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BURNING PHYLOGENIES: FIRE, MOLECULAR EVOLUTIONARY RATES, AND DIVERSIFICATION

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Mediterranean-type ecosystems are among the most remarkable plant biodiversity "hot spots" on the earth, and fire has traditionally been invoked as one of the evolutionary forces explaining this exceptional diversity. In these ecosystems, adult plants of some species are able to survive after fire (*resprouters*), whereas in other species fire kills the adults and populations are only maintained by an effective post-fire recruitment (*seeders*). Seeders tend to have shorter generation times than resprouters, particularly under short fire return intervals, thus potentially increasing their molecular evolutionary rates and, ultimately, their diversification. We explored whether seeder lineages actually have higher rates of molecular evolution and diversification than resprouters. Molecular evolutionary rates in different DNA regions were compared in 45 phylogenetically paired congeneric taxa from fire-prone Mediterranean-type ecosystems with contrasting seeder and resprouter life histories. Differential diversification was analyzed with both topological and chronological approaches in five genera (*Banksia, Daviesia, Lachnaea*, *Leucadendron*, and *Thamnochortus*) from two fire-prone regions (Australia and South Africa). We found that seeders had neither higher molecular rates nor higher diversification than resprouters. Such lack of differences in molecular rates between seeders and resprouters—which did not agree with theoretical predictions—may occur if (1) the timing of the switch from seeding to resprouting (or vice versa) occurs near the branch tip, so that most of the branch length evolves under the opposite life-history form; (2) resprouters suffer more somatic mutations and therefore counterbalancing the replication-induced mutations of seeders; and (3) the rate of mutations is not related to shorter generation times because plants do not undergo determinate germ-line replication. The absence of differential diversification is to be expected if seeders and resprouters do not differ from each other in their molecular evolutionary rate, which is the fuel for speciation. Although other factors such as the formation of isolated populations may trigger diversification, we can conclude that fire acting as a throttle for diversification is by no means the rule in fire-prone ecosystems

KEY WORDS: Fire, genetic differentiation, resprouter, seeder, speciation.

Why speciation occurs is a question of paramount importance in biology. The fact that species diversity is not constant either across lineages or across regions has attracted the attention of evolutionary biologists since Darwin's (1859) seminal work. Diversification has traditionally been thought to be associated with the rate of molecular evolution (Laird et al. 1969; Barraclough and Savolainen 2001; Verdú 2002; Wright et al. 2006) although a lack of association has also been suggested when speciation is not limited by genetic divergence (Coyne 1994; Barraclough and Savolainen 2001).

Fast rates of molecular evolution have been claimed to be produced in taxa with short generation times because of the inverse correlation between generation time and the number of germ-line cell divisions, and therefore replication induced mutations, per time unit (Laird et al. 1969; Ohta 1993; see Whittle and Johnson 2003 for the limitations to this hypothesis). Aside from generation time, other mechanisms that speed up evolutionary rates include population size and speciation rate (Muse and Gaut 1997). For example, molecular evolutionary rates may be viewed as a consequence of species diversification driven by the isolation of populations and the concomitant reduction in the effective population size, which could potentially accelerate the rate of fixation of alternative alleles (Ohta 1993; Bromham and Cardillo 2003).

Fire-prone ecosystems can be viewed as natural laboratories for studying changes in both molecular evolutionary rates and diversification. Indeed, plants from fire-prone environments provide astonishing examples of species-rich genera that suggest the occurrence of a recent, massive diversification (e.g., *Aspalathus*, Linder 2003; *Arctostaphylos,* Wells 1969*; Erica*, Ojeda 1998; Linder 2003; *Ceanothus*, Hardig et al. 2000; *Ehrharta*, Verboom et al. 2003; *Banksia, Eucalyptus*, *Casuarina*, Mast and Givnish 2002, Crisp et al. 2004; *Leucadendron*, Barker et al. 2004; *Thamnochortus*, Hardy and Linder 2005). In these ecosystems, two basic and contrasting life histories allow plants to cope with recurrent fires (Keeley and Zedler 1978; Pausas et al. 2004): one corresponds to species in which adults survive and resprout after fire (*resprouter* life history), and the other includes species in which adults die after fire and populations rely on seeds (often seed dispersal and/or germination is fire-stimulated) for their persistence (*seeder* life history). Although both seeder and resprouter life histories confer resilience to fire at the population level, their effects on the population dynamics are altogether different (Bond and van Wilgen 1996; Pausas 1999a; Ojeda et al. 2005). These two post-fire life histories are widespread and dominant in the woody flora of the different Mediterranean-type ecosystems, and species without any of these two traits are rare and localized in particular environments (Bond and van Wilgen 1996; Keeley and Fotheringham 2000; Verdú 2000; Pausas et al. 2004). Such clear-cut differences in life-history traits led Wells (1969) to suggest that seeders span numerous generations increasing the opportunity for natural selection to act, which would subsequently imply that seeders will diversify more rapidly. After Wells's contribution, further studies have argued that the exceptional species richness in some fire-prone ecosystems results partly from the high speciation rates (Cowling and Pressey 2001; Mast and Givnish 2002; Barraclough 2006).

In fire-prone ecosystems, seeder species are prone to extinction if fire regimes fall outside their range of tolerance, in terms of time to maturity, adult longevity, and seed bank persistence (the "immaturity risk" *sensu* Keeley and Fotheringham 2000). This is because seeders requiring a longer period to reach maturity than the mean fire interval are eliminated rapidly (Zedler et al. 1983; Clark 1991; Pausas 1999b). In such environments with short fire intervals, long life spans of seeders have no selective advantage. As in these species the trade-off between survival and reproduction is biased toward the latter (Bond and van Wilgen 1996), they tend to have higher fecundity than resprouters (Lamont and Wiens 2003; Pausas et al. 2004). Accordingly, short fire intervals would lead seeders to reproduce earlier, to have higher fecundity, and to live a shorter time (Clark 1991; Le Maitre 1992; Bell and Pate 1996; Lamont and Wiens 2003; Pausas et al. 2004; Pausas and Verdú 2005). Consequently, and because generation time is a function of age at maturity, fecundity, and life span (Leslie 1966), seeders would necessarily have a shorter generation time than resprouters, thus potentially increasing their molecular evolutionary rates and, ultimately, their diversification.

On the other hand, diversification mediated by isolation of populations is expected to be more marked in seeders (Cowling and Pressely 2001). This is because fluctuations in population size under recurrent fire are stronger in seeders as the recruitment of a new cohort is preceded by the death of the parent generation (Higgins et al. 2000; Ojeda et al. 2005), thus favoring genetic drift processes (Engen et al. 2005). Besides, this dynamics avoids generation overlapping, which would further increase genetic differentiation among populations (Wade and McCauley 1988; Nunney 1993; Ellner and Hairston 1994).

In contrast, we would not expect differences in molecular rates between seeders and resprouters if the latter accumulate somatic mutations due to higher longevity (Lamont and Wiens 2003) or if there is no link between generation time and mutation rates, as has been reported in some plant species (Whittle and Johnson 2003). Similarly, diversification rates are not expected to differ between seeders and resprouters if mutation rates are not driving diversification (Barraclough and Savolainen 2001).

It remains unknown whether seeders have higher molecular and diversification rates than resprouters. The recent increase in molecular information and phylogenetic methods (Chan and Moore 2002; Paradis 2005; Ree 2005) provides an adequate framework for testing the role of fire in the molecular evolutionary rates and the diversification of species in fire-prone ecosystems.

In this article, we explored whether seeder lineages have (1) higher molecular evolutionary rates, and (2) higher diversification, than resprouter lineages. The first hypothesis was tested by comparing the nucleotide substitution rates in phylogenetically paired taxa with contrasting seeder and resprouter life histories (Muse and Weir 1992). The second hypothesis was explored using two diversification approaches that exploit different sources of phylogenetic information (Chan and Moore 2002). The branching pattern (topological approach) and the branch length/duration (chronological approach) of the observed phylogenetic trees were compared against trees generated under stochastic diversification models (Chan and Moore 2005; Ree 2005).

Methods

MOLECULAR EVOLUTIONARY RATES

We searched DNA sequences of different nuclear (ITS*,* *ncp*GS) and chloroplast (*atp*B-*rbc*L, *mat*K, *ndh*F, *psb*A-*trn*H, *rp*L16, *rp*S16, *rp*L20-*rp*S18, *trn*T-L, *trn*L, *trn*L-F, *trn*V) regions available in the Genbank for congeneric pairs species (plus the outgroup) with contrasting post-fire regeneration life histories (resprouters vs. seeders) (see online Supplementary Appendix S1). Data on life-history strategies were obtained from Beyers (2001), Bond and Midgley (2003), Crisp and Cook (2003), Barker et al. (2004), Pausas et al. (2004), and Hardy and Linder (2005). To ensure phylogenetic independence of the pairs (Felsenstein 2004), they were selected as follows: (1) for species belonging to genera in which the phylogeny was unknown, only one congeneric pair per genus was considered, and a species from another closely related genus was chosen as an outgroup; (2) for genera with published phylogenies, we were able to select more than one pair by checking that the path between the two members of each pair did not intersect with the path of another pair; in this case, the outgroup was also from the same genus (Felsenstein 2004 p. 443). We finally obtained 45 pairs belonging to all the Mediterranean-type ecosystems in which fire has been thought to act as a selective pressure favoring the existence of seeders (Australia, South Africa, California, and the Mediterranean Basin; Cowling et al. 2005; Pausas et al. 2006). Because fire does not appear to have had an important evolutionary role in Chilean flora (Muñoz and Fuentes 1989; Cowling et al. 1996; Keeley 1995), no Chilean taxa were included.

DNA sequences for each species pair (plus the outgroup) were aligned using Clustal X (Thompson et al. 1997) and corrected by visual inspection in SE-AL 2.0 a.11 (Rambaut 1996). The branch lengths were estimated as the expected nucleotide substitutions per site by means of the Relative Rate Test method under maximum likelihood as implemented in the HyPhy software (Kosakovsky Pond et al. 2005). Because time is identical for the two species of each phylogenetic pair, branch lengths can be considered as evolutionary molecular rates. Then, for each DNA region we compared the number of pairs in which seeders had longer branch lengths than resprouters. Looking at the sign rather the magnitude of the difference between branch lengths, we weighted each pair equally avoiding problems with the difference in the branch lengths of the two compared species per pair (Whittle and Johnson 2003). A GTR+I+G model of DNA evolution was selected for all the comparisons (see Wright et al. 2006 for a similar procedure). We also calculated the molecular rates using Kimura's two parameter model of DNA evolution (Kimura 1980), which yielded similar results (data not shown). Because the demographic processes invoked here to explain higher mutation rates in seeders (genetic drift, isolation of populations, bottlenecks) are expected to affect all the genome (Lewontin and Krakauer 1973; Luikart et al. 2003), we also checked the consistency of the results across DNA regions within each paired comparison. In other words, we checked whether seeders had longer branch lengths in all the DNA regions studied. We used only the sign, not the magnitude, of the difference between seeder and resprouter branch lengths because each DNA region may evolve at different rates. This allowed us to include both coding and noncoding regions in our analysis because they behave in a similar qualitative manner (Barraclough and Savolainen 2001).

DIVERSIFICATION RATES

We searched the literature to find information on plant genera in fire-prone ecosystems with available data on both phylogeny and post-fire life-history traits. After excluding some genera because of either poor phylogenetic resolution (*Arctostaphylos*, *Ceanothus*, *Casuarina, Eucalyptus*, *Protea*) or poor information on the life history of many species (*Genista*), we finally selected five genera: *Banksia* (Proteaceae; Bond and Midgley 2003; Mast and Givnish 2002), *Daviesia* (Fabaceae; Crisp and Cook 2003), *Lachnaea* (Thymeleaceae; Beyers 2001; Robinson 2005), *Leucadendron* (Proteaceae; Barker et al. 2004), and *Thamnochortus* (Restionaceae; Hardy and Linder 2005). These genera encompass different biogeographical regions such as Australia (*Banksia* and *Daviesia*) and South Africa (*Lachnaea, Leucadendron*, and *Thamnochortus*) and different life forms such as herbaceous (*Thamnochortus*) and woody species. Nearly all the species of each genus are included in the phylogenies except for *Daviesia* that included only 50% of the species. Incomplete taxon sampling would bias our results only if the sampling was biased regarding our fire-persistence life-history trait, which is not expected to occur given the sampling design (Crisp and Cook 2003). On the other hand, the phylogeny of *Banksia* included a nested clade corresponding to five of the 93 *Dryandra* species. Thus, our results can be interpreted only to the currently considered genus *Banksia* until future molecular studies provide the whole phylogeny of the *Banksia*/*Dryandra* complex.

As only the consensus or one of the most parsimonious trees were published in the studies cited above, we repeated the phylogenetic reconstruction to recover the most probable trees and thus to account for different sources of uncertainty (topology and branch lengths). Thus, we reconstructed the phylogenies of these five genera in a Bayesian analysis with the help of MrBayes 3.1.2 and the Phylemon web server (Huelsenbeck and Ronquist

2001, Tárraga et al. 2007) by using the Markov Chain Monte Carlo (MCMC) procedure to sample trees reconstructed from the Genbank DNA sequence data deposited by the original authors. For each genus, we first aligned the sequences with Clustal X and then calculated the best-fit model of DNA evolution selected by the hierarchical-likelihood ratio test, as implemented in the HyPhy software (Posada and Crandall 1998; Kosakovsky Pond et al. 2005). The analyses were run for a number of generations that ensured convergence of the chains (ranging from 200,000 to 3,000,000 generations) and trees were sampled every 10 generations. We determined that chains had converged onto the stationary distribution when the average standard deviation of split frequencies was < 0.01. We also checked that the log-likelihood values stopped increasing with the number of generations. Following the MrBayes manual, the first 25% of the trees were discarded as the burnin of the chain. This arbitrary amount ensured by far that convergence had been reached. Default settings of MrBayes were used. We recovered consensus trees (online Supplementary Appendix S2) that were identical or strongly similar to those published by the authors. Differences consisted only in a few interchanges between two close tips. Chronological diversification methods require an estimation of the temporal duration of the branches. Because all the phylogenies significantly departed from a molecular-clock evolution ($P << 0.05$ for all the Likelihood Ratio Test; HyPhy software), we used the nonparametric rate smoothing method of Sanderson (1997), as implemented in the APE package (Paradis et al. 2004), to transform each tree in a chronogram. The branch lengths of the chronograms are clock-like and scaled to an arbitrary age of the tree of 1.

The topological test of diversification was run in a subset consisting of the 1000 trees with the highest posterior probability found during the MCMC search, using the Symmetree package (Chan and Moore 2005). This method tests if the whole tree experienced significant variation in diversification rates without considering the life-history traits. We calculated two statistical indices: Colless index I_c (Colless 1982; Heard 1992) and M_π (Chan and Moore 2002, 2004). These indices test the departure of the observed tree topologies from trees generated under an equal-rates Markov model of clade growth (Yule 1924), and each one is sensitive to asymmetry arising at different phylogenetic scales of the tree. M_π is more sensitive to large-scale (i.e., near the root) asymmetry whereas I_c is more sensitive to small-scale (i.e., near the tips) asymmetry (Chan and Moore 2002). The phylogenetic uncertainty is directly accommodated in this analysis because the probability of diversification rate variation for each tree is weighted by the posterior probability of that tree (see Symmetree users guide). To check if shifts in the diversification rate corresponded to shifts to the seeder life history, we selected 20 asymmetric trees per genus among the trees with higher posterior probability. The nodes in which the diversification rate significantly shifted were identified with the Δ_1 and Δ_2 indices provided by the Symmetree package. We then inspected if a transition from resprouting to seeding, based on parsimony reconstruction (MacClade 4; Maddison and Maddison 2003), had also occurred in such nodes.

The chronological test of diversification was run in a subset of 3000 randomly selected trees from the Bayesian samples of trees in the Key Innovation Test (KIT) program (Ree 2005; available at http://bioinformatics.org/~rick/software.html). This method tests whether the evolutionary history of one trait is associated with diversification (i.e., cladogenesis) under the expectation that lineages with the candidate trait have shorter waiting times between cladogenesis events. The waiting times represent relative temporal durations in terms of units of evolutionary changes in the character such that the length of the tree is the total amount of change in the character expected over the whole phylogeny (Ree 2005). We used two different treelength priors (R. Ree, pers. comm.): (1) a prior of 1, following the expectation for a binary character without knowledge of the phylogeny, and (2) a prior corresponding to the average number of parsimony-inferred character changes (10 for *Daviesia*, 5 for *Leucadendron*, 8 for *Lachnaea*, and 9 for *Thamnochortus;* attempts to run the test with *Banksia* were computationally unsuccessful possibly because of the large number of parsimony reconstructed changes (25); parsimony-reconstructions were done in the MacClade 4 software [Maddison and Maddison 2003]). Waiting times of the observed trees are compared against a null distribution generated by a stochastic model of character evolution and cladogenesis (Yule 1924). This method is appropriate for our problem because it is specially well suited for (1) recently evolved traits in which insufficient time has passed for the shift in the diversification rate to asymmetrize the tree topology, and (2) traits with bidirectional evolution (e.g., resprouters may evolve toward seeders and vice versa). Furthermore, the method accommodates uncertainty in the phylogeny. The life-history state for a few species was uncertain and therefore we accommodated such uncertainty by rerunning the test with alternative states. As the results did not change, we only present one of the tests. We finally combined the probabilities of the five diversification tests to analyze whether there is a significant trend across the five genera. This was done by means of the *Z*-transform test that is superior to Fisher's combined probability test (Whitlock 2005).

Results

The expected signature of seeders having higher molecular rates than resprouters consistently across different genome regions occurred only in seven of 26 congeneric pairs (Table 1). In the remaining comparisons, the molecular rate either depended on the target DNA region (15 of 26 comparisons; N-d in Table 1) or was consistently higher for resprouters (four out of 24; N-o in Table 1).

Table 1. Comparison of evolutionary molecular rates of congeneric resprouter and seeder species across different DNA regions. Resprouter = species that resprout after fire; Seeder = species that do not resprout (killed by fire) and whose population persist from seed bank. The fifth column indicates the life-history trait (R, S) of the species with the higher molecular rate for the different DNA regions (e, equal rate; -, no data available). DNA regions used are (and the order; the fifth column): *atp*B-*rbc*L, *mat*K, *ndh*F, *psb*A-*trn*H, *rp*L16, *rp*S16, *rp*L20-*rp*S18, *trn*T-L, *trn*L, *trn*L-F, *trn*V, *ncp*GS, and ITS. An asterisk indicates *rbc*L. For full details see online Supplementary Appendix S1. The last column shows whether the expected consistency across DNA regions of higher molecular rates for the seeder species occurs (Y) or not (N); in the latter case, the inconsistencies may be due to a different trend among the different DNA regions (N-d) or to an observed higher molecular rate in resprouters, that is, an opposite trend to the hypothesis (N-o).

Genus	Resprouter (R)	Seeder (S)	Outgroup	Higher	Consistency across DNA regions
Allocasuarina	*A. glareicola*	*A. distyla*	*A. nana*	R············	-
Arctostaphylos	*A. glandulosa*	*A. glauca*	*Arbutus unedo*	············R	-
Baeckea	*B. linifolia*	*B. crassifolia*	*Callistemon polandii*	SS···········	Y
Banksia	*B. audax*	*B. benthamiana*	*B. laevigata*	···e···R·····	N-d
Banksia	*B. elegans*	*B. cuneata*	*B. candolleana*	···R···S-R···	N-d
Banksia	*B. grossa*	*B. lanata*	*B. micrantha*	···S·········	-
Banksia	*B. incana*	*B. laricina*	*B. dolichostyla*	···S·········	-
Banksia	*B. oblongifolia*	*B. spinulosa collina*	*B. ericifolia*	···R·········	-
Banksia	*B. repens*	*B. blechnifolia*	*B. elderiana*	···e···S·····	N-d
Casuarina	*C. glauca*	*C. cristata*	*Ceuthostoma palawaense*	·R···········	-
Ceanothus	*C. cordulatus*	*C. cuneatus*	*Rhamnus californica*	·S··········S	Y
Cryptandra	*C. amara*	*C. lanosiflora*	*Spyridium parviflorum*	············R	-
Daviesia	*D. ulicifolia stenophylla*	*D. acicularis*	*D. wyattiana*	············R	-
Daviesia	*D. alternifolia*	*D. cordata*	*D. divaricata*	············R	-
Daviesia	*D. angulata*	*D. teretifolia*	*D. dilatata*	············S	-
Daviesia	*D. epiphyllum*	*D. obovata*	*D. genistifolia*	············S	-
Erica	*E. arborea*	*E. tristis*	*Daboecia cantabrica*	RR··········R	N-o
Eucalyptus	*E. glaucina*	*E. nitens*	*Corymbia eximia*	············S	-
Genista	*G. scorpius*	*G. triacanthos*	*Cytisus scoparius*	········S···R	N-d
Hibiscus	*H. tiliaceus*	*H. splendens*	*Abutilon fraseri*	····R·······R	-
Juniperus	*J. oxycedrus*	*J. sabina*	*Cupressus sempervirens*	············S	-
Kunzea	*K. ericoides*	*K. ambigua*	*Melaleuca viridiflora*	SS···········	Y
Lachnaea	*L. glomerata*	*L. diosmoides*	*L. burchellii*	S*····R······R	N-d
Lachnaea	*L. grandiflora*	*L. leipoldti*	*L. pusilla*	R···········R	N-o
Lachnaea	*L. naviculifolia*	*L. eriocephala*	*L. pedicellata*	S···S···S···S	Y
Leucadendron	*L. spissifolium spissifolium*	*L. laureolum*	*L. pondoense*	············S	-
Leucadendron	*L. brunioides*	*L. stellare*	*L. linifolium*	············R	-
Leucadendron	*L. salignum*	*L. discolor*	*L. modestum*	············S	-
Melaleuca	*M. nodosa*	*M. hypericifolia*	*Leptospermum scoparium*	············R	-
Mirbelia	*M. pungens*	*M. speciosa*	*Bossiaea linophylla*	·········S··S	Y
Ochrosperma	*O. lineare*	*O. citiodorum*	*Syncarpia glomulifera*	·R···········	-
Phillyrea	*Ph. angustifolia*	*Ph. latifolia*	*Olea europaea*	·····R···S···	N-d
Pinus	*P. canariensis*	*P. pinaster*	*P. halepensis*	eR···R···SS·S	N-d
Pinus	*P. virginiana*	*P. clausa*	*P. contorta*	SS···········	Y
Pinus	*P. rigida*	*P. pungens*	*P. muricata*	RS···e···R···	N-d
Protea	*P. revoluta*	*P. laevis*	*P. acaulos*	S····S··eR·S·	N-d
Protea	*P. scolopendriifolia*	*P. pruinosa*	*P. cynaroides*	e····R··ee·R·	N-d
Protea	*P. speciosa*	*P. stokoei*	*P. scabra*	e····R··ee·e·	N-d
Pultenaea	*P. myrtoides*	*P. glabra*	*Chorizema aciculare*	R·····S·····R	N-d
Thamnochortus	*Th. cinereus*	*Th. rigidus*	*Th. glaber*	Re·······R···	N-d
Thamnochortus	*Th. erectus*	*Th. insignis*	*Th. guthierae*	SS·······S···	Y
Thamnochortus	*Th. Lucens*	*Th. dumosus*	*Th. pellucidus*	SR·······e···	N-d
Thamnochortus	*Th. sporadicus*	*Th. punctatus*	*Th. platypteris*	RR·······R···	N-o
Thamnochortus	*Th. spicigerus*	*Th. fraternus*	*Th. pluristachyus*	RR·······e···	N-d
Ulex	*U. europaeus*	*U. parviflorus*	*Calicotome spinosa*	·········R··R	N-o

Table 2. Number of congeneric pairs within each DNA region in which the molecular evolutionary rate is higher for each life-history trait. The last column shows whether the hypothesis of seeders having higher molecular rates is supported or not, that is, Y indicates that there are more congeneric pairs in which the molecular rate is higher for seeders than for resprouters; N otherwise.

DNA region	R	S	Equal	Supported
*atp*B-*rbc*L	6	8	3	N
*mat*K	8	6	1	N
*ndh*F	1	0	0	N
*psb*A-*trn*H	2	2	2	N
*rp*L16	1	0	0	N
*rp*S16	4	2	0	N
*rp*L20-*rp*S18	1	0	1	N
*trn*T-L	1	2	0	Y
*trn*L	0	0	3	N
*trn*L-F	5	7	4	N
*trn*V	1	1	0	N
*ncp*GS	1	1	1	N
ITS	12	10	0	N

When comparing the congeneric pairs within the same DNA region, seeders had higher molecular rates in only one of the 13 DNA regions studied (Table 2). This is not to say that resprouters have higher molecular rates because many comparisons yielded equal branch lengths between seeders and resprouters (Table 2).

Only in one genus (*Lachnaea*) was there no evidence of asymmetric branching pattern (i.e., differential diversification) as the proportion of trees departing significantly from symmetry is negligible (Table 3). Some uncertainty remains for the rest of genera (*Banksia*, *Daviesia*, *Leucadendron*, and *Thamnochortus*), in which an important proportion of trees showed a topology indicating significant diversification. However, such diversification is not associated with the seeder life-history trait, as none of the nodes in which a shift in the diversification rate was detected corresponded with shifts to the seeder life history.

Supporting this conclusion, the chronological tests also showed that seeders did not have shorter waiting times between cladogenesis events than resprouters for any of the genera studied (Fig. 1). The same conclusions were obtained when the treelength prior was set to 1 (Fig. 1) or to the average number of parsimony-inferred character changes (data not shown). As expected from these results, the combination of the five probabilities did not show any significant overall pattern ($z = 0.13$; $P = 0.55$; z-transform test). Thus, these results did not provide any evidence to associate seeder life history with diversification.

Discussion

Mediterranean-type ecosystems are included among the main biodiversity hot spots around the world (Myers et al. 2000), and fire is considered one of the main drivers of diversification in these ecosystems (Cowling and Pressey 2001). Recurrent fire (with an average frequency of about 15–50 years; Cowling et al. 1996) has been suggested to trigger dramatic diversification by selecting taxa with short generation times and/or driving the isolation of populations. Because seeders have shorter generation times and their populations are more prone to between-fire fluctuations (Higgins et al. 2000), they are expected to diversify more. Nevertheless, we failed to find a consistent association of fire life histories with diversification rates by either topological or chronological methods. Consequently, seeders do not diversify more than resprouters. When the null hypothesis is not rejected, as is the case in this study, doubts can arise about the methodological approach and/or the data collection. However, the fact that the different lines of evidence reported herein point to this lack of association, enables us to suggest that diversification is not associated with the seeder life history in taxa from fire-prone ecosystems.

The absence of differential diversification is to be expected if seeders and resprouters do not differ from each other in their molecular evolutionary rate, which is the fuel for speciation under the evolutionary rate hypothesis (Barraclough and Savolainen 2001; Evans and Gaston 2005). Indeed, we have not found any evidence of seeders having faster evolutionary rates than resprouters when comparing either between DNA regions or between taxa. This result concurs with growing empirical evidence challenging

Table 3. Range of *P*-values for the two symmetry statistics used to test topological diversification in the five genera under study. The percentage of significant *P*-values (i.e., $P < 0.05$) is also shown. N refers to the number of taxa included in each phylogeny. See Methods section for a description of the statistics.

		Ic		M_Σ	
	N	*P*-range	%	*P*-range	%
Banksia	89	0.000–0.988	29.6	0.000-0.879	61.0
Daviesia	46	0.002–0.256	79.5	6×10^{-5}–0.576	75.7
Lachnaea	38	0.0094–0.6562	0.2	0.013–0.820	4.9
Leucadendron	62	0.000–0.6181	88.4	0.000–0.1643	98.4
Thamnochortus	30	0.029–0.450	19.95	0.038–0.774	0.9

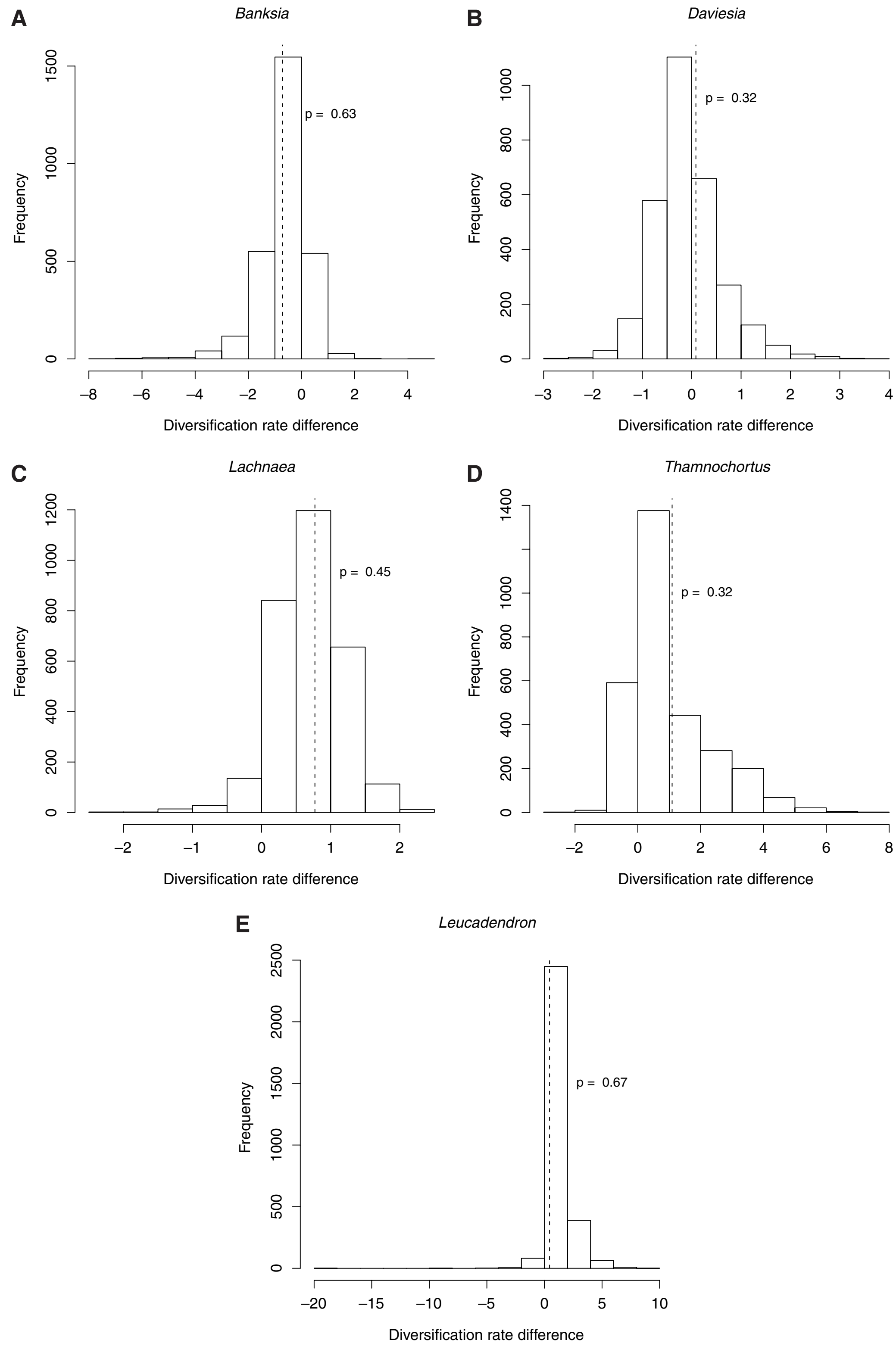

Figure 1. Frequency distributions of the differences in diversification rates between seeders and resprouters under the null model of chronological diversification for the five genera studied. The dashed line refers to the observed value; its associated *P*-value is also indicated.

the generation-time hypothesis in plants (Bousquet et al. 1992; Whittle and Johnson 2003, but see Catalán et al. 2006). Three different explanations could address the lack of differences in evolutionary rates between the two life-history strategies.

First, differences in evolutionary rates within a seeder–resprouter species pair may be masked if the timing of the switch from seeding to resprouting (or vice versa) occurs near the branch tip, so that most of the branch length evolves under the opposite life-history form (Whittle and Johnson 2003). An indication that recent switches are possible is the existence of species with both seeder and resprouter populations (e.g., *Banksia marginata*, George 1996; Aspalathus linearis, van der Bank et al. 1999; *Ceanothus tomentosus*, Schwik and Ackerly 2005; several southern African *Erica* species, Verdaguer and Ojeda 1998). However, such species are rare in nature. Second, in recurrently burned ecosystems, somatic mutations in resprouters might counterbalance the replication-induced mutations in seeders (Lamont and Wiens 2003), although empirical evidence to support this hypothesis is scarce. Third, the relationship between mutations and generation time in plants is unclear because plants do not undergo determinate germ-line replication (Gaut et al. 1996, 1997; Whittle and Johnson 2003).

Speciation may not be limited only by molecular evolutionary rates; it may also be influenced by other factors such as the formation of isolated populations (Ohta 1993; Bromham and Cardillo 2003) and/or strong reductions in genetic variability within fluctuating populations (Ellner and Hairston 1994; Engen et al. 2005). Such fluctuations and concomitant genetic erosion are more marked in organisms with nonoverlapping generations (Ellner and Hairston 1994; Waples 2002), such as seeder plant species from fire-prone environments (Bond and van Wilgen 1996; Ojeda et al. 2005), thus making them more prone to genetic drift and differentiation processes (Wade and McCauley 1988). However, these diversifying seeder populations or potentially “incipient species” (sensu Levin 2000) are also more prone to extinction, because they do not store reproductive potential over generations (Higgins et al. 2000; Ojeda et al. 2005; see Warner and Chesson 1985 for a general overview). This counteracting higher extinction would then account for the seeming lack of overall diversification in seeder lineages from the fire-prone, Mediterranean-type ecosystems reported in this study. Although this possibility deserves further investigation, we can conclude that fire acting as a throttle for diversification is by no means the rule in fire-prone ecosystems.

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Supplementary Material

The following supplementary material is available for this article:

Appendix S1. Evolutionary molecular rates for the resprouter–seeder congeneric pairs studied.
Appendix S2. Consensus trees for the phylogeny of the five genera used to study diversification (*Banksia, Daviesia, Lachnaea, Thamnochortus, Leucadendron*).

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