)	0.5
Proteales	actinomorphic	actinomorphic	1	actinomorphic	1	actinomorphic	0.99	equivocal (high rate)	0.5
Ranunculales	actinomorphic	actinomorphic	1	actinomorphic	1	actinomorphic		1 equivocal (high rate)	0.5
Rosales	actinomorphic	actinomorphic	1	actinomorphic	1	actinomorphic	0.99	equivocal (high rate)	0.5
Santalales	actinomorphic	actinomorphic	1	actinomorphic	1	actinomorphic	0.99	equivocal (high rate)	0.5
Sapindales	actinomorphic	actinomorphic	1	actinomorphic	1	actinomorphic	0.99	equivocal (high rate)	0.5
Saxifragales	actinomorphic	actinomorphic	1	actinomorphic	1	actinomorphic		1 equivocal (high rate)	0.5
Solanales	actinomorphic	actinomorphic	1	actinomorphic	1	actinomorphic	0.96	equivocal (high rate)	0.5
Vitales	actinomorphic	<b>actinomorphic</b>	1	<b>actinomorphic</b>	1	<b>actinomorphic</b>	<b>0.87</b>	equivocal (high rate)	0.5
Zingiberales	zygomorphic	zygomorphic	1	zygomorphic	0	zygomorphic	0.95	equivocal (high rate)	0.5
Zygophyllales	actinomorphic	actinomorphic	1	actinomorphic	0.97	actinomorphic	0.73	equivocal (high rate)	0.5

Appendix IV.5: Parsimony and Mk1 with imposed rates for the 54 order nodes

**Fusion**                      **Confirmed state change**                      *Suspected state change*  
 q= transition rate                      high rate= high transition rate equilibrium

RESCORED DATA	Parsimony	State for q=0.0001	State probability	State for q=0.001	State probability	State for q=0.01	State probability	State for q=0.1	State probability
Alismatales	free	free		1 free		1 free	0.99	equivocal (high rate)	0.5
Apiales	free, fused	fused	0.97	1 fused	0.97	1 fused	0.93	equivocal (high rate)	0.5
Aquifoliales	fused	fused		1 fused		1 fused	0.91	equivocal (high rate)	0.5
Arecales	free	free		1 free		1 free	0.81	equivocal (high rate)	0.5
Asparagales	free	free		1 free		1 free	0.96	equivocal (high rate)	0.5
Asterales	fused	fused		1 fused		1 fused		1 equivocal (high rate)	0.5
Austrobaileyales	free	free		1 free		1 free	0.91	equivocal (high rate)	0.5
Berberidopsidales	free	free		1 free	0.99	1 free	0.93	equivocal (high rate)	0.5
Boraginales	fused	fused		1 fused		1 fused	0.99	equivocal (high rate)	0.5
Brassicales	free, fused	free, fused	0.69	1 free	0.77	1 free	0.63	equivocal (high rate)	0.5
Bruniales	free, fused	fused		1 fused	0.99	1 fused	0.92	equivocal (high rate)	0.5
Buxales	free	free		1 free		1 free	0.82	equivocal (high rate)	0.5
Caneliales	free, fused	free	0.92	1 free	0.92	1 free	0.91	equivocal (high rate)	0.5
Caryophyllales	fused	fused	0.99	1 fused	0.92	1 free	0.65	equivocal (high rate)	0.5
Celastrales	free	free		1 free		1 free	0.93	equivocal (high rate)	0.5
Commelinales	free, fused	free		1 free	0.99	1 free	0.87	equivocal (high rate)	0.5
Cornales	fused	fused	0.99	1 fused	0.93	1 fused	0.6	equivocal (high rate)	0.5
Crossosomatales	fused	free	0.73	1 free	0.82	1 free	0.73	equivocal (high rate)	0.5
Cucurbitales	free	free		1 free		1 free	0.79	equivocal (high rate)	0.5
Dioscoreales	free	free	0.99	1 free	0.9	1 free	0.69	equivocal (high rate)	0.5
Dipsacales	fused	fused		1 fused		1 fused	0.97	equivocal (high rate)	0.5
Ericales	fused	fused		1 fused		1 fused	0.95	equivocal (high rate)	0.5
Escalloniales	fused	fused		1 fused		1 fused	0.96	equivocal (high rate)	0.5
Fabales	free	free		1 free	0.99	1 free	0.81	equivocal (high rate)	0.5
Fagales	free, fused	free	0.98	1 free	0.98	1 free	0.91	equivocal (high rate)	0.5
Garryales	free, fused	free		1 free	0.98	1 free	0.83	free (high rate)	0.51
Gentianales	fused	fused		1 fused		1 fused	0.98	equivocal (high rate)	0.5
Geraniales	fused	free	0.86	1 free	0.98	1 free	0.93	equivocal (high rate)	0.5
Huerteales	free, fused	free	0.84	1 free	0.93	1 free	0.78	equivocal (high rate)	0.5
Icacinales	fused	fused		1 fused		1 fused	0.93	equivocal (high rate)	0.5
Lamiales	fused	fused		1 fused		1 fused	0.99	equivocal (high rate)	0.5
Laurales	free	free		1 free		1 free	0.95	equivocal (high rate)	0.5
Liliales	free	free		1 free		1 free	0.96	equivocal (high rate)	0.5
Magnoliales	free	free		1 free		1 free	0.96	equivocal (high rate)	0.5
Malpighiales	free	free		1 free		1 free	0.95	equivocal (high rate)	0.5
Malvales	free, fused	fused		1 fused	0.95	1 fused	0.74	equivocal (high rate)	0.5
Myrtales	fused	free	0.85	1 free	0.95	1 free	0.81	equivocal (high rate)	0.5
Nymphaeales	free	free		1 free		1 free	0.89	equivocal (high rate)	0.5
Oxalidales	free	free		1 free	0.97	1 free	0.89	equivocal (high rate)	0.5
Pandanales	free	free	0.99	1 free	0.95	1 free	0.74	equivocal (high rate)	0.5
Paracryphiales	free	free		1 free	0.96	1 free	0.73	equivocal (high rate)	0.5
Petrosaviales	free	free		1 free	0.97	1 free	0.79	equivocal (high rate)	0.5
Piperales	free, fused	free	0.96	1 free	0.94	1 free	0.8	equivocal (high rate)	0.5
Poales	free, fused	free	0.98	1 free	0.98	1 free	0.91	equivocal (high rate)	0.5
Proteales	free	free		1 free		1 free	0.97	equivocal (high rate)	0.5
Ranunculales	free	free		1 free		1 free	0.98	equivocal (high rate)	0.5
Rosales	free	free		1 free		1 free	0.89	equivocal (high rate)	0.5

Santalales	fused	fused		1	fused	0.99	fused	0.72	equivocal (high rate)	0.5
Sapindales	free, fused	free	0.88		free	0.97	free	0.71	equivocal (high rate)	0.5
Saxifragales	fused	<b>fused</b>	<b>0.76</b>		<b>fused</b>	<b>0.7</b>	<b>free</b>	<b>0.68</b>	equivocal (high rate)	0.5
Solanales	fused	fused		1	fused		1	0.99	equivocal (high rate)	0.5
Vitales	fused	fused		1	fused	0.96	fused	0.69	equivocal (high rate)	0.5
Zingiberales	fused	fused		1	fused		1	0.93	equivocal (high rate)	0.5
Zygophyllales	free	free		1	free		1		1 free (high rate)	0.6

ORIGINAL DATA	Parsimony	State for q=0.0001	State probability	State for q=0.001	State probability	State for q=0.01	State probability	State for q=0.1	State probability	
Alismatales	free	free		1	free	1	0.99	equivocal (high rate)	0.5	
Apiales	free	<b>free</b>	<b>0.99</b>	<b>free</b>	<b>0.89</b>	<b>equivocal</b>	<b>0.5</b>	equivocal (high rate)	0.5	
Aquifoliales	fused	fused		1	fused	0	0.74	equivocal (high rate)	0.5	
Arecales	free	free		1	free	1	0.87	equivocal (high rate)	0.5	
Asparagales	free	free		1	free	1	0.97	equivocal (high rate)	0.5	
Asterales	fused	fused		1	fused	0	0.93	equivocal (high rate)	0.5	
Austrobaileyales	free	free		1	free	1	0.91	equivocal (high rate)	0.5	
Berberidopsidales	free	free		1	free	1	0.95	equivocal (high rate)	0.5	
Boraginales	fused	fused		1	fused	0	0.99	equivocal (high rate)	0.5	
Brassicales	free	free		1	free	1	0.94	equivocal (high rate)	0.5	
Bruniales	free, fused	fused	0.92	fused	0.09	fused	0.73	equivocal (high rate)	0.5	
Buxales	free	free		1	free	1	0.84	equivocal (high rate)	0.5	
Canellales	free	free	0.99	free	0.99	free	0.95	equivocal (high rate)	0.5	
Caryophyllales	free, fused	free	0.97	free	0.97	free	0.9	equivocal (high rate)	0.5	
Celastrales	free	free		1	free	1	0.95	equivocal (high rate)	0.5	
Commelinales	free	free		1	free	0.99	0.93	equivocal (high rate)	0.5	
Cornales	free	free		1	free	1	0.8	equivocal (high rate)	0.5	
Crossosomatales	free, fused	free	0.85	free	0.84	free	0.78	equivocal (high rate)	0.5	
Cucurbitales	free	free		1	free	1	0.81	equivocal (high rate)	0.5	
Dioscoreales	free	free	0.99	free	0.9	free	0.7	equivocal (high rate)	0.5	
Dipsacales	fused	fused		1	fused	0.99	fused	0.85	equivocal (high rate)	0.5
Ericales	free, fused	fused	0.92	fused	0.92	fused	0.67	equivocal (high rate)	0.5	
Escalloniales	fused	fused		1	fused	1	0.81	equivocal (high rate)	0.5	
Fabales	free	free		1	free	1	0.88	equivocal (high rate)	0.5	
Fagales	free	free		1	free	1	0.95	equivocal (high rate)	0.5	
Garryales	free, fused	free		1	free	0.98	free	0.86	free (high rate)	0.51
Gentianales	fused	fused		1	fused	1	0.97	equivocal (high rate)	0.5	
Geraniales	free	free		1	free	1	0.97	equivocal (high rate)	0.5	
Huerteales	free	free		1	free	0.99	free	0.89	equivocal (high rate)	0.5
Icacinales	fused	fused		1	fused	1	0.81	equivocal (high rate)	0.5	
Lamiales	fused	fused		0	fused	1	0.99	equivocal (high rate)	0.5	
Laurales	free	free		1	free	1	0.96	equivocal (high rate)	0.5	
Liliales	free	free		1	free	1	0.97	equivocal (high rate)	0.5	
Magnoliales	free	free		1	free	1	0.96	equivocal (high rate)	0.5	
Malpighiales	free	free		1	free	1		1 equivocal (high rate)	0.5	
Malvales	free, fused	fused	0.99	fused	0.92	fused	0.59	equivocal (high rate)	0.5	
Myrtales	free, fused	free	0.98	free	0.98	free	0.88	equivocal (high rate)	0.5	
Nymphaeales	free	free		1	free	1	0.9	equivocal (high rate)	0.5	
Oxalidales	free	free		1	free	0.97	free	0.93	equivocal (high rate)	0.5
Pandanales	free	free	0.99	free	0.95	free	0.75	equivocal (high rate)	0.5	
Paracryphiales	free	free		1	free	0.97	free	0.78	equivocal (high rate)	0.5
Petrosaviales	free	free		1	free	0.97	free	0.79	equivocal (high rate)	0.5
Piperales	free	free		1	free		0.86	equivocal (high rate)	0.5	

Poales	free	free		1 free		1 free	0.97	equivocal (high rate)	0.5
Proteales	free	free		1 free		1 free	0.99	equivocal (high rate)	0.5
Ranunculales	free	free		1 free		1 free		1 equivocal (high rate)	0.5
Rosales	free	free		1 free		1 free	0.98	equivocal (high rate)	0.5
Santalales	fused	<b>fused</b>		<b>1 fused</b>	<b>0.97</b>	<b>free</b>	<b>0.57</b>	equivocal (high rate)	0.5
Sapindales	free	free		1 free		1 free	0.96	equivocal (high rate)	0.5
Saxifragales	free	free		1 free	0.98	free	0.9	equivocal (high rate)	0.5
Solanales	fused	fused		1 fused		1 fused	0.97	equivocal (high rate)	0.5
Vitales	free, fused	fused	0.52	fused	0.52	fused	0.52	equivocal (high rate)	0.5
Zingiberales	free, fused	fused	0.67	fused	0.65	fused	0.54	equivocal (high rate)	0.5
Zygophyllales	free	free		1 free		1 free		1 free (high rate)	0.6

Appendix IV.5: Parsimony and Mk1 with imposed rates for the 54 order nodes

Phyllotaxy      **Confirmed state change**      *Suspected state change*  
 q= transition rate      high rate= high transition rate equilibrium

RESCORED DATA	Parsimony	State for q=0.0001	State probability	State for q=0.001	State probability	State for q=0.01	State probability	State for q=0.1	State probability
Alismatales	whorled	whorled		1 whorled		1 whorled	0.99	equivocal (high rate)	0.5
Apiales	whorled	whorled		1 whorled		1 whorled		1 equivocal (high rate)	0.5
Aquifoliales	whorled	whorled		1 whorled		1 whorled	0.98	equivocal (high rate)	0.5
Arecales	whorled	whorled		1 whorled		1 whorled	0.94	equivocal (high rate)	0.5
Asparagales	whorled	whorled		1 whorled		1 whorled		1 equivocal (high rate)	0.5
Asterales	whorled	whorled		1 whorled		1 whorled		1 equivocal (high rate)	0.5
Austrobaileyales	spiral	<b>spiral</b>	<b>0.99</b>	<b>spiral</b>	<b>0.84</b>	<b>whorled</b>	<b>0.78</b>	equivocal (high rate)	0.5
Berberidopsidales	whorled	whorled	0.99	whorled	0.92	whorled	0.58	equivocal (high rate)	0.5
Boraginales	whorled	whorled		1 whorled		1 whorled	0.99	equivocal (high rate)	0.5
Brassicales	whorled	whorled		1 whorled		1 whorled		1 equivocal (high rate)	0.5
Bruniales	whorled	whorled		1 whorled		1 whorled	0.97	equivocal (high rate)	0.5
Buxales	whorled	whorled		1 whorled	0.96	whorled	0.71	equivocal (high rate)	0.5
Caneliales	whorled	whorled		1 whorled		1 whorled	0.96	equivocal (high rate)	0.5
Caryophyllales	whorled	whorled		1 whorled		1 whorled		1 equivocal (high rate)	0.5
Celastrales	whorled	whorled		1 whorled		1 whorled	0.95	equivocal (high rate)	0.5
Commelinales	whorled	whorled		1 whorled		1 whorled		1 equivocal (high rate)	0.5
Cornales	whorled	whorled		1 whorled		1 whorled	0.99	equivocal (high rate)	0.5
Crossosomatales	whorled	whorled		1 whorled		1 whorled	0.94	equivocal (high rate)	0.5
Cucurbitales	whorled	whorled		1 whorled		1 whorled	0.99	equivocal (high rate)	0.5
Dioscoreales	whorled	whorled		1 whorled		1 whorled	0.96	equivocal (high rate)	0.5
Dipsacales	whorled	whorled		1 whorled		1 whorled	0.99	equivocal (high rate)	0.5
Ericales	whorled	whorled		1 whorled		1 whorled		1 equivocal (high rate)	0.5
Escalloniales	whorled	whorled		1 whorled		1 whorled	0.97	equivocal (high rate)	0.5
Fabales	whorled	whorled		1 whorled		1 whorled	0.99	equivocal (high rate)	0.5
Fagales	whorled	whorled		1 whorled		1 whorled	0.99	equivocal (high rate)	0.5
Garryales	whorled	whorled		1 whorled		1 whorled	0.99	whorled (high rate)	0.51
Gentianales	whorled	whorled		1 whorled		1 whorled	0.99	equivocal (high rate)	0.5
Geraniales	whorled	whorled		1 whorled		1 whorled	0.98	equivocal (high rate)	0.5
Huerteales	whorled	whorled		1 whorled		1 whorled	0.96	equivocal (high rate)	0.5
Icacinales	whorled	whorled		1 whorled		1 whorled	0.93	equivocal (high rate)	0.5
Lamiales	whorled	whorled		1 whorled		1 whorled	0.99	equivocal (high rate)	0.5
Laurales	whorled	whorled		1 whorled		1 whorled	0.97	equivocal (high rate)	0.5
Liliales	whorled	whorled		1 whorled		1 whorled	0.99	equivocal (high rate)	0.5
Magnoliales	whorled	whorled		1 whorled		1 whorled	0.89	equivocal (high rate)	0.5
Malpighiales	whorled	whorled		1 whorled		1 whorled		1 equivocal (high rate)	0.5
Malvales	whorled	whorled		1 whorled		1 whorled	0.99	equivocal (high rate)	0.5
Myrtales	whorled	whorled		1 whorled		1 whorled		1 equivocal (high rate)	0.5
Nymphaeales	whorled, spiral	whorled		1 whorled	0.99	whorled	0.89	equivocal (high rate)	0.5
Oxalidales	whorled	whorled		1 whorled		1 whorled	0.99	equivocal (high rate)	0.5
Pandanales	whorled	whorled		1 whorled		1 whorled	0.98	equivocal (high rate)	0.5
Paracryphiales	whorled	whorled		1 whorled		1 whorled	0.94	equivocal (high rate)	0.5
Petrosaviales	whorled	whorled		1 whorled		1 whorled	0.87	equivocal (high rate)	0.5
Piperales	whorled	whorled		1 whorled		1 whorled	0.97	equivocal (high rate)	0.5
Poales	whorled	whorled		1 whorled		1 whorled		1 equivocal (high rate)	0.5
Proteales	whorled	whorled		1 whorled		1 whorled	0.98	equivocal (high rate)	0.5
Ranunculales	whorled	whorled		1 whorled		1 whorled		1 equivocal (high rate)	0.5
Rosales	whorled	whorled		1 whorled		1 whorled	0.99	equivocal (high rate)	0.5

Santalales	whorled	whorled	1	whorled	1	whorled	0.99	equivocal (high rate)	0.5
Sapindales	whorled	whorled	1	whorled	1	whorled	0.99	equivocal (high rate)	0.5
Saxifragales	whorled	whorled	1	whorled	1	whorled	1	equivocal (high rate)	0.5
Solanales	whorled	whorled	1	whorled	1	whorled	0.99	equivocal (high rate)	0.5
Vitales	whorled	whorled	1	whorled	1	whorled	0.87	equivocal (high rate)	0.5
Zingiberales	whorled	whorled	1	whorled	1	whorled	1	equivocal (high rate)	0.5
Zygophyllales	whorled	whorled	1	whorled	1	whorled	0.9	equivocal (high rate)	0.5

ORIGINAL DATA	Parsimony	State for q=0.0001	State probability	State for q=0.001	State probability	State for q=0.01	State probability	State for q=0.1	State probability
Alismatales	whorled	whorled	1	whorled	1	whorled	0.99	equivocal (high rate)	0.5
Apiales	whorled	whorled	1	whorled	1	whorled	1	equivocal (high rate)	0.5
Aquifoliales	whorled	whorled	1	whorled	1	whorled	0,98	equivocal (high rate)	0.5
Arecales	whorled	whorled	1	whorled	1	whorled	0,94	equivocal (high rate)	0.5
Asparagales	whorled	whorled	1	whorled	1	whorled	1	equivocal (high rate)	0.5
Asterales	whorled	whorled	1	whorled	1	whorled	1	equivocal (high rate)	0.5
Austrobaileyales	spiral	<b>spiral</b>	<b>0,99</b>	<b>spiral</b>	<b>0,84</b>	<b>whorled</b>	<b>0,78</b>	equivocal (high rate)	0.5
Berberidopsidales	whorled	whorled	0,99	whorled	0,92	whorled	0,58	equivocal (high rate)	0.5
Boraginales	whorled	whorled	1	whorled	1	whorled	0,99	equivocal (high rate)	0.5
Brassicales	whorled	whorled	1	whorled	1	whorled	1	equivocal (high rate)	0.5
Bruniales	whorled	whorled	1	whorled	1	whorled	0,97	equivocal (high rate)	0.5
Buxales	whorled	whorled	1	whorled	1	whorled	0,78	equivocal (high rate)	0.5
Canellales	whorled	whorled	1	whorled	1	whorled	0,97	equivocal (high rate)	0.5
Caryophyllales	whorled	whorled	1	whorled	1	whorled	1	equivocal (high rate)	0.5
Celastrales	whorled	whorled	1	whorled	1	whorled	0,95	equivocal (high rate)	0.5
Commelinales	whorled	whorled	1	whorled	1	whorled	1	equivocal (high rate)	0.5
Cornales	whorled	whorled	1	whorled	1	whorled	0,99	equivocal (high rate)	0.5
Crossosomatales	whorled	whorled	1	whorled	1	whorled	0,95	equivocal (high rate)	0.5
Cucurbitales	whorled	whorled	1	whorled	1	whorled	0,99	equivocal (high rate)	0.5
Dioscoreales	whorled	whorled	1	whorled	1	whorled	0,96	equivocal (high rate)	0.5
Dipsacales	whorled	whorled	1	whorled	1	whorled	0,99	equivocal (high rate)	0.5
Ericales	whorled	whorled	1	whorled	1	whorled	1	equivocal (high rate)	0.5
Escalloniales	whorled	whorled	1	whorled	1	whorled	0,97	equivocal (high rate)	0.5
Fabales	whorled	whorled	1	whorled	1	whorled	0,99	equivocal (high rate)	0.5
Fagales	whorled	whorled	1	whorled	1	whorled	0,99	equivocal (high rate)	0.5
Garryales	whorled	whorled	1	whorled	1	whorled	0,99	whorled (high rate)	0.51
Gentianales	whorled	whorled	1	whorled	1	whorled	0,99	equivocal (high rate)	0.5
Geraniales	whorled	whorled	1	whorled	1	whorled	0,98	equivocal (high rate)	0.5
Huerteales	whorled	whorled	1	whorled	1	whorled	0,96	equivocal (high rate)	0.5
Icacinales	whorled	whorled	1	whorled	1	whorled	0,93	equivocal (high rate)	0.5
Lamiales	whorled	whorled	1	whorled	1	whorled	0,99	equivocal (high rate)	0.5
Laurales	whorled	whorled	1	whorled	1	whorled	0,97	equivocal (high rate)	0.5
Liliales	whorled	whorled	1	whorled	1	whorled	0,99	equivocal (high rate)	0.5
Magnoliales	whorled	whorled	1	whorled	1	whorled	0,89	equivocal (high rate)	0.5
Malpighiales	whorled	whorled	1	whorled	1	whorled	1	equivocal (high rate)	0.5
Malvales	whorled	whorled	1	whorled	1	whorled	0,99	equivocal (high rate)	0.5
Myrtales	whorled	whorled	1	whorled	1	whorled	1	equivocal (high rate)	0.5
Nymphaeales	whorled, spiral	whorled	1	whorled	0,99	whorled	0,89	equivocal (high rate)	0.5
Oxalidales	whorled	whorled	1	whorled	1	whorled	0,99	equivocal (high rate)	0.5
Pandanales	whorled	whorled	1	whorled	1	whorled	0,98	equivocal (high rate)	0.5
Paracryphiales	whorled	whorled	1	whorled	1	whorled	0,94	equivocal (high rate)	0.5
Petrosaviales	whorled	whorled	1	whorled	1	whorled	0,87	equivocal (high rate)	0.5
Piperales	whorled	whorled	1	whorled	1	whorled	0,97	equivocal (high rate)	0.5

Poales	whorled	whorled	1	whorled	1	whorled	1	equivocal (high rate)	0.5
Proteales	whorled	whorled	1	whorled	1	whorled	0,98	equivocal (high rate)	0.5
Ranunculales	whorled	whorled	1	whorled	1	whorled	1	equivocal (high rate)	0.5
Rosales	whorled	whorled	1	whorled	1	whorled	0,99	equivocal (high rate)	0.5
Santalales	whorled	whorled	1	whorled	1	whorled	0,99	equivocal (high rate)	0.5
Sapindales	whorled	whorled	1	whorled	1	whorled	0,99	equivocal (high rate)	0.5
Saxifragales	whorled	whorled	1	whorled	1	whorled	1	equivocal (high rate)	0.5
Solanales	whorled	whorled	1	whorled	1	whorled	0,99	equivocal (high rate)	0.5
Vitales	whorled	whorled	1	whorled	1	whorled	0,87	equivocal (high rate)	0.5
Zingiberales	whorled	whorled	1	whorled	1	whorled	1	equivocal (high rate)	0.5
Zygophyllales	whorled	whorled	1	whorled	1	whorled	0,9	equivocal (high rate)	0.5

Appendix IV.5: Parsimony and Mk1 with imposed rates for the 54 order nodes

**Pentamery**      **Confirmed state change**      *Suspected state change*  
 q= transition rate      high rate= high transition rate equilibrium

RESCORED DATA	Parsimony	State for q=0.0001	State probability	State for q=0.001	State probability	State for q=0.01	State probability	State for q=0.1	State probability
Alismatales	non-penta	non-penta		1 non-penta		1 non-penta	0.99	1 equivocal (high rate)	0.5
Apiales	pentamerous	pentamerous		1 pentamerous		1 pentamerous		1 equivocal (high rate)	0.5
Aquifoliales	pentamerous	pentamerous		1 pentamerous		1 pentamerous	0.96	1 equivocal (high rate)	0.5
Arecales	non-penta	non-penta		1 non-penta		1 non-penta	0.94	1 equivocal (high rate)	0.5
Asparagales	non-penta	non-penta		1 non-penta		1 non-penta		1 equivocal (high rate)	0.5
Asterales	pentamerous	pentamerous		1 pentamerous		1 pentamerous		1 equivocal (high rate)	0.5
Austrobaileyales	?	?	?	?	?	?	?	?	?
Berberidopsidales	pentamerous	?	?	?	?	?	?	?	?
Boraginales	pentamerous	pentamerous		1 pentamerous		1 pentamerous	0.97	1 equivocal (high rate)	0.5
Brassicales	pentamerous	pentamerous		1 pentamerous		1 pentamerous	0.96	1 equivocal (high rate)	0.5
Bruniales	pentamerous	pentamerous		1 pentamerous		1 pentamerous	0.97	1 equivocal (high rate)	0.5
Buxales	non-penta	non-penta		1 non-penta		1 non-penta	0.72	1 equivocal (high rate)	0.5
Caneliales	non-penta	non-penta		1 non-penta		1 non-penta	0.95	1 equivocal (high rate)	0.5
Caryophyllales	pentamerous	pentamerous		1 pentamerous		1 pentamerous		1 equivocal (high rate)	0.5
Celastrales	pentamerous	pentamerous		1 pentamerous		1 pentamerous	0.88	1 equivocal (high rate)	0.5
Commelinales	non-penta	non-penta		1 non-penta		1 non-penta		1 equivocal (high rate)	0.5
Cornales	pentamerous	pentamerous		1 pentamerous		1 pentamerous	0.93	1 equivocal (high rate)	0.5
Crossosomatales	pentamerous	pentamerous		1 pentamerous		1 pentamerous	0.92	1 equivocal (high rate)	0.5
Cucurbitales	pentamerous	pentamerous		1 pentamerous		1 pentamerous	0.87	1 equivocal (high rate)	0.5
Dioscoreales	non-penta	non-penta		1 non-penta		1 non-penta	0.96	1 equivocal (high rate)	0.5
Dipsacales	pentamerous	pentamerous		1 pentamerous		1 pentamerous	0.97	1 equivocal (high rate)	0.5
Ericales	pentamerous	pentamerous		1 pentamerous		1 pentamerous		1 equivocal (high rate)	0.5
Escalloniales	pentamerous	pentamerous		1 pentamerous	0.99	1 pentamerous	0.93	1 equivocal (high rate)	0.5
Fabales	pentamerous	pentamerous		1 pentamerous		1 pentamerous	0.98	1 equivocal (high rate)	0.5
Fagales	non-penta	<b>non-penta</b>	<b>0.99</b>	<b>non-penta</b>	<b>0.89</b>	<b>pentamerous</b>	<b>0.68</b>	1 equivocal (high rate)	0.5
Garryales	non-penta, pentamerous	non-penta		1 non-penta	0.98	non-penta	0.83	non-penta (high rate)	0.51
Gentianales	pentamerous	pentamerous		1 pentamerous		1 pentamerous	0.97	1 equivocal (high rate)	0.5
Geraniales	pentamerous	pentamerous		1 pentamerous		1 pentamerous	0.96	1 equivocal (high rate)	0.5
Huerteales	pentamerous	pentamerous		1 pentamerous		1 pentamerous	0.93	1 equivocal (high rate)	0.5
Icacinales	pentamerous	pentamerous		1 pentamerous	0.97	pentamerous	0.74	1 equivocal (high rate)	0.5
Lamiales	pentamerous	pentamerous		1 pentamerous		1 pentamerous	0.98	1 equivocal (high rate)	0.5
Laurales	non-penta	non-penta		1 non-penta		1 non-penta	0.99	1 equivocal (high rate)	0.5
Liliales	non-penta	non-penta		1 non-penta		1 non-penta	0.99	1 equivocal (high rate)	0.5
Magnoliales	non-penta	non-penta		1 non-penta		1 non-penta	0.94	1 equivocal (high rate)	0.5
Malpighiales	pentamerous	pentamerous		1 pentamerous		1 pentamerous		1 equivocal (high rate)	0.5
Malvales	pentamerous	pentamerous		1 pentamerous		1 pentamerous	0.97	1 equivocal (high rate)	0.5
Myrtales	pentamerous	pentamerous		1 pentamerous		1 pentamerous	0.91	1 equivocal (high rate)	0.5
Nymphaeales	non-penta	non-penta		1 non-penta		1 non-penta	0.9	1 equivocal (high rate)	0.5
Oxalidales	pentamerous	pentamerous		1 pentamerous		1 pentamerous	0.98	1 equivocal (high rate)	0.5
Pandanales	non-penta	non-penta		1 non-penta		1 non-penta	0.96	1 equivocal (high rate)	0.5
Paracryphiales	pentamerous	pentamerous		1 pentamerous	0.96	pentamerous	0.71	1 equivocal (high rate)	0.5
Petrosaviales	non-penta	non-penta		1 non-penta		1 non-penta	0.87	1 equivocal (high rate)	0.5
Piperales	non-penta	non-penta		1 non-penta		1 non-penta	0.96	1 equivocal (high rate)	0.5
Poales	non-penta	non-penta		1 non-penta		1 non-penta		1 equivocal (high rate)	0.5
Proteales	non-penta, pentamerous	non-penta	0.97	non-penta	0.97	non-penta	0.8	1 equivocal (high rate)	0.5
Ranunculales	non-penta	non-penta		1 non-penta		1 non-penta	0.95	1 equivocal (high rate)	0.5
Rosales	pentamerous	pentamerous		1 pentamerous		1 pentamerous	0.92	1 equivocal (high rate)	0.5

Santalales	pentamerous	pentamerous		1	pentamerous	0.98	pentamerous	0.83	equivocal (high rate)	0.5		
Sapindales	pentamerous	pentamerous		1	pentamerous		1	pentamerous	0.97	equivocal (high rate)	0.5	
Saxifragales	pentamerous	pentamerous		1	pentamerous		1	pentamerous	0.95	equivocal (high rate)	0.5	
Solanales	pentamerous	pentamerous		1	pentamerous		1	pentamerous	0.98	equivocal (high rate)	0.5	
Vitales	pentamerous	pentamerous	0.99		pentamerous	0.94		pentamerous	0.64	equivocal (high rate)	0.5	
Zingiberales	non-penta	non-penta		1	non-penta		1	non-penta		1	equivocal (high rate)	0.5
Zygophyllales	pentamerous	pentamerous		1	pentamerous		1	pentamerous	0.9		equivocal (high rate)	0.5

ORIGINAL DATA	Parsimony	State for q=0.0001	State probability	State for q=0.001	State probability	State for q=0.01	State probability	State for q=0.1	State probability			
Alismatales	non-penta	non-penta		1	non-penta		1	non-penta	0.99	equivocal (high rate)	0.5	
Apiales	pentamerous	pentamerous		1	pentamerous		1	pentamerous		1	equivocal (high rate)	0.5
Aquifoliales	pentamerous	pentamerous		1	pentamerous		1	pentamerous	0.91		equivocal (high rate)	0.5
Arecales	non-penta	non-penta		1	non-penta		1	non-penta	0.94		equivocal (high rate)	0.5
Asparagales	non-penta	non-penta		1	non-penta		1	non-penta		1	equivocal (high rate)	0.5
Asterales	pentamerous	pentamerous		1	pentamerous		1	pentamerous	0.99		equivocal (high rate)	0.5
Austrobaileyales	?	?	?	?	?	?	?	?	?	?	?	?
Berberidopsidales	non-penta, pentamerous	?	?	?	?	?	?	?	?	?	?	?
Boraginales	pentamerous	pentamerous		1	pentamerous		1	pentamerous	0.97		equivocal (high rate)	0.5
Brassicales	pentamerous	pentamerous		1	pentamerous		1	pentamerous	0.85		equivocal (high rate)	0.5
Bruniales	pentamerous	pentamerous		1	pentamerous		1	pentamerous	0.97		equivocal (high rate)	0.5
Buxales	non-penta	non-penta		1	non-penta		1	non-penta	0.74		equivocal (high rate)	0.5
Caneliales	non-penta	non-penta		1	non-penta		1	non-penta	0.97		equivocal (high rate)	0.5
Caryophyllales	pentamerous	pentamerous		1	pentamerous		1	pentamerous	0.99		equivocal (high rate)	0.5
Celastrales	pentamerous	pentamerous		1	pentamerous		1	pentamerous	0.86		equivocal (high rate)	0.5
Commelinales	non-penta	non-penta		1	non-penta		1	non-penta		1	equivocal (high rate)	0.5
Cornales	pentamerous	pentamerous		1	pentamerous	0.97		pentamerous	0.86		equivocal (high rate)	0.5
Crossosomatales	pentamerous	pentamerous		1	pentamerous		1	pentamerous	0.91		equivocal (high rate)	0.5
Cucurbitales	non-penta	non-penta	0.98		non-penta	0.86		equivocal	0.5		equivocal (high rate)	0.5
Dioscoreales	non-penta	non-penta		1	non-penta		1	non-penta	0.96		equivocal (high rate)	0.5
Dipsacales	pentamerous	pentamerous		1	pentamerous		1	pentamerous	0.97		equivocal (high rate)	0.5
Ericales	pentamerous	pentamerous		1	pentamerous		1	pentamerous	0.99		equivocal (high rate)	0.5
Escalloniales	pentamerous	pentamerous		1	pentamerous	0.99		pentamerous	0.93		equivocal (high rate)	0.5
Fabales	pentamerous	pentamerous		1	pentamerous		1	pentamerous	0.95		equivocal (high rate)	0.5
Fagales	non-penta	<b>non-penta</b>		<b>1 non-penta</b>	<b>0.99</b>		<b>1 pentamerous</b>	<b>0.53</b>			equivocal (high rate)	0.5
Garryales	non-penta, pentamerous	non-penta		1	non-penta	0.98		non-penta	0.83		non-penta (high rate)	0.51
Gentianales	pentamerous	pentamerous		1	pentamerous		1	pentamerous	0.94		equivocal (high rate)	0.5
Geraniales	pentamerous	pentamerous		1	pentamerous		1	pentamerous	0.9		equivocal (high rate)	0.5
Huerteales	pentamerous	pentamerous		1	pentamerous		1	pentamerous	0.88		equivocal (high rate)	0.5
Icacinales	pentamerous	pentamerous		1	pentamerous	0.97		pentamerous	0.74		equivocal (high rate)	0.5
Lamiales	pentamerous	pentamerous		1	pentamerous		1	pentamerous	0.96		equivocal (high rate)	0.5
Laurales	non-penta	non-penta		1	non-penta		1	non-penta	0.99		equivocal (high rate)	0.5
Liliales	non-penta	non-penta		1	non-penta		1	non-penta	0.99		equivocal (high rate)	0.5
Magnoliales	non-penta	non-penta		1	non-penta		1	non-penta	0.95		equivocal (high rate)	0.5
Malpighiales	pentamerous	pentamerous		1	pentamerous		1	pentamerous		1	equivocal (high rate)	0.5
Malvales	pentamerous	pentamerous		1	pentamerous		1	pentamerous	0.89		equivocal (high rate)	0.5
Myrtales	pentamerous	pentamerous		1	pentamerous		1	pentamerous	0.79		equivocal (high rate)	0.5
Nymphaeales	non-penta	non-penta		1	non-penta		1	non-penta	0.9		equivocal (high rate)	0.5
Oxalidales	pentamerous	pentamerous		1	pentamerous		1	pentamerous	0.93		equivocal (high rate)	0.5
Pandanales	non-penta	non-penta		1	non-penta		1	non-penta	0.96		equivocal (high rate)	0.5
Paracryphiales	pentamerous	pentamerous		1	pentamerous	0.96		pentamerous	0.71		equivocal (high rate)	0.5
Petrosaviales	non-penta	non-penta		1	non-penta		1	non-penta	0.87		equivocal (high rate)	0.5
Piperiales	non-penta	non-penta		1	non-penta		1	non-penta	0.97		equivocal (high rate)	0.5

Poales	non-penta	non-penta		1 non-penta		1 non-penta		1 equivocal (high rate)	0.5
Proteales	non-penta	non-penta		1 non-penta		1 non-penta	0.85	equivocal (high rate)	0.5
Ranunculales	non-penta	non-penta		1 non-penta		1 non-penta	0.96	equivocal (high rate)	0.5
Rosales	non-penta	<b>non-penta</b>	<b>0.99</b>	<b>non-penta</b>	<b>0.84</b>	<b>pentamerous</b>	<b>0.73</b>	equivocal (high rate)	0.5
Santalales	non-penta, pentamerous	non-penta	0.84	non-penta	0.82	non-penta	0.51	equivocal (high rate)	0.5
Sapindales	pentamerous	pentamerous		1 pentamerous		1 pentamerous	0.86	equivocal (high rate)	0.5
Saxifragales	pentamerous	pentamerous		1 pentamerous	0.99	pentamerous	0.82	equivocal (high rate)	0.5
Solanales	pentamerous	pentamerous		1 pentamerous		1 pentamerous	0.98	equivocal (high rate)	0.5
Vitales	pentamerous	pentamerous	0.99	pentamerous	0.94	pentamerous	0.61	equivocal (high rate)	0.5
Zingiberales	non-penta	non-penta		1 non-penta		1 non-penta		1 equivocal (high rate)	0.5
Zygophyllales	pentamerous	pentamerous		1 pentamerous		1 pentamerous	0.82	equivocal (high rate)	0.5

## Appendix IV.5: Parsimony and Mk1 with imposed rates for the 54 order nodes

**Differentiation**      **Confirmed state change**      *Suspected state change*  
q= transition rate      high rate= high transition rate equilibrium

RESCORED DATA	Parsimony	State for q=0.0001	State probability	State for q=0.001	State probability	State for q=0.01	State probability	State for q=0.1	State probability
Alismatales	undifferentiated	<b>undifferentiated</b>		<b>undifferentiated</b>	<b>0.75</b>	<b>differentiated</b>	<b>0.85</b>	equivocal (high rate)	0.5
Apiales	differentiated	differentiated		1 differentiated		1 differentiated		1 equivocal (high rate)	0.5
Aquifoliales	differentiated	differentiated		1 differentiated		1 differentiated	0.98	equivocal (high rate)	0.5
Arecales	undifferentiated, differentiated	<i>differentiated</i>	0.74	<i>differentiated</i>	0.81	<i>differentiated</i>	0.74	equivocal (high rate)	0.5
Asparagales	undifferentiated, differentiated	<b>undifferentiated</b>	<b>0.91</b>	<b>undifferentiated</b>	<b>0.63</b>	<b>differentiated</b>	<b>0.73</b>	equivocal (high rate)	0.5
Asterales	differentiated	differentiated		1 differentiated		1 differentiated		1 equivocal (high rate)	0.5
Austrobaileyales	undifferentiated, differentiated	<b>undifferentiated</b>	<b>0.96</b>	<b>undifferentiated</b>	<b>0.59</b>	<b>differentiated</b>	<b>0.79</b>	equivocal (high rate)	0.5
Berberidopsidales	differentiated	differentiated	0.99	differentiated	0.92	differentiated	0.58	equivocal (high rate)	0.5
Boraginales	differentiated	differentiated		1 differentiated		1 differentiated	0.99	equivocal (high rate)	0.5
Brassicales	differentiated	differentiated		1 differentiated		1 differentiated		1 equivocal (high rate)	0.5
Bruniales	differentiated	differentiated		1 differentiated		1 differentiated	0.97	equivocal (high rate)	0.5
Buxales	differentiated	differentiated		1 differentiated		1 differentiated	0.83	equivocal (high rate)	0.5
Canellales	differentiated	<i>differentiated</i>	0.85	<i>differentiated</i>	0.65	<i>differentiated</i>	0.9	equivocal (high rate)	0.5
Caryophyllales	differentiated	differentiated		1 differentiated		1 differentiated	0.9	equivocal (high rate)	0.5
Celastrales	differentiated	differentiated		1 differentiated		1 differentiated	0.95	equivocal (high rate)	0.5
Commelinales	differentiated	differentiated		1 differentiated		1 differentiated	0.97	equivocal (high rate)	0.5
Cornales	differentiated	differentiated		1 differentiated		1 differentiated	0.98	equivocal (high rate)	0.5
Crossosomatales	differentiated	differentiated		1 differentiated		1 differentiated	0.92	equivocal (high rate)	0.5
Cucurbitales	differentiated	differentiated	0.99	differentiated	0.99	differentiated	0.86	equivocal (high rate)	0.5
Dioscoreales	undifferentiated	<b>undifferentiated</b>		<b>undifferentiated</b>	<b>0.91</b>	<b>differentiated</b>	<b>0.59</b>	equivocal (high rate)	0.5
Dipsacales	differentiated	differentiated		1 differentiated		1 differentiated	0.99	equivocal (high rate)	0.5
Ericales	differentiated	differentiated		1 differentiated		1 differentiated		1 equivocal (high rate)	0.5
Escalloniales	differentiated	differentiated		1 differentiated		1 differentiated	0.97	equivocal (high rate)	0.5
Fabales	differentiated	differentiated		1 differentiated		1 differentiated	0.97	equivocal (high rate)	0.5
Fagales	undifferentiated	<b>undifferentiated</b>	<b>0.99</b>	<b>undifferentiated</b>	<b>0.89</b>	<b>differentiated</b>	<b>0.63</b>	equivocal (high rate)	0.5
Garryales	differentiated	differentiated		1 differentiated	0.98	differentiated	0.82	equivocal (high rate)	0.5
Gentianales	differentiated	differentiated		1 differentiated		1 differentiated	0.99	equivocal (high rate)	0.5
Geraniales	differentiated	differentiated		1 differentiated		1 differentiated	0.98	equivocal (high rate)	0.5
Huerteales	differentiated	differentiated	0.99	differentiated	0.88	differentiated	0.61	equivocal (high rate)	0.5
Icacinales	differentiated	differentiated		1 differentiated		1 differentiated	0.9	equivocal (high rate)	0.5
Lamiales	differentiated	differentiated		1 differentiated		1 differentiated	0.99	equivocal (high rate)	0.5
Laurales	undifferentiated	<b>undifferentiated</b>	<b>0.94</b>	<b>undifferentiated</b>	<b>0.53</b>	<b>differentiated</b>	<b>0.8</b>	equivocal (high rate)	0.5
Liliales	undifferentiated, differentiated	<b>undifferentiated</b>	<b>0.93</b>	<b>undifferentiated</b>	<b>0.61</b>	<b>differentiated</b>	<b>0.82</b>	equivocal (high rate)	0.5
Magnoliales	undifferentiated	<b>undifferentiated</b>	<b>0.93</b>	<b>differentiated</b>	<b>0.53</b>	<b>differentiated</b>	<b>0.87</b>	equivocal (high rate)	0.5
Malpighiales	differentiated	differentiated		1 differentiated		1 differentiated		1 equivocal (high rate)	0.5
Malvales	differentiated	differentiated		1 differentiated		1 differentiated	0.96	equivocal (high rate)	0.5
Myrtales	differentiated	differentiated		1 differentiated		1 differentiated	0.99	equivocal (high rate)	0.5
Nymphaeales	undifferentiated	<b>undifferentiated</b>	<b>0.96</b>	<b>undifferentiated</b>	<b>0.57</b>	<b>differentiated</b>	<b>0.8</b>	equivocal (high rate)	0.5
Oxalidales	differentiated	differentiated		1 differentiated		1 differentiated	0.99	equivocal (high rate)	0.5
Pandanales	undifferentiated	undifferentiated		1 undifferentiated		1 undifferentiated	0.84	equivocal (high rate)	0.5
Paracryphiales	differentiated	differentiated		1 differentiated	0.96	differentiated	0.72	equivocal (high rate)	0.5
Petrosaviales	differentiated	differentiated	0.97	differentiated	0.82	differentiated	0.79	equivocal (high rate)	0.5
Piperales	undifferentiated	<b>undifferentiated</b>	<b>0.98</b>	<b>undifferentiated</b>	<b>0.77</b>	<b>differentiated</b>	<b>0.63</b>	equivocal (high rate)	0.5
Poales	differentiated	differentiated		1 differentiated	0.99	differentiated	0.88	equivocal (high rate)	0.5
Proteales	differentiated	differentiated		1 differentiated		1 differentiated	0.97	equivocal (high rate)	0.5
Ranunculales	differentiated	differentiated		1 differentiated		1 differentiated	0.98	equivocal (high rate)	0.5
Rosales	differentiated	differentiated		1 differentiated	0.96	differentiated	0.78	equivocal (high rate)	0.5
Santalales	differentiated	differentiated		1 differentiated		1 differentiated	0.98	equivocal (high rate)	0.5

Sapindales	differentiated	differentiated	1	differentiated	1	differentiated	0.99	equivocal (high rate)	0.5
Saxifragales	differentiated	differentiated	1	differentiated	1	differentiated	0.99	equivocal (high rate)	0.5
Solanales	differentiated	differentiated	1	differentiated	1	differentiated	0.99	equivocal (high rate)	0.5
Vitales	differentiated	differentiated	1	differentiated	1	differentiated	0.87	equivocal (high rate)	0.5
Zingiberales	differentiated	differentiated	1	differentiated	1	differentiated	0.94	equivocal (high rate)	0.5
Zygophyllales	differentiated	differentiated	1	differentiated	1	differentiated	0.99	differentiated (high rate)	0.54

ORIGINAL DATA	Parsimony	State for q=0.0001	State probability	State for q=0.001	State probability	State for q=0.01	State probability	State for q=0.1	State probability
Alismatales	undifferentiated	<b>undifferentiated</b>		<b>undifferentiated</b>	<b>0.82</b>	<b>differentiated</b>	<b>0.82</b>	equivocal (high rate)	0.5
Apiales	differentiated	differentiated		1 differentiated		1 differentiated		1 equivocal (high rate)	0.5
Aquifoliales	differentiated	differentiated		1 differentiated		1 differentiated	0.98	equivocal (high rate)	0.5
Arecales	undifferentiated, differentiated	<i>differentiated</i>	0.74	<i>differentiated</i>	0.75	<i>differentiated</i>	0.72	equivocal (high rate)	0.5
Asparagales	undifferentiated, differentiated	<b>undifferentiated</b>	<b>0.91</b>	<b>undifferentiated</b>	<b>0.7</b>	<b>differentiated</b>	<b>0.68</b>	equivocal (high rate)	0.5
Asterales	differentiated	differentiated		1 differentiated		1 differentiated		1 equivocal (high rate)	0.5
Austrobaileyales	undifferentiated	<b>undifferentiated</b>	<b>0.97</b>	<b>undifferentiated</b>	<b>0.68</b>	<b>differentiated</b>	<b>0.78</b>	equivocal (high rate)	0.5
Berberidopsidales	differentiated	differentiated	0.99	differentiated	0.92	differentiated	0.58	equivocal (high rate)	0.5
Boraginales	differentiated	differentiated		1 differentiated		1 differentiated	0.99	equivocal (high rate)	0.5
Brassicales	differentiated	differentiated		1 differentiated		1 differentiated		1 equivocal (high rate)	0.5
Bruniales	differentiated	differentiated		1 differentiated		1 differentiated	0.97	equivocal (high rate)	0.5
Buxales	differentiated	differentiated		1 differentiated		1 differentiated	0.82	equivocal (high rate)	0.5
Caneliales	differentiated	<i>differentiated</i>	0.85	<i>differentiated</i>	0.58	<i>differentiated</i>	0.88	equivocal (high rate)	0.5
Caryophyllales	differentiated	differentiated		1 differentiated		1 differentiated	0.9	equivocal (high rate)	0.5
Celastrales	differentiated	differentiated		1 differentiated		1 differentiated	0.95	equivocal (high rate)	0.5
Commelinales	differentiated	differentiated		1 differentiated		1 differentiated	0.96	equivocal (high rate)	0.5
Cornales	differentiated	differentiated		1 differentiated		1 differentiated	0.98	equivocal (high rate)	0.5
Crossosomatales	differentiated	differentiated		1 differentiated		1 differentiated	0.89	equivocal (high rate)	0.5
Cucurbitales	differentiated	differentiated		1 differentiated	0.99	differentiated	0.86	equivocal (high rate)	0.5
Dioscoreales	undifferentiated	<b>undifferentiated</b>		<b>undifferentiated</b>	<b>0.93</b>	<b>differentiated</b>	<b>0.56</b>	equivocal (high rate)	0.5
Dipsacales	differentiated	differentiated		1 differentiated		1 differentiated	0.99	equivocal (high rate)	0.5
Ericales	differentiated	differentiated		1 differentiated		1 differentiated		1 equivocal (high rate)	0.5
Escalloniales	differentiated	differentiated		1 differentiated		1 differentiated	0.97	equivocal (high rate)	0.5
Fabales	differentiated	differentiated		1 differentiated		1 differentiated	0.96	equivocal (high rate)	0.5
Fagales	undifferentiated	<b>undifferentiated</b>	<b>0.99</b>	<b>undifferentiated</b>	<b>0.89</b>	<b>differentiated</b>	<b>0.62</b>	equivocal (high rate)	0.5
Garryales	differentiated	differentiated		1 differentiated	0.98	differentiated	0.82	equivocal (high rate)	0.5
Gentianales	differentiated	differentiated		1 differentiated		1 differentiated	0.99	equivocal (high rate)	0.5
Geraniales	differentiated	differentiated		1 differentiated		1 differentiated	0.98	equivocal (high rate)	0.5
Huerteales	differentiated	differentiated	0.99	differentiated	0.88	differentiated	0.61	equivocal (high rate)	0.5
Icacinales	differentiated	differentiated		1 differentiated		1 differentiated	0.9	equivocal (high rate)	0.5
Lamiales	differentiated	differentiated		1 differentiated		1 differentiated	0.99	equivocal (high rate)	0.5
Laurales	undifferentiated	<b>undifferentiated</b>	<b>0.96</b>	<b>undifferentiated</b>	<b>0.64</b>	<b>differentiated</b>	<b>0.76</b>	equivocal (high rate)	0.5
Liliales	undifferentiated, differentiated	<b>undifferentiated</b>	<b>0.94</b>	<b>undifferentiated</b>	<b>0.69</b>	<b>differentiated</b>	<b>0.78</b>	equivocal (high rate)	0.5
Magnoliales	undifferentiated	<b>undifferentiated</b>	<b>0.95</b>	<b>undifferentiated</b>	<b>0.57</b>	<b>differentiated</b>	<b>0.86</b>	equivocal (high rate)	0.5
Malpighiales	differentiated	differentiated		1 differentiated		1 differentiated		1 equivocal (high rate)	0.5
Malvales	differentiated	differentiated		1 differentiated		1 differentiated	0.96	equivocal (high rate)	0.5
Myrtales	differentiated	differentiated		1 differentiated		1 differentiated	0.99	equivocal (high rate)	0.5
Nymphaeales	undifferentiated	<b>undifferentiated</b>	<b>0.97</b>	<b>undifferentiated</b>	<b>0.66</b>	<b>differentiated</b>	<b>0.79</b>	equivocal (high rate)	0.5
Oxalidales	differentiated	differentiated		1 differentiated		1 differentiated	0.99	equivocal (high rate)	0.5
Pandanales	undifferentiated	undifferentiated		1 undifferentiated		1 undifferentiated	0.85	equivocal (high rate)	0.5
Paracryphiales	differentiated	differentiated		1 differentiated	0.96	differentiated	0.72	equivocal (high rate)	0.5
Petrosaviales	differentiated	differentiated	0.97	differentiated	0.8	differentiated	0.77	equivocal (high rate)	0.5
Piperales	undifferentiated	<b>undifferentiated</b>	<b>0.98</b>	<b>undifferentiated</b>	<b>0.82</b>	<b>differentiated</b>	<b>0.62</b>	equivocal (high rate)	0.5
Poales	differentiated	differentiated		1 differentiated	0.95	differentiated	0.83	equivocal (high rate)	0.5
Proteales	differentiated	differentiated		1 differentiated		1 differentiated	0.97	equivocal (high rate)	0.5

Ranunculales	differentiated	differentiated		1 differentiated		1 differentiated	0.97	equivocal (high rate)	0.5
Rosales	differentiated	differentiated	0.99	differentiated	0.94	differentiated	0.75	equivocal (high rate)	0.5
Santalales	differentiated	differentiated		1 differentiated		1 differentiated	0.98	equivocal (high rate)	0.5
Sapindales	differentiated	differentiated		1 differentiated		1 differentiated	0.99	equivocal (high rate)	0.5
Saxifragales	differentiated	differentiated		1 differentiated		1 differentiated	0.98	equivocal (high rate)	0.5
Solanales	differentiated	differentiated		1 differentiated		1 differentiated	0.99	equivocal (high rate)	0.5
Vitales	differentiated	differentiated		1 differentiated		1 differentiated	0.87	equivocal (high rate)	0.5
Zingiberales	differentiated	differentiated		1 differentiated		1 differentiated	0.94	equivocal (high rate)	0.5
Zygophyllales	differentiated	differentiated		1 differentiated		1 differentiated	0.99	differentiated (high rate)	0.54

Appendix IV.6: Parsimony steps, Mk1 and Mk2 transition rates, Mk2 rate symmetry ratios, logLikelihoods and AICs for the full tree an partitions

**Symmetry** q= transition rate; 0= actinomorphic; 1= zygomorphic

RESCORED DATA	Angiosperms	ANA+Mangoliidae	Monocotyledoneae	Basal eudicots	Superrosidae+Dillenales	Superasteridae	Mixed partions
Parsi steps	209	3	40	23	55	88	
Mk1 q	0,0064	0,0008	0,1041	0,2640	0,0043	0,0084	
Mk1 logLik	-691,03	-14,41	-114,34	-68,61	-189,38	-279,59	-666,33
Mk1 AIC	1384,06	30,82	230,68	139,22	380,76	561,18	1342,66
Mk2 q01	0,0061	0,0058	0,0663	0,0116	0,0044	0,01	
Mk2 q10	0,0257	0,1116	0,1294	0,0341	0,0217	0,03	
symmetry ratio	0,24	0,05	0,51	0,34	0,20	0,28	
Mk2 logLik	-612,85	-11,91	-105,67	-64,78	-169,21	-248,46	-600,03
Mk2 AIC	1229,70	27,82	215,34	133,56	342,42	500,92	1220,06
delta AIC	154,36	3,00	15,34	5,66	38,34	60,26	122,60

Delta AIC angio-mixed (Mk1)= 41,4

Delta AIC angio-mixed (Mk2)= 9,64

too high for state inference

ORIGINAL DATA	Angiosperms	ANA+Mangoliidae	Monocotyledoneae	Basal eudicots	Superrosidae+Dillenales	Superasteridae	Mixed total
Parsi steps	190	3	38	20	54	75	
Mk1 q	0,0055	0,0008	0,1261	0,0305	0,0042	0,0064	
Mk1 logLik	-643,88	-14,32	-112,28	-65,35	-186,83	-248,54	-627,32
Mk1 AIC	1289,76	30,64	226,56	132,70	375,66	499,08	1264,64
Mk2 q01	0,0056	0,0060	0,0992	0,0080	0,0041	0,0074	
Mk2 q10	0,0247	0,1104	0,2400	0,0314	0,0211	0,0280	
symmetry ratio	0,23	0,05	0,41	0,25	0,19	0,26	
Mk2 logLik	-577,57	-11,80	-102,41	-59,42	-166,75	-225,53	-565,91
Mk2 AIC	1159,14	27,60	208,82	122,84	337,50	455,06	1151,82
delta AIC	130,62	3,04	17,74	9,86	38,16	44,02	112,82

Delta AIC Angio-mixed (Mk1)= 25,12

Delta AIC Angio-mixed (Mk2)= 7,32

too high for state inference

Appendix IV.6: Parismony steps, Mk1 and Mk2 transition rates, Mk2 rate symmetry ratios, logLikelihoods and AICs for the full tree an partitions

**Fusion** q= transition rate; 0= free; 1= fused

RESCORED DATA	Angiosperms	ANA+Mangoliidae	Monocotyledoneae	Basal eudicots	Superrosidae+Dillenales	Superasteridae	Mixed partions
Parsi steps	212	19	38	16	77	61	
Mk1 q	0,0102	7,1730	0,0149	0,0090	0,0717	0,0059	
Mk1 logLik	-550,34	-45,05	-93,93	-56,13	-158,64	-176,43	-530,18
Mk1 AIC	1102,68	92,10	189,86	114,26	319,28	354,86	1070,36
Mk2 q01	0,0108	4,0140	0,0127	0,0071	0,0717	0,0106	
Mk2 q10	0,0093	7,8470	0,0194	0,0435	0,0709	0,0057	
symmetry ratio	0,86	0,51	0,65	0,16	0,99	0,54	
Mk2 logLik	-549,44	-41,60	-91,99	-46,02	-158,63	-174,69	-512,93
Mk2 AIC	1102,88	87,20	187,98	96,04	321,26	353,38	1045,86
delta AIC	0,20	4,90	1,88	18,22	1,98	1,48	24,50

Delta AIC Anglo-mixed (Mk1)= 32,32

Delta AIC Anglo-mixed (Mk2)= 57,02

too high for state inference

ORIGINAL DATA	Angiosperms	ANA+Mangoliidae	Monocotyledoneae	Basal eudicots	Superrosidae+Dillenales	Superasteridae	Mixed partions
Parsi steps	154	13	34	6	53	47	
Mk1 q	0,0072	0,0058	0,0120	0,0026	0,0112	0,0057	
Mk1 logLik	-429,57	-37,00	-85,49	-31,30	-128,23	-141,02	-423,04
Mk1 AIC	861,14	76,00	172,98	64,60	258,46	284,04	856,08
Mk2 q01	0,0069	0,3555	0,0121	0,0028	0,0306	0,0096	
Mk2 q10	0,0084	0,9481	0,0222	0,0506	0,0470	0,0028	
symmetry ratio	0,82	0,37	0,55	0,06	0,65	0,29	
Mk2 logLik	-428,80	-32,23	-81,91	-22,80	-125,76	-138,92	-401,62
Mk2 AIC	861,60	68,46	167,82	49,60	255,52	281,84	823,24
delta AIC	0,46	7,54	5,16	15,00	2,94	2,20	32,84

Delta AIC Anglo-mixed (Mk1)= 5,06

Delta AIC Anglo-mixed (Mk2)= 38,36

too high for state inference

Appendix IV.6: Parsimony steps, Mk1 and Mk2 transition rates, Mk2 rate symmetry ratios, logLikelihoods and AICs for the full tree an partitions

**Phyllotaxy** q= transition rate; 0= whorled; 1= spiral

RESCORED DATA	Angiosperms	ANA+Mangoliidae	Monocotyledoneae	Basal eudicots	Superrosidae+Dillenales	Superasteridae	Mixed partitions
Parsi steps	30	11	0	12	4	3	
Mk1 q	0,0006	0,0035	0,0000	0,0050	0,0002	0,0002	
Mk1 logLik	-149,96	-35,07	-0,69	-39,30	-23,85	-18,74	-117,65
Mk1 AIC	301,92	72,14	3,38	80,60	49,70	39,48	245,30
Mk2 q01	0,0003	0,0034	0,0000	0,0034	0,0008	0,0002	
Mk2 q10	0,0146	0,0030	0,0000	0,0179	0,0719	0,0182	
symmetry ratio	0,02	0,88	695370,37	0,19	0,01	0,01	
Mk2 logLik	-123,39	-36,06	0,00	-34,50	-22,24	-17,22	-110,02
Mk2 AIC	250,78	76,12	4,00	73,00	48,48	38,44	240,04
delta AIC	51,14	3,98	0,62	7,60	1,22	1,04	5,26

Delta AIC Anglo-mixed (Mk1)= 56,62

Delta AIC Anglo-mixed (Mk2)= 10,74

no state changes in clade

ORIGINAL DATA	Angiosperms	ANA+Mangoliidae	Monocotyledoneae	Basal eudicots	Superrosidae+Dillenales	Superasteridae	Mixed partitions
Parsi steps	28	11	0	11	3	3	
Mk1 q	0,0005	0,0036	0,0000	0,0045	0,0002	0,0002	
Mk1 logLik	-139,04	-34,08	-0,69	-35,69	-18,37	-18,74	-107,57
Mk1 AIC	280,08	70,16	3,38	73,38	38,74	39,48	225,14
Mk2 q01	0,0002	0,0035	0,0000	0,0035	0,0005	0,0002	
Mk2 q10	0,0152	0,0030	0,0000	0,0222	0,0663	0,0182	
symmetry ratio	0,01	0,86	695370,37	0,16	0,01	0,01	
Mk2 logLik	-114,17	-34,07	0,00	-30,95	-17,53	-17,22	-99,77
Mk2 AIC	232,34	72,14	4,00	65,90	39,06	38,44	219,54
delta AIC	47,74	1,98	0,62	7,48	0,32	1,04	5,60

Delta AIC Anglo-mixed (Mk1)= 54,94

Delta AIC Anglo-mixed (Mk2)= 12,80

no state changes in clade

Appendix IV.6: Parismony steps, Mk1 and Mk2 transition rates, Mk2 rate symmetry ratios, logLikelihoods and AICs for the full tree an partitions

**Pentamery** q= transition rate; 0= non-pentamerous; 1= pentamerous

<b>RESCORED DATA</b>	Angiosperms	ANA+Mangoliidae	Monocotyledoneae	Basal eudicots	Superrosidae+Dilliales	Superasteridae	Mixed partions
Parsi steps	227	6	1	9	103	107	
Mk1 q	0,0069	0,0024	0,0001	0,0042	0,0117	0,0110	
Mk1 logLik	-658,73	-21,56	-7,43	-34,05	-251,51	-284,84	-599,39
Mk1 AIC	1319,46	45,12	16,86	70,10	505,02	571,68	1208,78
Mk2 q01	0,0028	0,0041	0,0005	0,0020	0,0250	0,0421	
Mk2 q10	0,0074	0,0224	0,0719	0,0139	0,0136	0,0165	
symmetry ratio	0,38	0,18	0,01	0,14	0,54	0,39	
Mk2 logLik	-646,16	-19,90	-6,08	-29,38	-241,21	-261,06	-557,63
Mk2 AIC	1296,32	43,80	16,16	62,76	486,42	526,12	1135,26
delta AIC	23,14	1,32	0,70	7,34	18,60	45,56	73,52

Delta AIC Anglo-mixed (Mk1)= 110,68

Delta AIC Anglo-mixed (Mk2)= 161,06

<b>ORIGINAL DATA</b>	Angiosperms	ANA+Mangoliidae	Monocotyledoneae	Basal eudicots	Superrosidae+Dilliales	Superasteridae	Mixed partions
Parsi steps	199	3	1	7	89	98	
Mk1 q	0,0064	0,0012	0,0001	0,0032	0,0119	0,0107	
Mk1 logLik	-584,45	-13,04	-7,43	-29,16	-216,12	-256,56	-522,31
Mk1 AIC	1170,90	28,08	16,86	60,32	434,24	515,12	1054,62
Mk2 q01	0,0018	0,0041	0,0005	0,0013	0,0170	0,0283	
Mk2 q10	0,0077	0,0537	0,0719	0,0120	0,0113	0,0128	
symmetry ratio	0,23	0,08	0,01	0,11	0,66	0,45	
Mk2 logLik	-563,30	-10,85	-6,08	-24,38	-213,30	-244,00	-498,61
Mk2 AIC	1130,60	25,70	16,16	52,76	430,60	492,00	1017,22
delta AIC	40,30	2,38	0,70	7,56	3,64	23,12	37,40

Delta AIC Anglo-mixed (Mk1)= 116,28

Delta AIC Anglo-mixed (Mk2)= 113,38

Appendix IV.6: Parismony steps, Mk1 and Mk2 transition rates, Mk2 rate symmetry ratios, logLikelihoods and AICs for the full tree an partitions

**Differentiation** q= transition rate; 0= undifferentiated; 1= differentiated

<b>RESCORED DATA</b>	Angiosperms	ANA+Mangoliidae	Monocotyledoneae	Basal eudicots	Superrosidae+Dillenales	Superasteridae	Mixed partions
Parsi steps	165	20	43	14	54	33	
Mk1 q	0,0042	0,0139	0,0130	0,0057	0,0037	0,0021	
Mk1 logLik	-524,02	-39,05	-104,21	-42,52	-177,89	-132,72	-496,39
Mk1 AIC	1050,04	80,10	210,42	87,04	357,78	267,44	1002,78
Mk2 q01	0,0095	0,0144	0,0136	0,0022	0,0106	0,0100	
Mk2 q10	0,0029	0,0136	0,0125	0,0060	0,0036	0,0014	
symmetry ratio	0,31	0,94	0,92	0,37	0,34	0,14	
Mk2 logLik	-501,41	-39,03	-104,14	-41,91	-173,76	-124,44	-483,28
Mk2 AIC	1006,82	82,06	212,28	87,82	351,52	252,88	986,56
delta AIC	43,22	1,96	1,86	0,78	6,26	14,56	16,22

Delta AIC Anglo-mixed (Mk1)= 47,26

Delta AIC Anglo-mixed (Mk2)= 20,26

<b>ORIGINAL DATA</b>	Angiosperms	ANA+Mangoliidae	Monocotyledoneae	Basal eudicots	Superrosidae+Dillenales	Superasteridae	Mixed partions
Parsi steps	161	19	42	14	53	32	
Mk1 q	0,0042	0,0139	0,0130	0,0057	0,0036	0,0020	
Mk1 logLik	-514,86	-37,05	-102,44	-42,52	-175,52	-129,59	-487,12
Mk1 AIC	1031,72	76,10	206,88	87,04	353,04	261,18	984,24
Mk2 q01	0,0091	0,0143	0,0133	0,0022	0,0098	0,0090	
Mk2 q10	0,0029	0,0137	0,0123	0,0060	0,0035	0,0015	
symmetry ratio	0,32	0,96	0,92	0,37	0,36	0,17	
Mk2 logLik	-493,56	-37,04	-102,42	-41,91	-171,74	-122,76	-475,87
Mk2 AIC	991,12	78,08	208,84	87,82	347,48	249,52	971,74
delta AIC	40,60	1,98	1,96	0,78	5,56	11,66	12,50

Delta AIC Anglo-mixed (Mk1)= 47,48

Delta AIC Anglo-mixed (Mk2)= 19,38

Appendix IV.7 : Results and discussion for original data.

*Full tree and partitions under Mk1 and Mk2.* When compared with the rescored data, fusion and pentamery are both more prone to give different results between reconstructions. These characters are the ones with the largest number of species that have states affected by rescaling. The nodes in which there are difference between the two type of data tend to be ancestors of clades in which there are several rescored species. As in the rescored results, the most frequently found difference in inferred state are between the Mk2 full and partitioned trees and the Mk1 and Mk2 full trees. One of the differences compared to the rescored data is a more frequent occurrence of differences between the Mk1 and Mk2 partitioned trees.

Table IV.S1: Number of changes in inferred most likely ancestral state between models and tree types in the key node series. Each column compares the results of two of the model and tree type combinations and reports the number of nodes that change most likely state in each case. Mk1= one rate maximum likelihood model; Mk2= two-rate maximum likelihood model; full= full tree; part= partitioned tree.

15 nodes (full) 13 nodes (part)	<b>Mk1</b> (full) ≠ (part)	<b>Mk2</b> (full) ≠ (part)	<b>(full)</b> Mk1 ≠ Mk2	<b>(part)</b> Mk1 ≠ Mk2
fusion	2	2	0	4
symmetry	0	0	1	0
phyllotaxy	0	4	5	0
pentamery	0	4	0	4
differentiation	3	2	6	0

Table IV.S2: Number of changes in inferred most likely ancestral state between models and tree types in the order series. Each column compares the results of two of the model and tree

type combinations and reports the number of nodes that change most likely state in each case.

Mk1= one rate maximum likelihood model; Mk2= two-rate maximum likelihood model; full= full tree; part= partitioned tree.

54 nodes	<b>Mk1</b> (full) ≠ (part)	<b>Mk2</b> (full) ≠ (part)	<b>(full)</b> Mk1 ≠ Mk2	<b>(part)</b> Mk1 ≠ Mk2
fusion	1	7	1	6
symmetry	0	2	6	2
phyllotaxy	1	7	6	0
pentamery	3	10	4	9
differentiation	5	8	15	2

Most inferred states are identical to those inferred by parsimony or can be both states according to parsimony. At key node level (13-15 nodes), nodes in which at least one of the maximum likelihood estimates does not match the parsimony state are four in fusion, one in symmetry, five in phyllotaxy, four in pentamery, four in differentiation. This is a few more than with rescored data, but still in the same order of magnitude. At order node level (54 nodes) mismatch between parsimony and at least one of the tested maximum likelihood inferred states are five in fusion, four in symmetry, six in phyllotaxy, three in pentamery and 13 in differentiation. This is a higher but close total number than in the rescored data as well.

*Constrained Mk1 rates.* At key node level, only differentiation has nodes that change most probable states depending on transition rates (Table IV.S3). At order node level, fusion, phyllotaxy and pentamery all have fewer state-changing node than differentiation, while symmetry has none (Table IV.S4).

Table IV.S3: Number of nodes confirmed or suspected to state changes under different Mk1 rates (Key nodes). Confirmed: observed change in most probable state. Suspected: state change not observed, but shared evolution pattern.

<b>15 nodes</b>	<b>Total</b>	<b>Confirmed</b>	<b>Suspected</b>
fusion	0	0	0
symmetry	0	0	0
phyllotaxy	0	0	0
pentamery	0	0	0
differentiation	5	4	1

Table IV.S4: Number of nodes confirmed or suspected to state changes under different Mk1 rates (Order nodes). Confirmed: observed change in most probable state. Suspected: state change not observed, but shared evolution pattern.

<b>54 nodes</b>	<b>Total</b>	<b>Confirmed</b>	<b>Suspected</b>
fusion	2	2	0
symmetry	0	0	0
phyllotaxy	1	1	0
pentamery	2	2	0
differentiation	12	10	2

All the fusion nodes suspected to be state-changing with the rescored data show a what seems to be a simple probability decrease pattern with the original data. The state-changing node numbers for symmetry, phyllotaxy and differentiation remain the same and are the same nodes as in the rescored data.

*Original and rescored data.* When comparing the original and rescored versions of the Mk1 and Mk2 full and partitioned trees, the differences between the two are rare. They are the most frequently found in fusion and pentamery. The nodes that display differences tend to be ancestors of clades in which there are several rescored species. The Mk2 partitioned tree has the most frequent difference between the original data and the rescored data.

Table IV.S5: Number of changes in inferred most likely ancestral state between models and tree types in the key node series. Each column compares the results of two of the model and tree type combinations and reports the number of nodes that change most likely state between rescored and original data in each case. Mk1= one rate maximum likelihood model; Mk2= two-rate maximum likelihood model; full= full tree; part= partitioned tree; rescor= rescored used in article; orig= data as it was entered in PROTEUS.

15 nodes (full) 12 nodes (part)	<b>Mk1 (full)</b> (rescor) ≠ (orig)	<b>Mk2 (full)</b> (rescor) ≠ (orig)	<b>Mk1 (part)</b> (rescor) ≠ (orig)	<b>Mk2 (part)</b> (rescor) ≠ (orig)
fusion	1	1	0	4
symmetry	0	0	0	0
phyllotaxy	0	0	0	0
pentamery	0	0	0	4
differentiation	0	0	0	0

Table IV.S6: Number of changes in inferred most likely ancestral state between models and tree types in the order node series. Each column compares the results of two of the model and tree type combinations and reports the number of nodes that change most likely state between rescored and original data in each case. Mk1= one rate maximum likelihood model; Mk2= two-rate maximum likelihood model; full= full tree; part= partitioned tree; rescor= rescored used in article; orig= data as it was entered in PROTEUS.

54 nodes	<b>Mk1 (full)</b> (rescor) ≠ (orig)	<b>Mk2 (full)</b> (rescor) ≠ (orig)	<b>Mk1 (part)</b> (rescor) ≠ (orig)	<b>Mk2 (part)</b> (rescor) ≠ (orig)
fusion	2	2	2	7
symmetry	1	1	0	0
phyllotaxy	0	1	0	0
pentamery	3	1	0	10
differentiation	0	0	0	1

# Synthèse

Les fleurs, qui sont les structures reproductrices caractérisant le grand clade des Angiospermes, présentent des formes morphologiques très diverses. Parmi les nombreux attributs morphologiques des fleurs, certains sont souvent trouvés dans des clades fortement diversifiés en termes de nombre d'espèces. Ces attributs sont par conséquent suspectés d'être la cause de la diversité de ces clades. De tels attributs, dont l'apparition semble corrélée à une plus forte diversification des espèces, sont appelés innovations clés. Toutefois, prouver la nature d'innovation clé d'un attribut donné peut s'avérer compliqué, car d'autres facteurs peuvent expliquer une apparente corrélation entre un attribut morphologique et la diversité des espèces. Prouver qu'un attribut morphologique est une innovation clé inclut la reconstruction d'états ancestraux, qui permet d'identifier à quels endroits de l'arbre phylogénétique l'attribut est apparu.

Cette thèse fait partie du projet eFLOWER, dont les objectifs incluent la reconstruction de la morphologie des fleurs dans les nœuds profonds de l'arbre des angiospermes et quelles innovations florales sont liées à des augmentations majeures des taux de diversification. L'un des outils utilisés pour cet objectif est la base de données collaborative pour traits morphologiques floraux PROTEUS, qui a également été beaucoup utilisée pour cette thèse.

Cette thèse se focalise sur l'évolution des caractères floraux chez les angiospermes et leur impact sur la diversification des espèces. Le premier chapitre vise à tester la nature d'innovation clé de deux états de caractère, la symétrie florale bilatérale et la présence dans les hotspots à climat Méditerranéen, au sein de la famille des Proteaceae. Le second chapitre présente une reconstruction de l'évolution de la symétrie du périanthe sur un arbre des Angiospermes dont l'échantillonnage des espèces est orienté vers les changements d'états présumés. Il passe également en revue certaines propriétés de la symétrie bilatérale du périanthe. Le troisième chapitre relève d'une approche similaire, mais avec un échantillonnage basé sur cinq caractères du périanthe : la symétrie, la fusion, la phyllotaxie, la pentamérie et la différenciation. Il pose aussi la question de l'impact de l'hétérogénéité des taux de transition au sein des Angiospermes sur les états ancestraux reconstruits à différentes échelles taxonomiques.

## Chapitre 1 – La présence dans les hotspots à climat Méditerranéen et la symétrie florale affectent les taux de spéciation et d’extinction chez les Proteaceae

Le premier chapitre de ma thèse se focalise sur la famille des Proteaceae (1750 espèces), un clade de plantes à fleurs, dont la majorité des espèces se trouvent dans des hotspots à climat Méditerranéen, qui sont connus pour avoir une diversité spécifique très élevée. Un précédent article avait montré l’existence d’une corrélation entre la présence dans les hotspots et des taux de diversification élevés dans la famille. La famille des Proteaceae inclut par ailleurs de nombreuses espèces avec un périanthe présentant une symétrie bilatérale, un trait de caractère qui est corrélé avec de forts taux de diversification chez les Angiospermes. Deux méthodes d’analyse de la diversification des espèces ont été testées sur ces deux caractères pour vérifier si la présence dans les hotspots et la symétrie bilatérale avaient un impact sur la diversification des espèces au sein de la famille. La famille a été choisie pour ce test parce que la diversité de chaque genre et les relations de parenté entre eux sont bien connues. La morphologie florale des espèces est bien connue également ; les fleurs ont un plan d’organisation conservé (quatre tépales, quatre étamines opposées aux tépales et un ovaire unicarpellé).

La symétrie bilatérale est aussi connue sous le nom de zygomorphie et est un état de caractère caractéristique de plusieurs grandes familles d’Angiospermes, telles que les Fabaceae et les Orchidaceae. Il a été suggéré précédemment que la zygomorphie est une innovation-clé au sein des Angiospermes. La première méthode que j’ai utilisée pour analyser la diversification des espèces est MEDUSA, qui permet de détecter des changements dans le taux de diversification au sein d’un arbre phylogénétique. La seconde méthode que j’ai employée est le modèle BiSSE, qui permet de tester si un état de caractère donné est associé à des taux de diversification des espèces plus élevés qu’un autre état de caractère.

Les résultats de MEDUSA ont permis de mettre en évidence au moins cinq changements probables des taux de diversification au sein des Proteaceae : quatre augmentations et une diminution du taux de diversification. Ces résultats, bien que suggérant quels clades pouvaient avoir bénéficié d’une innovation-clé, sont indépendants de l’état de caractère des espèces et montrent seulement que des changements de taux de diversification ont eu lieu. Les résultats de BiSSE ont montré que les espèces trouvées dans les hotspots à climat Méditerranéen ont un taux de diversification significativement plus élevé que celui des espèces trouvées uniquement hors des hotspots. Ils ont également montré que le taux de transition de hotspot à région non-hotspot est beaucoup plus élevé que le taux de transition

allant de non-hotspot à hotspot. De plus, j'ai observé que le taux de diversification des clades non-hotspot était négatif et que le taux de transition net entre les hotspots et les régions non-hotspot était assez élevé pour compenser la perte d'espèces des régions non-hotspot. En d'autres termes, mes résultats suggèrent que les régions à non-hotspot sont globalement un puits macroévolutif pour les Proteaceae, c'est-à-dire un état de caractère qui cause une extinction globale chez les espèces qui le présentent, mais est acquis par de nouvelles espèces assez fréquemment (ici par migration) pour compenser la perte des espèces qui s'éteignent. Les résultats pour la symétrie du périanthe ont montré que la zygomorphie du périanthe n'a pas d'impact discernable sur la diversification, mais qu'il y a un changement plus fréquent d'actinomorphe (symétrie radiaire) à zygomorphe que de zygomorphe à actinomorphe. Nous en avons conclu que les Proteaceae étaient une exception à la règle (de l'impact de la symétrie sur la diversification chez les Angiospermes) et que d'autres caractéristiques de la famille pourraient avoir une plus grande importance pour expliquer la diversification des espèces que la zygomorphie des fleurs individuelles.

Cet article a été publié à peu près en même temps que deux articles discréditant l'approche BiSSE et d'autres méthodes qui en étaient dérivées. Le premier d'entre eux était accepté, mais pas encore publié au moment où le premier chapitre était en revue. Du point de vue de mon étude, ce premier article prévenait simplement d'un piège potentiel de BiSSE. Ce piège était de conclure qu'un état de caractère avait un impact sur le taux de diversification même quand il n'apparaissait que dans un seul clade qui se trouvait avoir un taux de diversification élevé. Pour donner du soutien à mes résultats, j'ai démontré via une reconstruction d'états ancestraux par maximum de vraisemblance que les espèces vivant dans des hotspots Méditerranéens et les espèces à périanthe zygomorphe avaient plusieurs origines indépendantes pour chacun des deux caractères. Cela montrait qu'aucun des aspects du piège potentiel ne s'appliquaient aux données de la présente étude. Un second article publié plus tard a démontré que l'approche BiSSE et ses variantes avaient tendance à conclure que des états de caractère neutres avaient un impact sur la diversification. Dans le cas de la présente étude, toutefois, la symétrie du périanthe (qui n'a aucun effet sur la diversification) a été démontrée comme étant neutre chez les Proteaceae et le climat non-hotspot s'est trouvé être un puits macroévolutif plutôt que d'être simplement un état dans lequel les espèces se diversifiaient moins vite que les espèces se trouvant dans un hotspot. Par conséquent, ces deux résultats reflètent probablement une réalité biologique.

Suite à la présente étude, j'ai reconstruit un nouvel arbre daté (chronogramme) des Proteaceae à l'échelle de l'espèce avec un échantillonnage proportionnel à la diversité de chaque genre, ce qui nous a permis d'établir un scénario plus fin pour l'évolution de la symétrie du périanthe au sein de la famille. Cette étude est plus tard devenue une partie d'un article co-signé notamment par mes directeurs de thèse Sophie Nadot et Hervé Sauquet et moi-même. J'ai construit le jeu de données au niveau des espèces avec l'aide de Peter Weston et j'étais en charge de la reconstruction phylogénétique et de la datation. La reconstruction par parcimonie a résulté en un minimum de 10 origines et 12 reversions. Le maximum de vraisemblance a résulté en 16 origines de la zygomorphie et six reversions vers l'actinomorphie.

## Chapitre 2 – La symétrie du périanthe a changé au moins 199 fois au cours de l'évolution des angiospermes

Le second chapitre de ma thèse avait initialement pour objectif de tester si la zygomorphie était bel et bien une innovation-clé chez les Angiospermes. Toute méthode que j'aurais pu utiliser pour cela nécessitait de connaître la distribution de la zygomorphie au sein de l'arbre phylogénétique des Angiospermes. Les optimisations existantes de la symétrie florale sur des arbres phylogénétiques demeuraient incomplètes, donc j'ai décidé de produire ma propre reconstruction. La première étape a donc consisté à identifier où la zygomorphie était apparue dans l'arbre, ainsi qu'où elle était retournée à l'état ancestral d'actinomorphie. Deux de mes impératifs pour ma stratégie d'échantillonnage de cette phylogénie étaient de refléter la diversité taxonomique des Angiospermes et d'inclure autant de changements présumés de la symétrie du périanthe que possible. J'ai choisi de représenter les familles monomorphes pour la symétrie du périanthe par une seule espèce, tandis que les familles dans lesquelles la symétrie du périanthe changeait au moins une fois entre actinomorphe et zygomorphe ont été représentées par le nombre d'espèces nécessaire pour montrer tous les changements subis par la famille. Pour garder la charge de travail raisonnable, les genres polymorphes n'ont jamais été représentés par plus d'une espèce dans chaque état. Le point de départ de mon échantillonnage était un précédent article, qui était la phylogénie datée incluant le plus grand nombre de familles d'angiospermes actuellement reconnues et dont les espèces avaient déjà été codées dans la base de données PROTEUS, dans le cadre du projet eFLOWER. Dans le processus de la construction de mon propre échantillon d'espèces, plusieurs espèces ont été ajoutées ou enlevées de cette phylogénie de départ pour diverses

raisons. Tout d'abord, j'ai ajouté des espèces venant de familles qui n'étaient pas échantillonnées dans l'arbre de départ. Ensuite, j'ai ajouté des représentants de l'autre état dans des familles qui étaient polymorphes pour la symétrie du périanthe, mais avaient des espèces dans un seul état dans l'arbre de départ. Dans d'autres cas, des données fiables sur la symétrie n'existaient pas pour les espèces de l'arbre de départ, ce qui m'a conduit à choisir un autre représentant de la même famille pour lequel ces données étaient disponibles. Plusieurs espèces de mon échantillon final n'avaient pas de séquences moléculaires et générer de nouvelles séquences moléculaires ne faisait pas partie des objectifs de ma thèse. En raison de cette combinaison de facteurs, je n'ai pas utilisé de séquences moléculaires pour faire un chronogramme et j'ai utilisé d'autres méthodes pour obtenir la topologie d'arbre phylogénétique.

Pour construire la topologie de l'arbre, j'ai d'abord utilisé la fonction d'export de la base de données PROTEUS pour produire un super-arbre correspondant aux relations entre ordres, entre familles et parfois à l'intérieur des familles basé sur les relations qui avaient été résumées sur l'Angiosperm Phylogeny Website (site de la phylogénie des angiospermes) en 2011. J'ai ensuite résolu manuellement la phylogénie interne de certaines familles en utilisant des études publiées récemment. Les groupements de familles étaient ceux d'APG III ; ils sont suffisamment similaires à ceux d'APG IV pour ne pas apporter de changement dans les inférences de cette étude. J'ai ensuite transformé cet arbre dépourvu de longueurs de branches en un arbre ultramétrique grâce à une fonction d'étirement des nœuds utilisée en écologie évolutive. Cette fonction requiert d'indiquer l'âge du plus grand nombre de nœuds possibles à partir d'une source externe et calcule l'âge des autres nœuds en les répartissant équitablement entre les nœuds pré-datés. Les dates entrées par l'utilisateur viennent du même article que celui qui a été utilisé comme point de départ de mon échantillonnage et ont été entrées pour 399 nœuds, à des niveaux allant du nœud menant à une famille à des nœuds plus profonds partagés entre mon arbre et celui de l'article de référence. Malheureusement, les taux de transition reconstruits sur l'arbre avec longueurs de branches (arbre daté) se sont révélés extrêmement élevés, ce qui a donné une reconstruction des états ancestraux pour la symétrie complètement équivoque pour tous les nœuds de la phylogénie (ayant tous des probabilités d'état identiques). Je n'ai donc pu reconstruire les états ancestraux qu'avec la méthode de parcimonie, ne nécessitant pas de longueurs de branches. Les résultats obtenus montrent un nombre de changements nettement supérieur à celui du dernier article présentant une estimation de ces transitions. Là où l'article en question avait proposé 70 origines de la

zygomorphie parmi les Angiospermes, j'ai trouvé 199 changements, dont 130 origines et 69 réversions. Le véritable nombre de changements pourrait être encore plus élevé, car ma méthode d'échantillonnage limite le nombre de changements par genre à un seul et les genres les plus grands pourraient en avoir plus. De plus, les méthodes qui prennent les longueurs de branches en compte permettent plus de changements sur les branches plus longues, ce qui fait du nombre de changements trouvé par parcimonie une sous-estimation probable (voir aussi les différences entre parcimonie et maximum de vraisemblance rapportés pour le dérivé du premier chapitre).

J'ai décidé d'accompagner cette étude d'une revue des aspects développementaux, génétiques et fonctionnels de la symétrie du périanthe. Ma revue de l'ontogénie met en évidence que la zygomorphie du périanthe pouvait se manifester à diverses étapes du développement, de l'initiation des sépales au stade pré-anthèse. Cette étape peut varier entre des clades fortement apparentés. De plus, certaines espèces qui sont actinomorphes à l'anthèse peuvent avoir une zygomorphie précoce (durant le développement) tout en n'étant pas fortement apparentées à des espèces zygomorphes. Ces deux faits montrent que le stade du développement auquel la zygomorphie apparaît est un trait labile. Ma revue du contrôle génétique de la zygomorphie a révélé que dans chaque clade au sein duquel cela a été étudié, le gène *CYCLOIDEA* ou l'un de ses proches parents a été recruté pour le contrôle de la zygomorphie. Enfin, à travers ma revue des avantages et des inconvénients de la zygomorphie, il apparaît que la zygomorphie pourrait aussi bien être un promoteur de la diversification des espèces que la cause d'une spécialisation trop extrême qui peut mener à l'extinction. La zygomorphie pourrait également être un état faisant de l'« auto-stop » dans certains clades où elle semble corrélée avec une diversité plus importante.

### Chapitre 3 – Est-ce que l'hétérogénéité des taux de transition affecte les reconstructions d'états ancestraux ? Un test empirique avec cinq caractères floraux

Dans le troisième chapitre, j'examine la manière dont les méthodes de reconstruction des états ancestraux (c'est-à-dire la méthode de parcimonie et la méthode du maximum de vraisemblance avec les modèles Mk1 et Mk2) et les différences de taux de transition entre différentes parties de l'arbre phylogénétique affectent les états ancestraux qui sont reconstruits. Et si cela s'avère être le cas, à quel point sont-ils affectés ? Pour apporter une réponse à cela, j'ai utilisé les mêmes méthodes que dans le précédent chapitre, mais avec cinq

caractères du périanthe au lieu d'un seul. Les caractères étaient la symétrie, la fusion, la phyllotaxie, la pentamérie et la différenciation. Trois des cinq caractères avaient une dichotomie naturelle dans leur forme, qui pouvait être facilement utilisée pour produire des états binaires : l'actinomorphie et la zygomorphie pour la symétrie, des organes libres et fusionnés pour la fusion, un périanthe différencié ou non différencié pour la différenciation. En revanche, le mérisme est un caractère multi-état par nature, qui peut être rendu binaire de diverses façons. J'ai décidé de faire une division entre les espèces chez lesquelles la pentamérie est présente (pentamères) et celles dont la pentamérie est absente (non-pentamères), la pentamérie étant le mérisme le plus commun parmi les Angiospermes. Mon échantillonnage de départ était celui du précédent chapitre. J'y ai ajouté des espèces se trouvant dans la base de données PROTEUS permettant de montrer les changements présumés des autres caractères en dehors de la symétrie. L'échantillon final était constitué de 1232 espèces représentant toutes les familles (416 dans le système APG IV) d'Angiospermes. APG IV avait été récemment publié au moment où ce travail a été effectué, apportant des changements aux définitions de certains clades ; les différences apportées à mes optimisations comparées à celles réalisées avec les définitions d'APG III étaient heureusement mineures. La fonction `bladj` du programme Phylocom m'a permis de donner des longueurs de branches à mon arbre ; il s'agit de la fonction utilisée pour dater l'arbre dans le second chapitre et implique donc le même processus que celui décrit dans l'introduction du précédent chapitre.

Relever tous les changements de cinq caractères au lieu d'un seul sur un même arbre s'est révélé avoir plusieurs avantages. L'ajout d'espèces nécessaires pour montrer les changements de caractères hors symétrie divise les branches de l'arbre montrant uniquement la symétrie. L'une des conséquences est qu'une famille avec un seul changement de symétrie et de nombreux changements entre l'état fusionné et l'état libre sera représentée par davantage d'espèces que si elle avait été échantillonnée en se basant uniquement sur la symétrie. Cela modifie également la distribution des états, tout en améliorant la résolution des reconstructions d'états ancestraux. Ce facteur a baissé les taux de transition estimés par maximum de vraisemblance comparé à l'arbre montrant uniquement la symétrie et m'a permis de l'utiliser pour des reconstructions d'états ancestraux prenant la longueur des branches en compte.

Pour évaluer la portée de l'hétérogénéité des taux à l'intérieur des Angiospermes, j'ai comparé les taux estimés quand le même modèle était suivi par tous les Angiospermes à ceux qui étaient estimés quand différentes sous-sections de l'arbre suivaient un modèle différent.

Pour cela, l'arbre a été divisé en cinq partitions, sur lesquelles l'évolution de chacun des cinq caractères a été optimisée séparément : un grade d'Angiospermes basales (le grade ANA et les Magnoliidae), les Monocotyledoneae, les Eudicotyledoneae basales (Ranunculales à Buxales), les Superrosidae (Dilleniales incluses) et les Superasteridae. La parcimonie et le maximum de vraisemblance avec les modèles Mk1 et Mk2 ont été utilisés pour reconstruire les états ancestraux aussi bien sur l'arbre complet des Angiospermes que sur les partitions (collectivement appelées arbre partitionné). Ici, la plupart des taux de transition estimés étaient assez bas pour que chaque nœud ait des probabilités d'état distinctes, contrairement à l'artéfact induit par des taux de transition trop élevés dans le précédent chapitre.

Pour tester l'impact du changement du modèle d'optimisation entre Mk1 et Mk2 et les optimisations sur l'arbre complet et l'arbre partitionné, j'ai comparé les états ancestraux inférés par les diverses optimisations sur deux jeux de nœuds. L'un de ces jeux consistait en quinze nœuds définissant les plus grands clades communément acceptés de l'arbre : les Angiospermae, les Mesangiospermae (toutes les Angiospermes excepté le grade ANA), les Magnoliidae, les Monocotyledoneae, les Commelinidae, les Eudicotyledoneae, les Pentapetalae, les Superrosidae, les Rosidae, les Malvidae, les Fabidae, les Superasteridae, les Asteridae, les Campanulidae et les Lamiidae. Il s'agit là des mêmes nœuds-clés que ceux de la première étude eFLOWER, permettant une comparaison immédiate entre les deux études. L'autre jeu de nœuds comprend les nœuds ancestraux des 54 ordres (parmi 64) comprenant plus d'une espèce. L'hétérogénéité des taux de transition entre les partitions des Angiospermes s'est révélée être forte ; pour un caractère donné, les partitions avaient toujours un taux de transition différent de celui de l'arbre complet et très souvent différent de celui des autres partitions. Malgré cela, dans les deux jeux de nœuds, une majorité d'entre eux ont gardé le même état quel que soit le modèle de maximum de vraisemblance. Cependant, une minorité de nœuds s'est révélée extrêmement sensible au modèle utilisé.

J'ai aussi réalisé une série de tests sur des taux Mk1 contraints sur l'arbre complet sur une petite échelle logarithmique allant de 0,0001 changements par million d'années (l'ordre de grandeur des taux les plus bas estimés lors de mon test précédent) à 0,1. Les états ancestraux se sont révélés globalement insensibles aux variations dans les taux de transition, mais une minorité de nœuds avait un état qui était variable en fonction du taux de transition.

Ces tests ont montré que même lorsqu'il est possible d'utiliser le maximum de vraisemblance sur l'arbre, les taux de transitions sont très élevés. Les tests sur différents taux

de transition Mk1 ont montré que cela pouvait poser des problèmes car, dans les régions dans lesquelles un caractère binaire change souvent d'état, les taux bas aboutissent à des états similaires à ceux reconstitués par la parcimonie, alors que l'autre l'état devient plus probable sous des taux de transition plus élevés. Cela peut mener à la reconstruction d'un état différent que celui qui aurait été reconstruit dans un échantillonnage reflétant la véritable répartition des états de caractère au sein du clade. De plus, les problèmes rencontrés par les arbres utilisés dans ce chapitre existent aussi pour les caractères qui sont naturellement très labiles. Cet élément devra être pris en compte dans de futures reconstructions d'états ancestraux.

Le premier chapitre a montré que les zones en dehors des hotspots à climat Méditerranéen sont des puits macroévolutifs pour les Proteaceae et que la zygomorphie florale n'avait aucun effet sur la diversification de cette famille. Le second chapitre a montré que ma méthode d'échantillonnage basée sur les changements d'états présumés permet de reconstituer les états ancestraux uniquement avec la parcimonie et que la zygomorphie peut aussi bien être un avantage qu'un inconvénient vis-à-vis de la diversification des espèces. Le troisième chapitre a montré qu'il y avait une forte hétérogénéité des taux de transition au sein des différents clades des Angiospermes et qu'elle causait parfois une différence dans les états ancestraux reconstruits comparé à un modèle homogène appliqué à toutes les Angiospermes.

Dans son ensemble, cette thèse montre qu'il existe diverses méthodes d'échantillonnage des espèces et de reconstruction des états ancestraux, qui peuvent être adaptées selon l'aspect de l'évolution des caractères que l'on veut montrer. La méthode d'échantillonnage orientée vers les transitions entre états de caractère en particulier est une approche inhabituelle dans l'étude de leur évolution. Cette thèse montre aussi deux choses qui sont nécessaires pour mieux comprendre l'évolution des fleurs. L'une est la nécessité de collecter le plus de données possibles sur les caractères floraux, car les modèles ont tendance à être construits en supposant que les états de caractère sont connus pour le clade entier. L'autre est la nécessité de construire des modèles plus flexibles vis-à-vis des informations disponibles, car ces dernières sont souvent très incomplètes vis-à-vis de la réalité de la famille, malgré les efforts d'échantillonnage. Ces deux points amènent aussi la question du niveau jusqu'auquel l'arbre phylogénétique des Angiospermes a besoin d'être résolu pour pouvoir montrer toute l'évolution d'un caractère donné. D'un côté, une résolution complète au niveau de l'espèce pourrait ne pas être strictement nécessaire, car beaucoup d'états de caractère restent inchangés dans des clades entiers de tailles diverses. De l'autre, certains caractères sont très labiles ou

ont un état présenté par un très petit nombre d'espèces au sein de certains clades, ce qui peut rendre une résolution très fine du clade nécessaire pour reconstruire son histoire évolutive.

**Titre :** Histoire évolutive des innovations-clés florales chez les Angiospermes

**Mots clés :** reconstruction d'état ancestraux, Angiospermes, évolution florale, taux de diversification, symétrie florale, Proteaceae.

**Résumé :** Les Angiospermes forment un clade connu pour sa grande diversité d'espèces et une répartition inégale de cette diversité en son sein. Ce travail se concentre sur la reconstruction d'états ancestraux de caractères floraux sur les phylogénies dans le but d'étudier leur impact sur la diversification. En premier lieu, nous nous sommes focalisés sur deux innovations-clés potentielles chez les Proteaceae, la symétrie bilatérale du périanthe et la présence dans les hotspots à climat méditerranéen. L'utilisation sur une phylogénie de modèles de diversification dépendants des états de caractère a permis de montrer que le premier état caractère n'a pas d'impact significatif sur la diversification des espèces dans ce groupe, contrairement au second. Ensuite, nous avons reconstruit les états ancestraux de la symétrie du périanthe à l'échelle de toutes les Angiospermes, avec une stratégie d'échantillonnage permettant de refléter toutes les transitions de ce caractère. Nous avons mis en évidence un minimum de 130 origines de la symétrie bilatérale, suivies de 69 réversions vers la symétrie radiaire. Enfin, la même méthode a été étendue à l'étude de quatre autres caractères du périanthe. Différents modèles de reconstruction d'états ancestraux ont été utilisés sur une phylogénie de 1232 espèces pour examiner l'influence des changements de modèle sur les états ancestraux reconstruits. Nos résultats montrent que les changements de modèles de reconstruction ont une influence sur l'état de certains nœuds seulement, jamais sur l'ensemble. Les différents résultats trouvés pour la symétrie, caractère commun aux trois chapitres, révèlent que notre méthode d'échantillonnage présente l'inconvénient d'estimer des taux de transition trop élevés pour donner des résultats concluants avec la méthode du maximum de vraisemblance. Les résultats sont en revanche beaucoup moins biaisés lorsque l'on examine plusieurs caractères simultanément.

**Title:** Evolutionary history of floral key innovations in angiosperms

**Keywords:** ancestral state reconstruction, angiosperms, floral evolution, diversification rates, floral symmetry, Proteaceae.

**Abstract:** Angiosperms are a clade known for its great species diversity and the uneven distribution of this diversity among its lineages. This work focuses on the ancestral state reconstruction of floral characters on phylogenies in the purpose of studying their impact on diversification. We first focused on two potential key innovations in Proteaceae, bilateral perianth symmetry and presence in Mediterranean-climate hotspots. Using character state dependent diversification models, we found that the first character state did not have any significant impact on species diversification in this group, contrary to the second one. We then reconstructed ancestral states for perianth symmetry in angiosperms as a whole, using a sampling strategy aimed at capturing all of the transitions in this character. We found a minimum of 130 origins of bilateral symmetry, followed by 69 subsequent reversals to radial symmetry. Lastly, the same approach was extended to the study of four other perianth characters. Different ancestral state reconstruction models were used on a phylogenetic tree of 1232 species to test the influence of model changes on reconstructed ancestral states. Our results show that changes in reconstruction models have an impact on the inferred ancestral state of some nodes, but not all of them. The various results obtained for symmetry, a character shared among the three chapters, indicate that our sampling method has the drawback of estimating transition rates too high to give conclusive results with maximum likelihood. On the other hand, results are much less biased when several characters are examined simultaneously.