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SYNDROME-DRIVEN DIVERSIFICATION IN A MEDITERRANEAN ECOSYSTEM

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Phylogenetic methods to detect lineage diversification have been traditionally used within a particular taxonomic clade, but rarely applied to detect local diversification. For understanding in situ diversification triggered by novel conditions it is necessary to focus on the time slice where such conditions occur. These new conditions may differentially affect the diversification rate of lineages with different morpho-functional syndromes. A prominent example of these processes occurs in the Mediterranean Basin, where climate arising along the Tertiary/Quaternary transition acted as an environmental filter. In this context, lineages with different syndromes (sclerophyllous and nonsclerophyllous) are hypothesized to have different local diversification rates after the rise of the Mediterranean conditions. We used macroevolutionary methods of time-dependent diversification on a calibrated local phylogeny accommodating topological and chronological uncertainty to test syndrome-driven diversification in Mediterranean shrublands from the eastern Iberian Peninsula. We found phylogenetic evidence of higher speciation associated with the nonsclerophyllous syndrome, although extinction rates were similar between syndromes. Consequently a syndrome-driven local diversification has occurred in shrublands under Mediterranean conditions. The results provide an example of how the integration of the environmental filter in a dated phylogeny may recreate the local history of lineages and help to explain assembly processes in Mediterranean ecosystems.

KEY WORDS: Diversification, extinction, Mediterranean Basin, Mediterranean climate, speciation, traits..

Phylogenies encapsulate information about the pattern and timing of diversification. Phylogenetic methods to detect lineage diversification have been traditionally used within a particular taxonomic clade, but rarely applied to detect local diversification (e.g., Anacker et al. 2011). Indeed, local diversification has been overlooked because species assemblages are mostly viewed as the outcome of the ecological filtering of species traits by the environment (Webb et al. 2002; Pausas and Verdú 2010). Filtering is an assembly process reducing the number of local species pool and therefore the phylogeny resulting from such subset of species (pruned phylogenies sensu Ackerly (2000)) should be the appropriate phylogenetic framework to understand local diversification. For instance, Anacker et al. (2011) combined diversification estimates from many small local phylogenies of plant genera living in Californian serpentines to obtain an estimation of diversification across genera. This approach necessarily limits the analysis to only a subset of clades. Here, we suggest that considering the whole phylogeny containing the local flora should allow us to better detect the topological and chronological signature in the phylogeny left by trait evolution and local diversification (Fig. 1). In addition, to detect in situ diversification triggered by the novel local conditions, it is necessary to focus on the time slice where such conditions occur (Simon et al. 1994). Fortunately, available methods allow to estimate separately diversification rates occurring in different time slices (FitzJohn 2012).

Diversification processes are not a response to a single pressure, but probably triggered by a constellation of traits conforming the so called syndromes. Many studies aimed to document trait-driven diversification have focused on a single trait, such as a conspicuous floral trait or body size, often considered a key innovation (e.g., Hodges and Arnold 1995; Hardman and Hardman 2008). Davies et al. (2004) have shown that the major shifts in

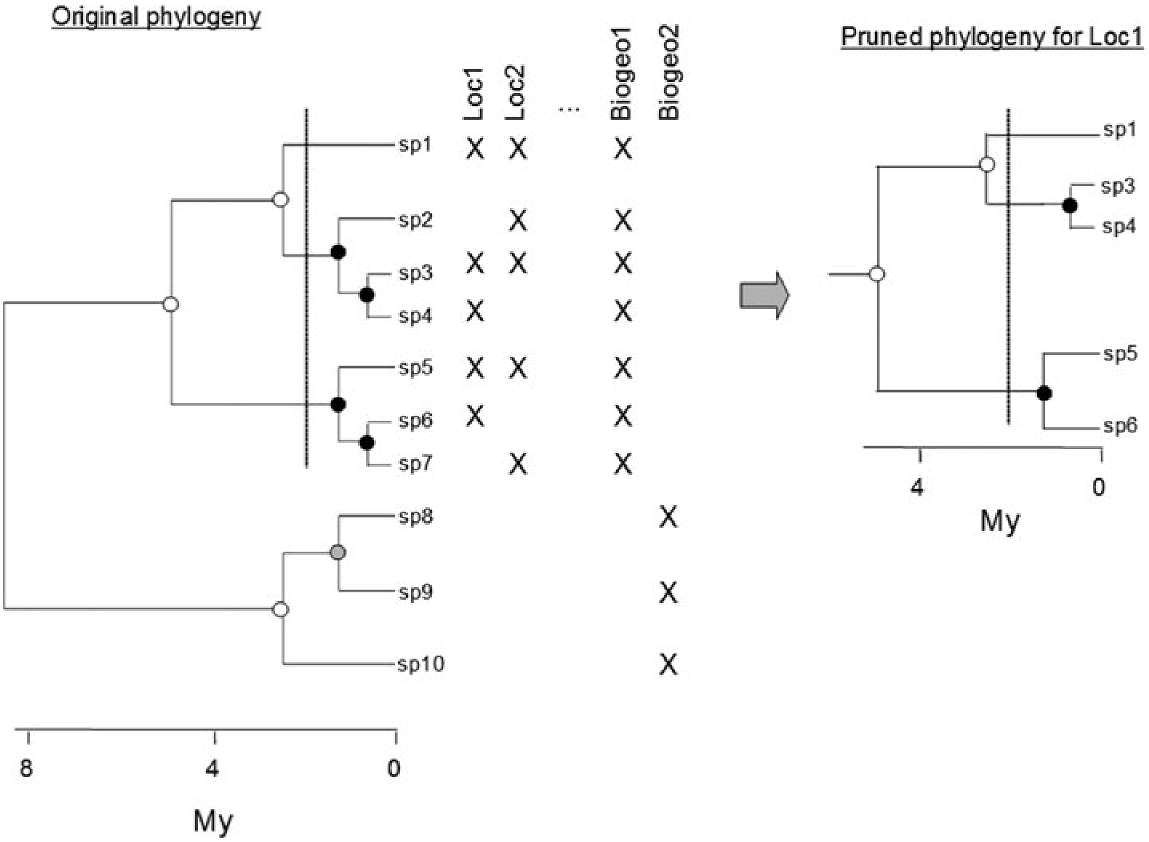

Figure 1. Schematic representation of the topological and chronological signatures left by both filtering and local diversification processes in the pruned phylogeny. The scheme represents two biogeographical regions (Biogeo1, Biogeo2) in which one (Biogeo1, e.g., the Mediterranean Basin) includes two localities (Loc1, Loc2). Crosses represent the presence of the species in the corresponding locality. Vertical dashed lines refer to the rise of Mediterranean conditions. Black nodes represent split events occurring under Mediterranean climatic conditions; white nodes represent splits occurring before the Mediterranean conditions; and the gray node represents a recent split in a non-Mediterranean ecosystem. Species sp3, sp4, sp5, and sp6 were originated by diversification within the Loc1, whereas species sp1 entered by the filtering process as shown in the pruned phylogeny. For Loc2, species sp2, sp3, sp5, and sp7 were originated by diversification whereas species sp1 by filtering.

angiosperm diversification rates have not been caused by a few key innovations but instead are reflecting the interactive effects of biological traits and the environment. For example, the spectacular diversification in Mediterranean biodiversity hotspots has been linked to multiple traits responding to different evolutionary pressures (e.g., poor soils, fire, and drought), which are often strongly tied to each other (Cowling and Pressey 2001; Keeley et al. 2009).

Specifically the complex pattern of diversification in the Mediterranean Basin woody flora is considered the result of the age of lineages, their intrinsic traits, and the differential environmental conditions experienced with the rise of the Mediterranean climate (Herrera 1992). The flora of this region can be grouped in two contrasting trait syndromes that are not only a consequence of adaptive processes, but also of phylogenetic constraints, historical effects, and sorting processes. One syndrome (plants with sclerophyllous, evergreen leaves, and small, unisexual greenish or brownish flowers with a reduced perianth, and large seeds dispersed by animals) occurs in clades whose characteristics predate the appearance of the Mediterranean climate while the other syndrome (plants with alternative character states) arose in clades that have evolved after the emergence of this distinctive climate (Herrera 1992; Ackerly 2009). Plant lineages with the latter syndrome (nonsclerophyllous hereafter) were hypothesized to experience higher diversification rates whereas lineages with the former syndrome (sclerophyllous hereafter) were thought to experience higher extinction rates (Herrera 1992). Most of the posterior work triggered by the seminal paper by Herrera (1992) focused on the relationship between the evolutionary age and the syndrome of the lineages (Verdú 2007; Verdú et al. 2002, 2003; Pausas and Verdú 2008), but few (if any) were centered in the association between the syndromes and diversification.

We use time-dependent macroevolutionary methods of lineage diversification to test whether the onset of Mediterranean climatic conditions differentially affected the rates of speciation and extinction of plant lineages with contrasted morphofunctional syndromes. More specifically, we estimated local speciation, extinction, and diversification rates of lineages belonging to the nonsclerophyllous and sclerophyllous syndromes after three alternative temporal cutoffs (10, 6, and 3.6 Ma) related to the increasing aridity associated with the rise of Mediterranean climate. The extraordinary knowledge of the temporal dynamics in Cenozoic plants for the Iberian Peninsula (Carrión and Leroy 2010) and the independent geological history from the rest of the continent makes this region an appropriate spatial framework to analyze local diversification. To assess the generality of our results, we replicate the diversification analysis in another Mediterranean flora (Sicily). Finally, we evaluate the possible bias in the estimation of local diversification rates by simulating different pruning levels in the phylogeny.

Materials and Methods

The analysis was conducted in the woody flora of the eastern Iberian Peninsula (Valencia region). The climate is typical Mediterranean with precipitation concentrated (>60%) in spring and autumn and with a dry summer (<20% of the annual precipitation) (see Pausas 2004 for more details). The Valencia region, with 1.2 million ha of wildland area, range from sea level to 1839 m asl. This coastal region includes a variety of natural areas where Mediterranean shrublands are dominant (see Folch 1986 for vegetation maps and description). We selected genera dominated by woody species (i.e., with 50% or more woody species); on average the 96% (SD = 11) of the species in each genus were woody. Genera with more that 50% of species associated with humid habitats (wetlands, water courses, etc.) were excluded. The fact of discarding nonwoody species from the studied genera did not generate a taxonomic sampling bias of species sensu Matthews et al. (2011)(*G*-test = 65.99, df = 95, $P = 0.99$). The resulting species list includes 312 woody species from 96 genera and 46 families (Table S1). This list represents 71% of the woody species inhabiting Mediterranean shrublands of the study area (Mateo and Crespo 2007).

We first compiled for all species and from local floras the following 10 binary traits (see Table S1): spinescence (yes/no), leaf type (sclerophyllous/otherwise), leaf habit (evergreen/deciduous, semideciduos, or summer-deciduous), flower size (perianth depth × width < 25 mm/otherwise), flower sexuality (unisexual/hermaphroditic), flower color (colored/brownish or greenish), perianth reduction (complete/at least one verticil absent or much reduced), pollinator type (insect/wind), seed size (length × width > 2.25 m^2/otherwise) main seed dispersal mode (endozoochorous/others). We then summarized this multidimensional trait information in the first ordination axis (dim1) of a multiple correspondence analysis (Fig. S1) using the ade4 software (Chessel et al. 2004). We defined the two contrasted syndromes using a discontinuity in dim1 scores. Two clear discontinuities occurred around the score 0 and 1 (Fig. 2); preliminary analyses using both cutoffs provided the same results, and therefore, only the cutoff at the score 1 was considered (Fig. 2). We discarded the possible taxonomic sampling bias associated with syndromes by checking that the residuals of the phylogenetic regression between the number of sampled (woody) and nonsampled (nonwoody) species of each genera were not explained by the syndrome ($F = 1.17$, $P = 0.281$; Matthews et al. 2001).

To obtain a phylogenetic tree reflecting the local diversification processes, we estimated the phylogenetic relationships between the 312 species with the help of Phylomatic2 (http://www.phylodiversity.net/phylomatic) and the associated GAWK script named *makemega*. We assembled a megatree using all phylogenetic information available in the phylomatic2 repository (accessed in March 2011) plus the necessary trees from published phylogenies to resolve the topology of the tree below family level (Table S2). The topology of our phylogenetic tree was then obtained after matching taxa names of our study species to those contained in the megatree, resulting in a tree with 35% of the nodes as polytomies (Fig. S2a). The assignment of branch lengths between all the nodes resulting from the resolution of polytomies is a crucial issue, especially in diversification studies. Nonmodel-based approaches, like that used by the BLADJ algorithm in phylocom (Webb et al. 2003) assign branch lengths by evenly distributing the undated nodes between the known parent age and the known daughter age. However, these based methods have strong biases making inferences on diversification from such approaches inappropriate (Kuhn et al. 2012). In contrast, the Bayesian polytomy resolution based on a birth-death process has been shown to be unbiased. This method is able to estimate the original diversification parameters of fully resolved trees with up to 60% of random nodes collapsed to polytomies (Kuhn et al. 2012). We used this Bayesian polytomy resolution method to simultaneously adjust branch lengths and resolve polytomies with the help of BEAST (Drummond and Rambaut 2007) and the PolytomyResolver script (Kuhn et al. 2012). This script allows to write a BEAST input file including the topological constraints and the specifications of a birth–death tree prior. We then fixed the 38% of the node ages using data obtained from dated phylogenies (see Appendix S1 for node ages, and Fig. S2 for the corresponding tree), and leaving the remaining nodes to be dated by BEAST using the default settings specified in the PolytomyResolver script. We first ran Markov Chain Monte Carlo analyses for 10^6 iterations, sampling trees every 10^3 iterations, then after a 25% burn-in, we randomly selected 100 fully resolved dated trees (see Fig. S2b, for an example). By doing this, we accounted for

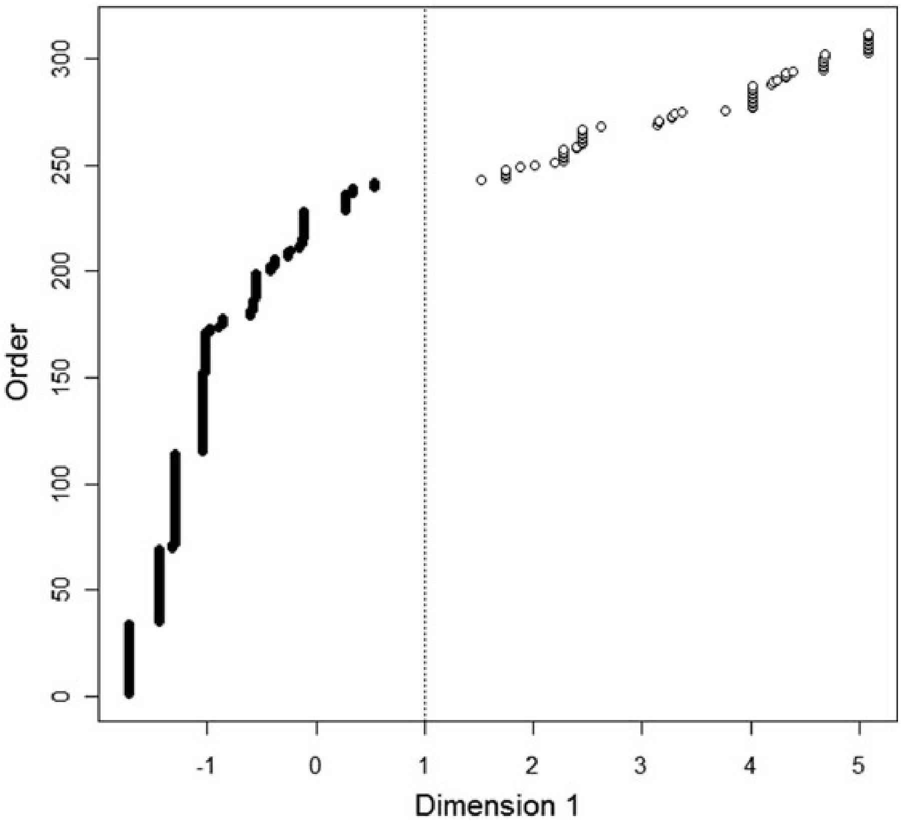

Figure 2. Species ordered by the multidimensional trait (dim1) indicating the cutoff considered (dotted vertical lines) to separate two character syndromes, those with scoring < 1 (242 species; nonsclerophyllous syndrome) and those with scoring >1 (70 species; sclerophyllous syndrome).

the uncertainty in the ages of the undated nodes for the subsequent diversification analysis. In summary, the process consisted of (1) assembling the topology of the phylogenetic tree with phylomatic, considering angiosperm phylogeny group and other published phylogenies below the family level (Table S2); and (2) estimating branch length by BEAST based on chronological constraints from several sources (Appendix S1) and following a birth–death process.

For each of the 100 trees, we tested differential speciation, extinction, and diversification rates between the two syndromes following the binary state speciation and extinction approach (BiSSE, Maddison et al. 2011; FitzJohn et al. 2009). BiSSE is a likelihood-based method that simultaneously estimates state-dependent diversification rates and rates of character change for binary traits. For each of the 100 trees, we compared the likelihood of a constant-rate BiSSE model against the likelihood of a model with two time slices separated at the time of the rise on the Mediterranean conditions by means of a likelihood ratio test (LR test). The time-dependent BiSSE model allows for estimation of different speciation and extinction parameters for each time slice (FitzJohn 2010); alternative quantitative approaches (e.g., QuaSSE) do not account for such time-dependent processes. If trait-driven diversification is associated with Mediterranean conditions, then the time-dependent model should fit better than the constant-rate BiSSE. BiSSE analyses were run with the diversitree library of the R software (FitzJohn 2012). Palynological evidence of major reduction of subtropical taxa and increase of sclerophyllous vegetation suggest that Mediterranean climates was established 3.2 million years ago (Mya), subsequently revised to 3.6 million years (My) (Suc 2009; Suc and Popescu 1984). However, there are evidence of increasing drying conditions predating 3.6 My (Tzedakis 1986), such as the observation on growth banding in *Porites* corals from central Crete representing seasonal environmental variability (~10 My, Brachert et al. (2006)); and the evaporitic deposits representing the dried out of saline lakes and concomitant vegetation changes around 6 My (Hsu et al. 1977). Consequently differential diversification between syndromes was estimated considering three alternative scenarios indicating different moments along the aridity trend of the Tertiary/Quaternary transition: 3.6, 6. and 10 My (cutoffs hereafter).

To evaluate the generality of our results, we repeated the trait-driven diversification analysis for another Mediterranean Basin flora. Specifically, we use the flora of Sicily (Raimondo et al. 2008) with the cutoff provided by the palynological evidences

Table 1. Local speciation (λ), local extinction (μ), and local diversification (*r*) estimates of nonsclerophyllous (*n*) and sclerophyllous (*s*) syndromes for the two temporal slices before (precut) and after (postcut) the studied temporal cutoffs. For the Valencia study, three alternative cutoffs related to the increasing aridity associated with the rise of Mediterranean climate are presented; for the Sicily study, only the most recent cutoff is presented. Data are mean and SD of the parameters obtained by maximum likelihood from 100 phylogenetic trees.

		Valencia			Sicily
Temporal slices	Parameter	10 My	6 My	3.6 My	3.6 My
Postcut	λ_n	0.383 ± 0.039	0.366 ± 0.045	0.336 ± 0.045	0.224 ± 0.032
	λ_s	0.108 ± 0.033	0.125 ± 0.034	0.127 ± 0.04	0.105 ± 0.036
	μ_n	0.421 ± 0.059	0.367 ± 0.083	0.251 ± 0.105	0.201 ± 0.118
	μ_s	0.21 ± 0.105	0.325 ± 0.131	0.335 ± 0.22	0.243 ± 0.155
	r_n	− 0.039 ± 0.023	− 0.001 ± 0.042	0.085 ± 0.066	0.023 ± 0.091
	r_s	− 0.102 ± 0.076	− 0.2 ± 0.102	− 0.208 ± 0.189	− 0.138 ± 0.155
Precut	λ_n	0.244 ± 0.068	0.217 ± 0.063	0.198 ± 0.054	0.139 ± 0.049
	λ_s	0.124 ± 0.082	0.153 ± 0.076	0.106 ± 0.067	0.084 ± 0.049
	μ_n	0.224 ± 0.07	0.2 ± 0.065	0.185 ± 0.055	0.121 ± 0.051
	μ_s	0.123 ± 0.084	0.155 ± 0.076	0.108 ± 0.067	0.083 ± 0.049
	r_n	0.02 ± 0.005	0.018 ± 0.006	0.013 ± 0.005	0.018 ± 0.005
	r_s	0.001 ± 0.002	− 0.002 ± 0.001	− 0.002 ± 0.001	0.002 ± 0.06

(3.6 Mya; Suc 2009; Suc and Popescu 1984). Following the same criteria of species selection described above for the Valencia region, we obtained a total of 240 woody species for this island. Characters state scoring, phylogeny reconstruction (Fig. S2c), and diversification estimates followed the same procedures as for the Valencia region.

A crucial factor to appropriately use macroevolutionary methods to detect local diversification is its sensitivity to different levels of phylogenetic pruning produced by the environmental filter (i.e., in our case, the Mediterranean climate). To evaluate to what extent our results may be biased by the use of such a pruned phylogeny, we simulated 100 trees in which character states were evolved following known diversification rates and then we infer those rates from the trees after pruning a varying number of species (Maddison et al. 2011 Fitzjohn et al. 2009). Two sets of simulations were performed. The first one was aimed to test whether our observed differences in speciation and extinction rate between character states can be detected after pruning the phylogeny. The second one tested if pruning may lead to infer the observed difference in speciation and extinction rates between character states when it does not really exist.

For the first set of simulations, we generated trees driven by unequal trait-diversification rates. We first defined a character with three states as follows: (1) non-Mediterranean (state 0); (2) Mediterranean sclerophyll (state *S*); 3) Mediterranean nonsclerophyll (state *N*). We then evolved a tree under the MuSSE (multistate speciation and extinction) model using the “diversitree” software (FitzJohn 2012). Simulated trees contained 10,000 species and speciation and extinction rates were kept constant at $\lambda_0 = \lambda_s = 0.127$; $\lambda_n = 0.34$; $\mu_0 = \mu_s = 0.033$; $\mu_n = 0.025$ across simulations (λ values based on Table 1; μ values were arbitrarily reduced to avoid negative diversification rates that precludes tree simulation). Character transition rates from Mediterranean sclerophyllous and nonsclerophyllous syndromes (states S and N) were kept constant across simulations ($q_{S0} = q_{SN} = q_{N0} = q_{NS} = 0.1$; following Maddison et al. 2011; Fitzjohn et al. 2009), whereas those from the non-Mediterranean state ($q_{0\mathrm{S}}$, $q_{0\mathrm{N}}$) were changed across simulations ($q_{0\mathrm{S}}$ from 0.00001 to 1.1; $q_{0\mathrm{N}}$ from 0.00006 to 2.2) to obtain a tree in which the frequency of state *N* is two to three times that of state *S*. We generated this difference in frequency between states because this uneven representation is common in the Mediterranean flora where the nonsclerophyllous syndrome is more frequent than the sclerophyllous one (Herrera 1992). Then, we pruned all the non-Mediterranean species from the resulting tree and estimated λ_S, λ_N, μ_S, and μ_N with the BiSSE model. The likelihood of this model was tested against the likelihood of a model in which $\lambda_S \sim \lambda_N$ (LR test) to evaluate whether BiSSE was capable of correctly detecting the characters state with the highest speciation rate when speciation rate asymmetry exists.

For the second set of simulations aimed to test whether pruning may lead to detect a difference in diversification rates between traits when it does not really exist, we followed the same above procedure but constraining the diversification parameters to be equal between states ($\lambda_0 = \lambda_S = \lambda_N = 0.127$; $\mu_0 = \mu_S = \mu_N = 0.033$; $\mathrm{q}_{S0} = \mathrm{q}_{SN} = \mathrm{q}_{N0} = \mathrm{q}_{NS} = 0.1$; q_{0S} from 0.0001 to 0.005; q_{0N} from 0.002 to 1.045).

Results

A multidimensional trait (dim1) of 312 species inhabiting shrublands of the eastern Iberian Peninsula ranges between −1.7 and 5.1 and explained 41% of the variance. High values of dim1 are associated with sclerophyllous plants having small unisexual uncolored flowers with reduced perianth and pollinated by wind (Fig. S1). Low values of dim1 are related to deciduous (including semideciduous and summer deciduous) species with large and conspicuous flowers pollinated by insects. We defined two contrasted syndromes using the main discontinuity in dim1 scores (i.e., 0.5–1.5 gap; Fig. 2), and obtained two set of species, one for low scoring values (nonsclerophyllous syndrome) and the other for high scoring values (sclerophyllous syndrome). The first group is richer in species (242 species) but with lower morpho-functional variance (shorter range of dim1) than the second group (70 species; Fig. 2).

The occurrence of local diversification is shown by the proportion of species whose most recent common ancestor was dated after the appearance of Mediterranean conditions (mean and SD for the 100 trees: 50.5 ± 2.0%, 59.9 ± 1.6%, and 68.1 ± 1.35% for cutoffs 3.6, 6, and 10 My, respectively). The time-dependent model in which diversification was estimated separately for the periods before and after the onset of Mediterranean conditions fitted significantly better (LR test, $P < 0.05$) for most of the phylogenetic trees than the constant-rate model in which time was not split. Specifically the differences were significant for 93%, 80%, 70% of the trees at the cutoffs of 10, 6, and 3.6 My, respectively. The differences between pre-Mediterranean versus post-Mediterranean time slices are related to the increased local speciation rates in the nonsclerophyllous relative to the sclerophyllous syndrome; this was true for the three cutoffs considered (Table 1). In contrast, extinction rates were not consistently different between pre- and post-Mediterranean time slices (Table 1).

In the time slice under Mediterranean conditions, local speciation rate was always greater for species with the nonsclerophyllous syndrome ($\lambda_n > \lambda_s$) in all scenarios (Fig. 3). The extinction rates for the sclerophyllous syndrome increased toward the recent cutoff, whereas the opposite trend was observed for the nonsclerophyllous syndrome (Table 1), changing the relative extinction rate across the different cutoffs (Fig. 3). Consequently, the more recent was the cutoff, the higher the difference in diversification rate between syndromes (Fig. 3). These results were consistent for the Sicily flora, where the proportion of species with a common ancestor dated within the Mediterranean slice was 39.6% (SD = 2.6). The model splitting the phylogeny in pre- and post-Mediterranean time slices fitted significantly better than the constant-rate model in 84% of the trees (LR test, $P < 0.05$). In the time slice under Mediterranean conditions, local speciation rate in the Sicily flora was always greater for species with the nonsclerophyllous syndrome ($\lambda_n > \lambda_s$), whereas extinction rates were similar between syndromes (Table 1, Fig. 4), yielding to a greater local diversification rate in the nonsclerophyllous syndrome.

Our first set of simulations mostly recovered a relative speciation rate between syndromes lower than one (solid symbols in Fig 5a), indicating that $\lambda_S < \lambda_N$, as expected from the initial values ($\lambda_S = 0.127$; $\lambda_N = 0.34$). Only in the case of very extreme pruning (~99%) the estimates are meaningless (open symbols in Fig. 5a). That is, the relative speciation rate between syndromes (λ_S/λ_N) is qualitatively well predicted in most cases (Fig. 5a). Quantitatively, the pruned phylogenies recovered a relative speciation rate close to the expected value ($\lambda_S/\lambda_N = 0.373$; dotted line in Fig 5a), except at extreme pruning values. Extinction rates were very poorly predicted across the whole range of pruning values, with most of the estimates around zero.

The second set of simulations show that pruning generally lead to detect a difference in the diversification rates between traits when it does not really exist (solid symbols in Fig 5b). This bias becomes larger with extreme pruning values in such a way that estimates of $\lambda_S >> \lambda_N$. Such a bias goes in the opposite direction to the values observed for the Mediterranean Basin flora where $\lambda_S < \lambda_N$. As in the other set of simulations, extinction rates were very poorly predicted across the whole range of pruning values, with most of the estimates around zero.

Discussion

Local species assemblages are not only the product of filtering processes, but also a framework where diversification occurs (Johnson and Stinchcombe 1977; Prinzing et al. 2004; Pausas and Verdú 2010). Here, we show that it is possible to separate the two processes by properly delimiting the historical context (Fig. 1). Local diversification estimated as the proportion of species whose most recent common ancestor was dated after the appearance of Mediterranean conditions ranged from 51% to 68% depending of the cutoff.

Our results support the evidence that diversification in woody plants of Mediterranean shrublands is associated with differential success of morpho-functional syndromes to the increasing aridity along the Tertiary/Quaternary transition (Herrera 1992). Such differential diversification has mainly occurred due to differences in local speciation rates. Lineages with the syndrome defined by nonsclerophyllous, deciduous leaves and large, hermaphroditic, colored flowers with a complete perianth, and small seeds abiotically dispersed (nonsclerophyllous syndrome) are more prone to speciate than lineages with the complementary syndrome (sclerophyllous syndrome).

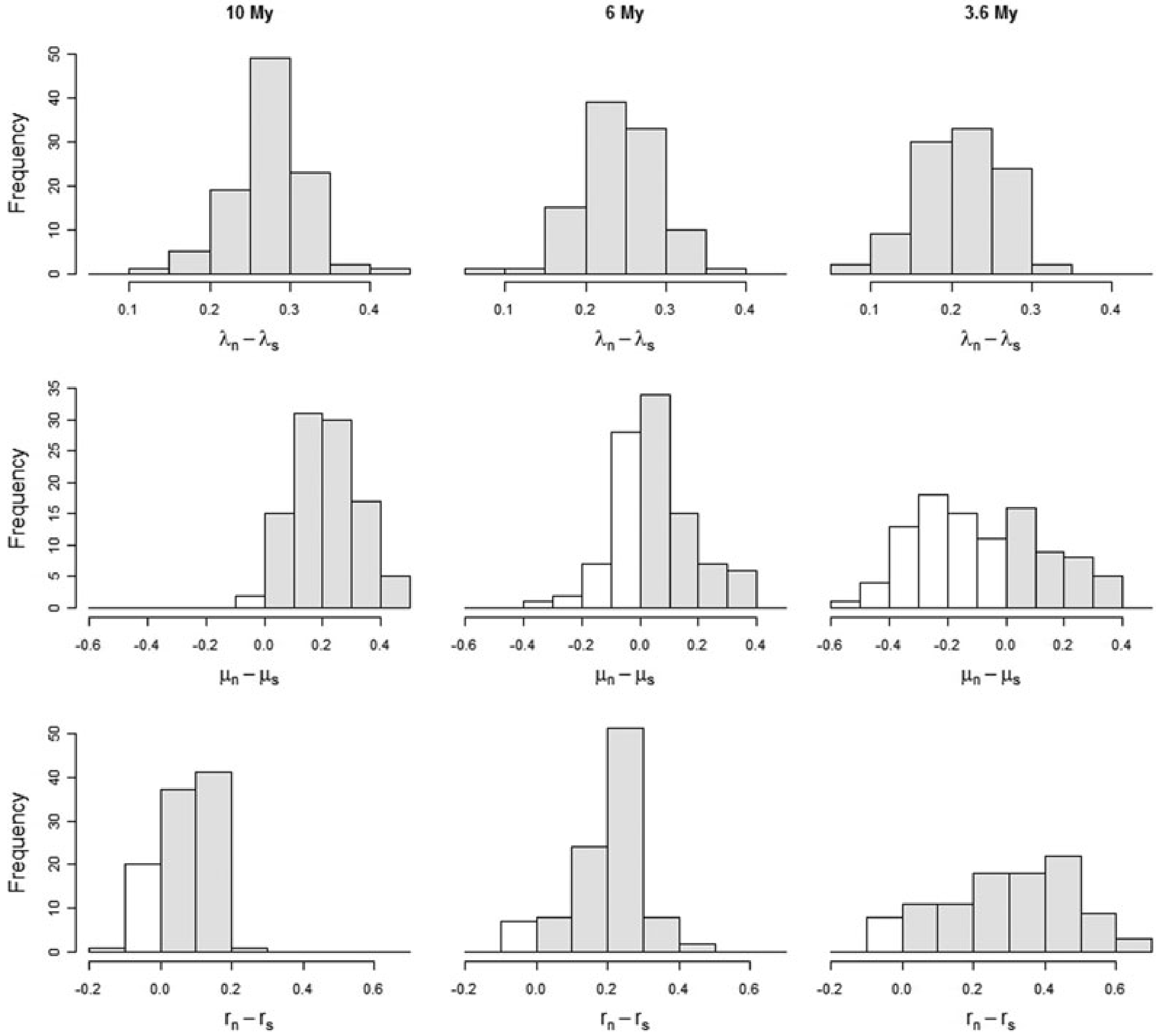

Figure 3. Frequency distribution of differences in local speciation (λ, upper panel), local extinction (μ, middle panel), and local diversification (r, bottom panel) rates between nonsclerophyllous (n) and sclerophyllous (s) syndromes in the Valencia woody flora. Columns refer to the different postcut temporal slices (cutoff of 10, 6, and 3.6 My) related to the increasing aridity associated with the rise of Mediterranean climate. Gray bars are values greater than zero.

The use of a pruned phylogeny for an accurate estimation of local speciation necessarily needs to exclude both non-Mediterranean species and species from other Mediterranean localities (Fig. 1). Currently there are methods of incorporating incomplete taxon sampling (FitzJohn et al. 2009), but they require an explicit definition of the regional species pool; this is not a trivial issue and can greatly influence the results (Swenson et al. 2005). In contrast, our approach should be independent of the definition of the regional species pool because it focuses in the temporal and spatial framework defined by the local conditions. Despite this advantage, pruning might produce inaccurate estimations of the *absolute* values of local speciation rates between syndromes; however, our simulations show that pruning does not greatly affect estimates of the *relative* local speciation rates. Except for very extreme pruned levels, our simulations are able to detect the differences in local speciation rates when they do exist. The simulations also show that pruning might lead to retrieve differences when they do not exist, but in those cases, the bias goes in the opposite direction from the observed values in the Mediterranean Basin. Overall these simulations provide confidence in our results. At the same time they highlight the importance of applying appropriate simulations to detect statistical biases that may be confounding the biological interpretation.

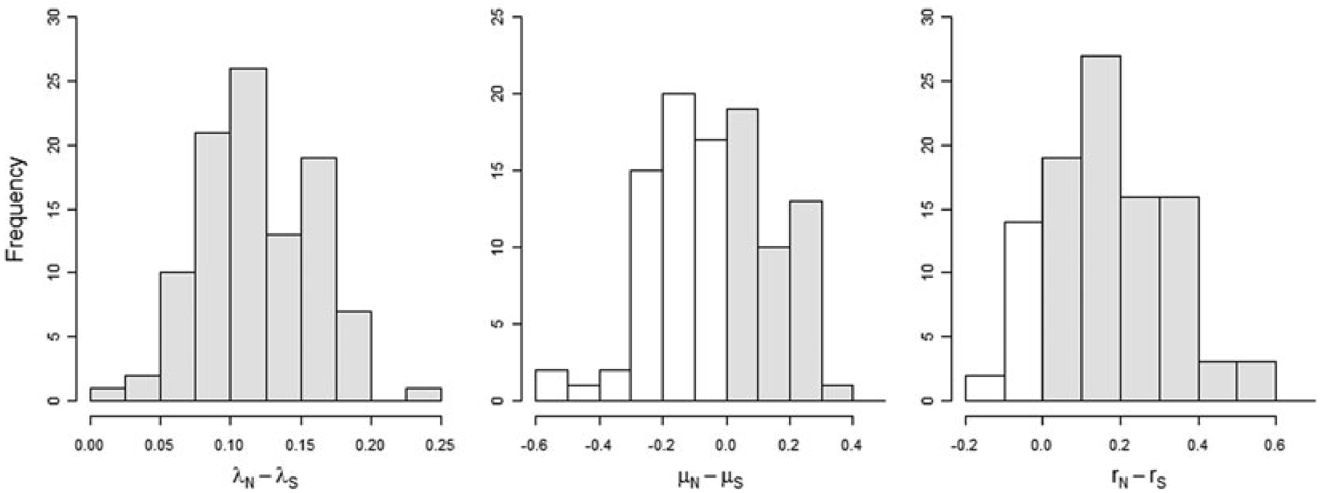

Figure 4. **Frequency distribution of differences in local speciation (λ, left panel), local extinction (μ, middle panel), and local diversification (*r*, right panel) rates between nonsclerophyllous (*n*) and sclerophyllous (*s*) syndromes, in the Sicily woody flora.**

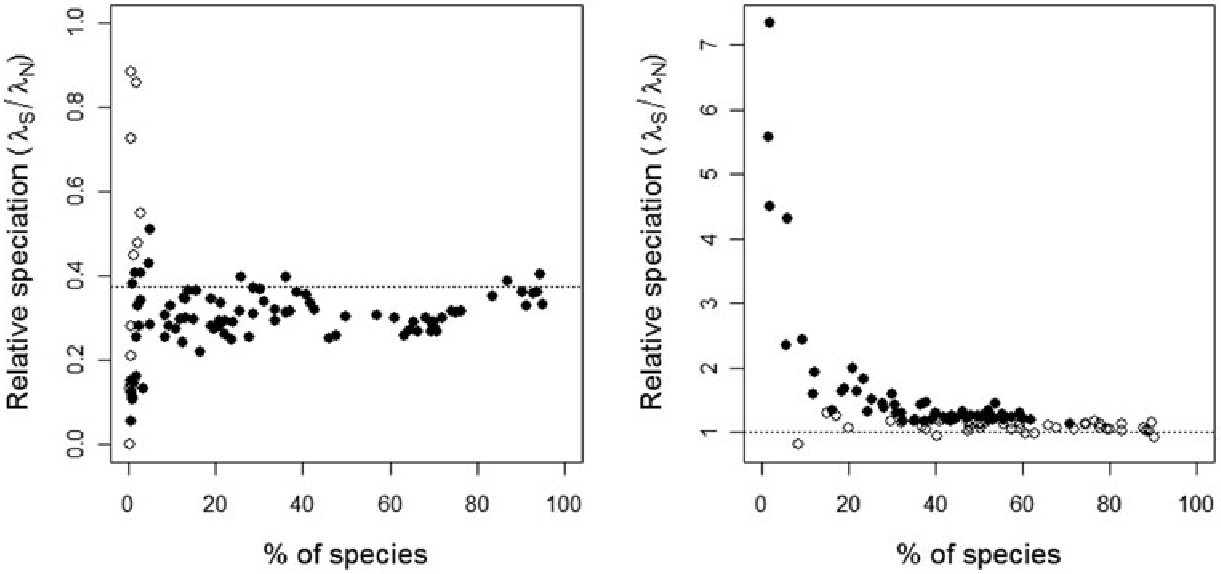

Figure 5. **Estimations of relative speciation rate between two syndromes *S* and *N* (λ_S/λ_N) in 100 trees with different pruning levels (proportion of species remaining in the tree after the pruning). Speciation rates between syndromes were simulated to be different ($\lambda_S/\lambda_N = 0.373$, left panel) and equal ($\lambda_S/\lambda_N = 1$, right panel). Horizontal dotted lines refer to the true relative speciation rate. Closed symbols indicate a relative speciation rate significantly different from one (LT test).**

The extinction rate of the sclerophyllous syndrome increased toward recent cutoffs (i.e., drier periods) reaching similar values to those for the nonsclerophyllous syndrome for the 3.6 My scenario. These estimations should be taken with caution because of the well-known problem of studying diversification using phylogenies with only extant taxa (Harvey et al. 1994; Paradis 2010; Rabosky 2010). Indeed our simulations also showed that extincton rates are poorly estimated. However, an independent line of evidence based on biogeographical and fossil information (Herrera 1992) supports the lack of differences in local extinction rates between the two syndromes.

Another criticism to the use of pruned phylogenies is the inaccurate estimation of the diversification rates of the pre-Mediterranean time slice. For instance, diversification within Loc1 (in Fig. 1) would exclude species diversifying in another biogeographical region (e.g., species sp8 to sp10 in Fig. 1) as well as species from other Mediterranean localities (e.g., species sp2 and sp7 in Fig. 1). In fact, the pre-Mediterranean conditions include a huge temporal scale far beyond the scope of this study where many environmental shifts have occurred. For this reason, diversification rates of the pre-Mediterranean time slice should be taken with caution. However, events prior to the cutoff have no implications for estimates of diversification rates after the cutoffs. In other words, splitting events occurring outside the temporal and spatial framework defined by the local conditions would be irrelevant for local diversification estimates. Admittedly, one shortcoming could be the case of a very recent (<3.6 Ma) diversification elsewhere followed by migration into the Mediterranean region. For instance, *Phlomis* is a lineage that has diversified outside the Mediterranean region (Mathiesen et al. 2011) and there is the possibility of recent diversification and immigration into the Mediterranean Basin, although evidence is lacking. In any case, including uncertainty in node age in diversification analyses confers robustness to our results. In addition, combining the geographical component with a trait-based approach under different time slices would help disentangling the relevance of migration processes in diversification estimates. To tackle this sort of analysis, future methods will need to combine the spatial component of diversification (GeoSSE model; Goldberg et al. 2011) with trait-driven diversification in a temporal context (e.g., time-dependent models; FitzJohn 2010).

The processes behind the differential speciation under Mediterranean conditions should be related to the trait states defining the nonsclerophyllous syndrome. For instance, there is evidence that traits like insect pollination (e.g., Kay and Sargent 2007) or unisexual flowers (e.g., Heilbuth 2000) may trigger diversification. However, other studies have found that traits characterizing the sclerophyllous syndrome are better linked to diversification, such as vertebrate seed dispersal (e.g., Tiffney 2006). A lack of clear relationship between pollination and dispersal traits and diversification has also been reported in other systems (e.g., Ricklefs and Renner 2010). This lack of congruence emphasizes the importance of a multidimensional trait approach for understanding diversification processes. In addition, it is important to consider that other traits may co-vary with our syndromes and be the ultimate responsible of the mechanistic process of changes in diversification rate (Donoghue 2005). For instance, the rise of Mediterranean climate increased the fire activity (Keeley et al. 2009) and traits defining our syndromes are related to postfire regeneration traits (Verdú 2007; Pausas et al. 2004) and to the age to maturity (Pausas and Verdú 2005). Indeed the nonsclerophyllous syndrome is more associated with species considered postfire seeders (i.e., killed by fire in which populations regenerate from a persistent seed bank) and to species with earlier maturation than species in the sclerophyllous syndrome. In fire-prone environments, these characteristics reduce generation time and the overlap between generations in species with the nonsclerophyllous syndrome and thus they provide more opportunities for diversification (Ellner and Hairston 1994; Verdú 2000).

A syndrome-driven diversification may result in an overrepresentation of the favored traits in the local flora. It should be noted that such overrepresentation of traits can also be favored by processes like environmental filtering of lineages possessing a particular combination of traits (Pausas and Verdú 2008, 2010). However, environmental filtering and trait-driven diversification should not be viewed as mutually exclusive processes. Indeed, one of the current challenges of community ecology is to include evolutionary dynamics into community assembly to disentangle the role of community dynamics in triggering diversification and trait divergence (Johnson and Stinchcombe 1977; Pausas and Verdú 2010). For example, recurrent fires in Mediterranean plant communities act as an environmental filter favoring the overrepresentation of particular set of traits (Verdú and Pausas 2002; Pausas and Verdú 2008). Specifically traits associated with the nonsclerophyllous syndrome permit a massive recruitment after fire finally leading to communities dominated by species with this syndrome. By changing from regional to local spatial scale, the phylogenetic structure of these communities was shown to reflect not only the filtering process, but also the force driving trait-diversification (Ojeda et al. 2011). This conclusion was reached by working in an already fire-filtered flora, suggesting that it is possible to separate processes occurring at different scales. This is exactly the approach we have done when studying differential diversification associated with character syndromes. By including the filter in a dated phylogeny, we are recreating the local history of lineages once they have been filtered from the regional flora. This new approach will help to fill the gap between community ecology and evolutionary biology.

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Supporting Information

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

Table S1. Trait data.

Table S2. References used for generating the phylogenetic trees.

Figure S1. Multidimensional trait ordination.

Figure S2. Phylogenetic trees.

Appendix S1. Node age estimation.