

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

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## Summary

- The Proteaceae is 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 the Proteaceae.
- A rate shift analysis was conducted on dated genus-level phylogenetic trees of the 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 5–10 major diversification rate shifts in the Proteaceae tree. The character-dependent analyses showed that speciation rates, extinction rates and net diversification rates of the Proteaceae were 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 with observations at the scale of angiosperms, floral symmetry is not a trait that strongly influences their evolutionary success.

## 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 the 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 & Heard, 1997). Although 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). Although this explanation is widely accepted (but see Pigot *et al.*, 2010 for possible ‘neutral’ explanations), the heritable trait(s) responsible for diversification rate variation is rarely known.

The Proteaceae is a flowering plant family with > 1700 species widely distributed throughout the Southern Hemisphere which provides a good illustration of uneven species distribution across genera: 25 of 81 genera are monospecific and 12 have only two species, whereas the largest genus, *Grevillea*, contains 359 species and the second largest, *Banksia*, has 169 species. Although the

Proteaceae has 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.

Members of the 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 members of the 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 the 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 the 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 & 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 has 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 the adjustment of the shape of the flower to that of the pollinating animal and thus enhance plant–pollinator specialization. In the Proteaceae, floral symmetry is remarkably variable, despite a well-preserved floral ground-plan of four tepals with opposite stamens and a single carpel (Weston, 2006). Zygomorphy is expressed in various ways in the family, from the 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 the Proteaceae.

The objective of the present study was to identify shifts in diversification rates throughout the 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 the Proteaceae, including all genera. Analyses of this phylogeny suggested that some clades within the family diversified significantly more rapidly than the family-wide background rate, and that clades present in Mediterranean-type hotspots diversified more rapidly (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, or to disentangle the separate effect on speciation and extinction rates of the occurrence in Mediterranean-type regions.

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

A complete species-level tree for the Proteaceae would be desirable to answer our questions, but it does not yet exist for the entire family. Recent studies have addressed similar questions using well-sampled, species-level phylogenies of two large genera of the Proteaceae (Valente *et al.*, 2010; Schnitzler *et al.*, 2011;

Cardillo & Pratt, 2013). Interestingly, these studies did not find support for increased diversification rates in Mediterranean climate hotspots. However, it remains unknown whether these results can be generalized across the entire family. Thus, here, we chose 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 identified shifts in diversification rates in the history of the Proteaceae, and analyzed whether 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* (five spp.) and *Nothorites* (one sp.) (Mast *et al.*, 2008), are not sampled in this tree. This phylogeny was constructed from eight molecular markers (the nuclear internal transcribed spacer (ITS) and seven plastid markers: *atpB*, *atpB-rbcL*, *matK*, *rbcL*, *rpl16* intron, *trnL* intron, *trnL-trnF*) using BEAST (Drummond & 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 (four 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 later). 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 on 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. Moreover, 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 the Proteaceae, but we also generated results with the other two models for comparison. The total numbers of species in each genus are provided in Supporting Information Table S1 and were taken from Sauquet *et al.* (2009). All analyses were performed in R using the multiMEDUSA function from the 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 binary-state speciation and extinction (BiSSE) 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 later). All BiSSE analyses were conducted using the diversitree package in R.

If the possible states of a given character are 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 three-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 six-parameter model in which speciation, extinction and transition rates were dependent on the character state ( $\lambda_0 \neq \lambda_1$ ,  $\mu_0 \neq \mu_1$ ,  $q_{01} \neq q_{10}$ ). We found that, for this dataset, 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 eight BiSSE models) multiple times for each analysis, and present the results for which the highest ML score was obtained. We then selected the best

model based on AIC and ran a Markov chain Monte Carlo (MCMC) 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 can 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) and BiSSE-ness (Magnuson-Ford & Otto, 2012), would allow these processes to be taken into account. However, GeoSSE has not yet been 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 (< 10%) and, for these species, most of the distribution was either clearly Mediterranean or not. Finally, 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 > 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 pseudoclades. Makinson (2000) recognized 33 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 sp.), ‘Grevillea2’ (112 spp.), ‘Grevillea3’ (90 spp.) and ‘Grevillea4’ (156 spp.). To approximate a polytomy giving birth to all four clusters, they were arranged in such a way that the clusters diverged from each other early within a short time span (0.5 million yr): (*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 S1). Therefore, we 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 in diversification rates in *Grevillea*. We then randomly resolved the relationships among these four clades and tested the robustness of our results with three alternative resolutions differing only in topology (same branch lengths): tree 1 = (*Grevillea1*, *Grevillea4*), (*Grevillea2*, *Grevillea3*); tree

2 = (*Grevillea*1, *Grevillea*2), (*Grevillea*3, *Grevillea*4); tree 3 = (*Grevillea*1, *Grevillea*3), (*Grevillea*2, *Grevillea*4). We also tested a different set of branch lengths in which divergences were more evenly distributed: (*Finschia*: 11.1947, ((*Grevillea*1: 3.7316, *Grevillea*4: 3.7316): 3.7315, (*Grevillea*2: 3.7316, *Grevillea*3: 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 numbers 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 the *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 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 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 is heterogeneity in diversification rates across the tree linked to other factors, and if one (or potentially few) large clades coincidentally happen 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 & FitzJohn, 2014). To assess whether our analyses are likely to be 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 of 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.

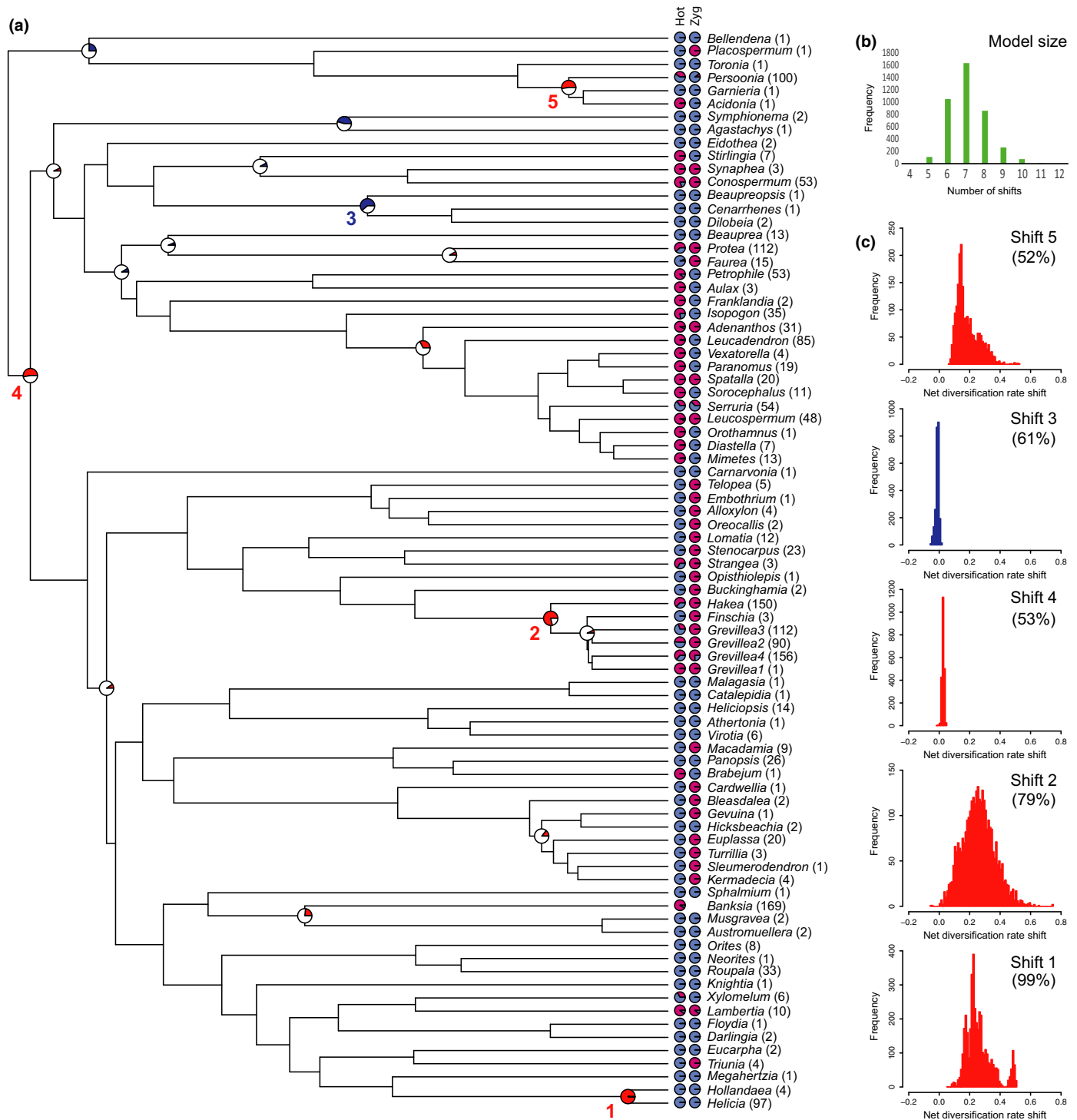
## Results

Our MEDUSA analyses of 4000 timetrees of the Proteaceae with the birth–death model identified 5–11 rate shifts per tree, with a

peak frequency of seven rate shifts (Fig. 1). Five diversification rate shifts were found in > 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 7 to 16, with a peak frequency of 10 rate shifts, and seven shifts supported in > 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 7 to 17, with a peak frequency of 11 rate shifts, and eight shifts supported in > 50% of trees. Of these eight shifts, only three were 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 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 1; Figs 2a,c, S1). Specifically, we found both speciation and extinction rates to be higher in Mediterranean hotspots than outside them (Table 1; Fig. 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. 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 1; Fig. 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 S3; Fig. S2). The mapping of ancestral states showed that the 'hotspot' and 'non-hotspot' states were not located in one single or a few clades, suggesting that our results are probably not a spurious outcome of pseudoreplication (Fig. 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 analysis with a split at shift 2, which yielded a positive diversification rate instead of a negative one outside hotspots, but was still less than a tenth of the diversification rate inside hotspots. We chose not to report the 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 (Tables 2, S2; Figs 2d, 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 2; Fig. S1). However, net diversification rates showed completely overlapping credibility

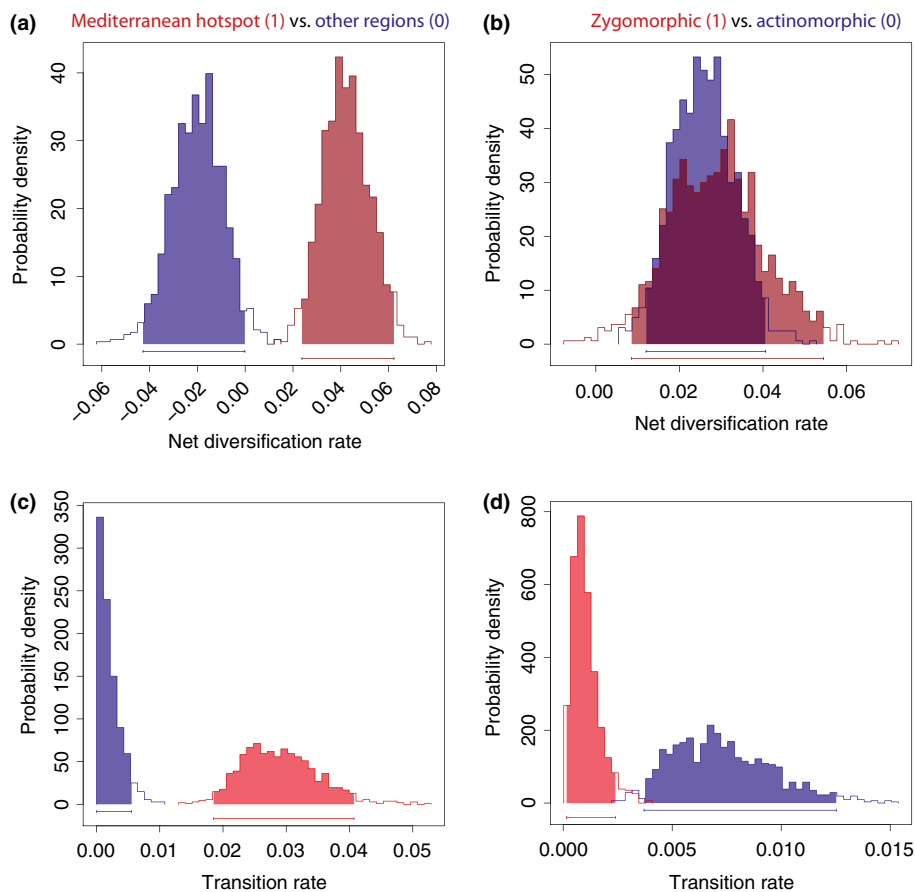


**Fig. 1** (a) Genus-level, time-calibrated phylogenetic tree of the Proteaceae from Sauquet *et al.* (2009), with significant shifts in diversification rates obtained from the multiMEDUSA analysis of 4000 trees randomly sampled from the BEAST posterior. The numbers of extant species in each genus are 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 in which 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 1** Parameter estimates obtained from the binary-state speciation and extinction (BiSSE) analysis testing the impact of occurring in Mediterranean hotspots on diversification rates in the Proteaceae (mean estimates followed by 95% confidence intervals)

	$\lambda$	$\mu$	$r$	$\varepsilon$	$q_{01}$	$q_{10}$
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)		

$\lambda$ , speciation rate;  $\mu$ , extinction rate;  $r$ , net diversification rate ( $\lambda - \mu$ );  $\varepsilon$ , relative extinction rate ( $\mu/\lambda$ );  $q$ , transition rate.

**Fig. 2** Parameter estimates obtained for the best binary-state speciation and extinction (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 S1 and S4.  $r$ , net diversification rate;  $q$ , transition rate.

intervals and therefore did not appear to differ significantly between actinomorphic and zygomorphic species (Fig. 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 S4–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. 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 S4; Fig. S2). Similar to the ‘hotspot’ analyses, the mapping of ancestral symmetry showed that zygomorphy appeared several times in the Proteaceae, yielding confidence that the BiSSE results were not spurious (Fig. S7). The results of the split BiSSE analyses corresponding to the main part of the tree consistently showed the

zygomorphic species to have a diversification rate slightly higher than that of actinomorphic species. However, the difference between the two diversification rates was small and similar to the ML results from the core BiSSE analysis, suggesting that they were not significantly different. We chose not to 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.

## Discussion

### Proteaceae diversification rates

The estimates of the background net diversification rate for the Proteaceae are slightly lower, but similar to, those reported previously for the family as a whole (Sauquet *et al.*, 2009). Diversification rates, however, are not homogeneous across the Proteaceae family (Fig. 1). Our analyses of rate shifts confirm the results of

**Table 2** Parameter estimates obtained in the binary-state speciation and extinction (BiSSE) analysis of the impact of floral symmetry on diversification rates in the Proteaceae (mean estimates followed by 95% confidence intervals)

	$\lambda$	$\mu$	$r$	$\varepsilon$	$q_{01}$	$q_{10}$
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)		

$\lambda$ , speciation rate;  $\mu$ , extinction rate;  $r$ , net diversification rate ( $\lambda - \mu$ );  $\varepsilon$ , relative extinction rate ( $\mu/\lambda$ );  $q$ , transition rate.

Sauquet *et al.* (2009), which showed that some clades within the Proteaceae have diversified much more rapidly than others. Previous studies with the MEDUSA model have identified a similar number of significant shifts for clades much larger than the Proteaceae. Among angiosperms, 13 diversification rate shifts have been found by Baker & Couvreur (2013) for palms (*c.* 2500 spp.) and six among the Malpighiales (*c.* 16 000 spp.) by Xi *et al.* (2012). Among the Metazoa, Moreau & Bell (2013) found 10 shifts among 12 500 ant species, and the original study by Alfaro *et al.* (2009) on the *c.* 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 5279 mammals (four and 149, respectively; see also Yu *et al.*, 2012). Although these previous studies also show that the identification of several rate shifts is not exceptional, we note that the other known groups in which a similar number of shifts have been found so far have much higher diversity than the Proteaceae.

### Diversification within vs outside Mediterranean hotspots

Our results confirm that the diversification of the Proteaceae in the two Mediterranean biodiversity hotspots, CFR and SWA, is 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 CFR could be the result of the 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 used here (Maddison *et al.*, 2007; FitzJohn *et al.*, 2009) allowed a finer analysis than in the study by 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 also appear to have 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 later). 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 the Proteaceae, a view also matched by the

fossil record (Dettmann & Jarzen, 1998). Specifically, negative net diversification rates would explain why tropical Proteaceae genera are species poor, whereas comparatively lower extinction rates would explain why old lineages of Proteaceae are more common in the Tropics than in Mediterranean hotspots. Finally, all of our analyses estimated high relative extinction rates (close to unity), 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 must be taken with caution, especially because of the difficulty in accurately estimating extinction rates from molecular phylogenies (Quental & Marshall, 2010; Rabosky, 2010). These results are also conditional on the assumption of constant rates in each region, which might be incorrect if diversification experienced slowdowns, either as a result of adaptive radiations or other processes, in these new environments (Moen & Morlon, 2014).

Several factors have been proposed to explain the higher net diversification rates of the 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, the establishment of 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 the 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 & 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 with 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 the detection of 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%) of 87 and 15 (9%) of 158 total sampled species, respectively. For comparison, there are 735 (42%) non-hotspot species in the Proteaceae and these are more widespread across the family tree than in these two genera (Fig. 1). It remains uncertain whether the BiSSE

approach has the power to detect 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 the Proteaceae. Similar analyses carried out on a densely sampled species-level phylogeny of the Proteaceae, when available, will help us to understand further this apparent contradiction (Cardillo & 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 *c.* 10 times lower than the mean rate for the transition to a non-Mediterranean region ( $q_{10} = 0.029$ ; CI = 0.019–0.041; Table 1; Fig. 2c). This result may be caused by the nature of prerequisites for moving from one area to the other: it may be easier for a species that is optimized for the absorption of 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 & Pratt, 2013), in which the authors suspected 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 would be able to last a significant amount of time in the region because of high competition. The non-hotspot regions, however, would be places in which 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 the 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 because the Proteaceae have 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 has no significant impact on the net diversification rates of the Proteaceae, 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 suggests 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 results 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 support both higher speciation and higher extinction rates for zygomorphic species in the family. Although we note that we should remain careful about the 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 the 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 that 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 the 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), whereas 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 & 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 has undergone 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 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 S5, 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

In this study, we used a genus-level phylogeny of the Proteaceae because a species-level phylogeny of the family is not yet available. Analyses at the species level would have shown more precise and potentially different results. For example, diversification shifts found at the root of some genera might instead have been identified within these genera; new diversification rate shifts may also have been detected (e.g. Yu *et al.*, 2012). Thus, similar analyses carried out on a detailed species-level phylogeny of the Proteaceae, and with other candidate traits potentially influencing diversification, will eventually allow a refined understanding of the factors that have influenced the success of the Proteaceae and



of particular clades within the family. Nevertheless, our study strongly suggests that the presence of species in Mediterranean hotspots is associated with higher diversification rates in the 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 & 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.

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## Supporting Information

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

**Fig. S1** Additional parameter estimates obtained for the best binary-state speciation and extinction (BiSSE) models selected for each trait of interest.

**Fig. S2** Parameter estimates obtained for the best binary-state speciation and extinction (BiSSE) models selected for each trait of interest when nodes are spread evenly within *Grevillea*.

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

**Fig. S4** Sensitivity of binary-state speciation and extinction (BiSSE) analyses of floral symmetry to various hypothetical proportions of zygomorphic species in *Banksia* (net diversification rate estimates).

**Fig. S5** Sensitivity of binary-state speciation and extinction (BiSSE) analyses of floral symmetry to various hypothetical proportions of zygomorphic species in *Banksia* (speciation rate estimates).

**Fig. S6** Sensitivity of binary-state speciation and extinction (BiSSE) analyses of floral symmetry to various hypothetical proportions of zygomorphic species in *Banksia* (extinction rate estimates).

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

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

**Table S2** Akaike information criterion (AIC) values and delta-AIC (in parentheses) obtained for each diversification model tested in the character-dependent analyses (binary-state speciation and extinction, BiSSE)

**Table S3** Parameter estimates obtained from the binary-state speciation and extinction (BiSSE) analysis testing the impact of occurring in Mediterranean hotspots on diversification rates in Proteaceae when nodes are spread evenly within *Grevillea*

**Table S4** Parameter estimates obtained in the binary-state speciation and extinction (BiSSE) analysis of the impact of floral symmetry on diversification rates in Proteaceae when nodes are spread evenly within *Grevillea*

**Table S5** Results from the split binary-state speciation and extinction (BiSSE) analyses on presence in Mediterranean hotspots

**Table S6** Results from the split binary-state speciation and extinction (BiSSE) analyses on floral symmetry

**Methods S1** Ancestral state reconstruction.

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