

Banksia born to burn

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Summary

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- Historical evidence of recurrent fire in many of the world's biomes suggests that fire may have had profound evolutionary influences on their extant floras. However, the role of fire as a selective force in the origin and evolution of plant traits remains controversial.
- Using Bayesian Monte-Carlo-Markov-Chain procedures and calibration points from the fossil record, we generated a dated phylogeny for the iconic Australian genus *Banksia*, and reconstructed the evolutionary/chronological position of five putatively fire-related traits.
- The fire-dependent trait, on-plant seed storage (serotiny), and associated fire-enhancing trait, dead floret retention, co-originated with the first appearance of *Banksia* 60.8 million yr ago (Palaeocene). Whether nonsprouting or resprouting is ancestral was indeterminable, but the first banksias were nonclonal. Derived traits, such as dead leaf retention (fire-enhancing) and clonality (underground budbanks; fire-avoiding), first appeared 26–16 million yr ago (Miocene) with the onset of seasonal drought and thus more frequent fire, and culminated in dead florets/bracts completely covering the persistent fruits in some species.
- Thus, fire may have been a selective force in the very origin of *Banksia* 40 million yr before the onset of climate seasonality in the Miocene, and continued to have an impact on the direction of evolution, favouring traits consistent with adaptation to an increasingly (sometimes less) fire-prone environment.

Introduction

Fire has been an element of some landscapes for hundreds of millions of years (Bowman *et al.*, 2009; Pausas & Keeley, 2009), creating plant communities different in structure and composition from those expected in its absence (Bond *et al.*, 2005). Bond & Scott (2010) even suggested that the development of novel fire regimes promoted the spread of angiosperms in the Cretaceous. In fire-prone sclerophyll shrublands of Australia, Mediterranean Europe, North America and South Africa, for example, most plant species possess one or more sophisticated traits, such as serotinous fruits and cones that only open in response to heat, resprouting from protected dormant buds, fire-stimulated flowering, and heat- or smoke-stimulated seed germination, that appear to be adaptive responses to fire (Pausas *et al.*, 2006; Pausas & Keeley, 2009). Other putative fire-related traits are less well studied, such as highly combustible leaves, retention of dead leaves and branches (Schwilck & Ackerly,

2001), and serotinous cones that retain their dead florets (Lamont & Cowling, 1984).

The occurrence of fire throughout the history of terrestrial vegetation (Scott & Glasspool, 2006; Bond & Scott, 2010) invites conjecture that fire must have had pronounced evolutionary effects on many biotas (Bowman *et al.*, 2009). However, the role of fire as an evolutionary selective force remains controversial. Zedler (1995) suggested that, when a species is exposed to frequent fire for long enough, a degree of fire-related selection is inevitable. However, Hopper (2009) argued that the literature on plant traits inferred to be adaptations to fire is replete with confusion between adaptation and exaptation, and that multiple evolutionary selective forces other than fire may be invoked as the cause of traits that function in coping with fire today. For example, post-burn resprouting is often considered to be a fire adaptation, but resprouting *per se* is a widespread trait in angiosperms even in nonfire-prone environments. Serotiny of cones and fruits is closely tied to

fire, and is regarded as a key fire-survival trait in many woody taxa (Lamont *et al.*, 1991), but Axelrod (1980) attributed serotiny to harsh climates, at least among pines. Traits such as the retention of dead leaves and florets have been cited as potential flammability-enhancing traits (Lamont & Cowling, 1984; Schwilk, 2003). Mutch (1970) hypothesized that 'natural selection has favoured development of characteristics that make plants in fire-prone environments more flammable', whereas Snyder (1984) dismissed this hypothesis, noting that flammability traits can have other equally plausible explanations.

Evolutionary interpretations of fire-related traits only make sense within the context of a phylogenetic/palaeo-environmental analysis (Bond & Midgley, 1995). Dating the tree of life has now become central to relating patterns of biodiversity to key processes in the Earth's history, and provides insights into questions such as how past fire regimes have contributed to the origin and evolution of the traits present today. Rates of trait evolution have changed over time, producing variations in the levels of morphological, functional and ecological diversity among groups. Testing for the presence of these rate shifts is a key component of determining their time of origin and understanding the driving factors in their evolution (O'Meara *et al.*, 2006).

The emerging ability to convert molecular phylogenies into time-based chronograms is revolutionizing our understanding of the historical and environmental contexts of the origin and speciation of taxa (e.g. Sauquet *et al.*, 2009; Valente *et al.*, 2010); moreover, the mapping of binary traits on a chronogram (i.e. reconstructing ancestral traits) has become a powerful method to test evolutionary hypotheses, and holds the key to understanding the pathway of trait evolution (Ronquist, 2004; Schaffer *et al.*, 2010). Rigorous phylogenetic analyses, although rare, have been providing new insights into species' and trait evolution in fire-prone environments (Schwilk & Ackerly, 2001; Bond *et al.*, 2005; Pausas & Verdú, 2005; Simon *et al.*, 2009). In our study, we utilized these new phylogenetic applications to tackle the following key questions regarding species' and trait evolution in fire-prone environments in *Banksia*, the archetypal genus for fire-response studies in Australia: (1) when did putative fire-related traits, namely serotiny, dead floret and leaf retention, and clonality (and their opposing states) first appear within *Banksia*?; (2) at what rate did *Banksia* taxa with these fire-related traits proliferate in particular geological periods associated with known climatic changes?; and (3) if the coevolution of fire-dependent and fire-enhancing traits can be detected, what does this imply about the timing of fire as a selective force? Answers to such questions are critical to understanding the evolution of fire-prone ecosystems and in the quest to identify traits uniquely adapted to fire.

Materials and Methods

Banksia

Although 75% of *Banksia* (Proteaceae) taxa are endemic to the southwest, this genus is ubiquitous across the nonarid parts of Australia. This genus consists of highly sclerophyllous, creeping shrubs to moderate-sized trees, and *Banksia sensu stricto* is probably the best understood plant group taxonomically, genetically, functionally and ecologically in Australia (Lamont *et al.*, 2007). There is also a near-complete molecular phylogeny (Mast & Givnish, 2002) and extensive dated macrofossils are available (Greenwood *et al.*, 2001). This genus has been in Australia for at least 60 million yr (My; Martin, 1994; Crisp *et al.*, 2004), and so can be expected to possess a range of traits to cope with fire (Lamont *et al.*, 2007). Mast & Thiele (2007) recently sank *Dryandra* into *Banksia* according to their preliminary molecular evidence, but Collins *et al.* (2008) disagreed with this treatment, supporting the long-held view that *Dryandra* evolved from *Banksia* but is clearly distinguished from it by sound morphological characters. Here, we adopt the taxonomy of Collins *et al.* (2008) for *Banksia*, and retain *Dryandra* as a separate taxonomic identity. The closely related (rainforest) species, *Musgravea heterophylla* and *Macadamia integrifolia*, were included as the ancestral outgroup (Mast & Givnish, 2002).

Molecular dating

DNA sequences for the five chloroplast DNA regions (*trnL* intron, *trnL/trnF* spacer, *rp116* intron, *psbA/trnH* spacer, *trnT/trnL* spacer), which were originally used to derive the *Banksia* phylogeny including 85 species/subspecies/varieties (Mast & Givnish, 2002), were obtained from GenBank (<http://www.ncbi.nih.gov>) and re-analysed to produce a molecular phylogeny. Five representative (serotinous, retaining bracts and dead leaves, and with variable growth forms) *Dryandra* species for which equivalent data were available were also included. As a recent and monophyletic outlier (Mast *et al.*, 2005; and confirmed here), and the studied traits being relatively general across the genus (George, 1996), the weak sampling of *Dryandra* was expected to have negligible effects on molecular dating and the primary task of ancestral trait reconstruction (Hawkins, 2006) in *Banksia*. The dating of lineages was carried out using BEAST V1.4.8 (Drummond & Rambaut, 2007), which estimates the tree structure and the date of node (stem age) simultaneously, using a Bayesian Monte-Carlo-Markov-Chain (MCMC) procedure and calibration points. The crown age of *Banksia* was calibrated to 41 million yr ago (Ma) (Crisp *et al.*, 2004) with a normal prior distribution and a standard deviation of 2.1 Ma to allow for temporal error. The normal prior distribution was used

because it best allows for uncertainty in the calibration estimates (Ho, 2007).

We constructed the base of the stem of the Banksieae (*Banksia* + *Dryandra*) to the first appearance of its distinctive pollen at 62 Ma (Macphail *et al.*, 1994; Martin, 1994), assuming the basal divergence between Banksieae and the outgroup (*Macadamia* and *Musgravea*) at 62 Ma with a normal prior distribution and a standard deviation of 3.1 Ma. BEAUTi (Drummond & Rambaut, 2007) was used to generate the BEAST input file. A Yule process was used to describe speciation, and an uncorrelated lognormal (UCLN) model was used to describe the relaxed molecular clock. Five independent MCMC runs were conducted, and each MCMC chain was run for 10^7 generations, sampling every 10^4 , and the log and time tree files were combined using LogCombiner (version 1.5.1). MCMC diagnostics were evaluated in Tracer v1.4.1 (Rambaut & Drummond, 2008). The consequent tree file containing 1000 plausible *Banksia* phylogenies with branch lengths (divergence time) was used for the reconstruction of ancestral states as described below. In addition, the phylogenies for each run were combined, and the maximum clade credibility tree was produced and annotated with the mean age of nodes and posterior clade probability for each node using TreeAnnotator V1.4.8 (Rambaut & Drummond, 2008). Figtree V1.1.2 was then used to display the tree with estimated node ages (Rambaut, 2008). The maximum clade credibility tree was used to illustrate the origin and evolution of the trait.

Ancestral traits

Five traits, and their opposing states (which we also term traits, consistent with the tradition in ecology), that appear to give a selective advantage in the presence of fire were investigated: resprouting (fire-tolerant/avoiding), clonality (fire-avoiding), serotiny (fire-dependent), and retention of dead florets and retention of dead leaves (fire-enhancing). Traits for all *Banksia* taxa, five *Dryandra* species and the outgroup were collated from the literature (Taylor & Hopper, 1988; George, 1996; Collins *et al.*, 2008; Western Australian Herbarium, 2009) and our own fieldwork (dead leaf retention has not been recognized previously). Study species were first categorized according to their whole-plant response to fire, that is, resprouting or its opposing state, fire-killed. Clonality may be viewed as the ultimate whole-plant adaptation to fire [most vulnerable to being burnt (ground-hugging), best protected budbank (soil), least seed production, survives the most fire-cycles]. Consequently, growth form was partitioned into clonal (vegetative reproduction via rhizomes or root suckers after fire, minimal seed production) and nonclonal (no vegetative reproduction, recovery via epicormic and/or lignotuberous buds and/or seeds after fire) taxa. *Banksia* produces cone-like inflores-

cences bearing numerous florets attached to a rachis from which up to 5% develop as woody follicles (Lamont *et al.*, 2007). Taxa were then classified as either retaining at least some of their seeds in the cones for several years or more (serotinous; Lamont *et al.*, 1991) or releasing all of their seeds at maturity (nonserotinous). Taxa were also grouped into those that retained at least some of their dead florets around the mature fruits, those whose mantle of dead florets was so thick and dense that the fruits were concealed (the involucre bracts serve this purpose in *Dryandra*), and those that shed their spent florets immediately. Finally, species were separated into those that retained at least some of their dead leaves on the parent stem and those that shed them once dead. Traits for individual species are provided in the accompanying figures.

We assumed that the current trait state for each species has remained unchanged since the time of origin of that species. The tree file containing 1000 plausible *Banksia* phylogenies with branch length (node age) generated by the above molecular dating procedure, and trait files containing the discrete state for each species, were used for the analyses. Bayesian MCMC methods were used to derive posterior distributions of log-likelihoods and the values of traits at the nodes of phylogenies (Pagel *et al.*, 2004). We used a continuous-time Markov model which assumes that trait states can evolve repeatedly between their possible states at any branch of the phylogenetic tree (Pagel, 1994; Pagel *et al.*, 2004) to construct the ancestral trait at each internal node to investigate trait evolution. A reversible jump (RJ)-MCMC was adopted (Pagel & Meade, 2007). In RJ-MCMC, the Markov chain searches the posterior distribution of different models of evolution as well as the posterior distributions of the parameters of these models. This model allows the trait to change from the state it is in at any given moment to any other state over infinitesimally small intervals of time. The rate parameters of the model estimate these transition rates (Pagel, 1994). The model traverses the tree estimating transition rates and the likelihood associated with different states at each node.

In the MCMC mode, a new set of rate parameters for the evolution model is proposed at each iteration of the Markov chain and implemented in *BayesMultiStates* by changing the current values by an amount given by the *ratedev* parameter representing the deviation from the normal distribution (Pagel & Meade, 2007). Thus, we first ran a few Markov chains for each trait and monitored the acceptance rate of newly proposed values of the rate parameters to determine a suitable value for the *ratedev* parameter. A value of *ratedev* was accepted if it generated an acceptance rate of 0.20–0.40 (Pagel & Meade, 2007). A *hyperprior* seeding an exponential distribution from a uniform 0–30 distribution was applied in Markov chain runs. With a burn-in of 10^4 , the Markov chains were run for 10^7 iterations, and every 10^3 iteration was recorded. The ancestral state was defined as

the trait with the higher average posterior probability determined by a t -test ($P < 0.05$). The existence of differences in trait transition rate (rate of transition from state A to state B, and rate of transition from state B to state A) for each attribute was tested by constraining the two rates to be the same (Pagel & Meade, 2007). The existence of differences in rate transition was accepted if this model was more than two log-likelihood units worse than the unconstrained model.

Trait proliferation rates

The net proliferation rate of a lineage with a particular trait state (hereafter referred to as the trait proliferation rate, TPR) was calculated as $TPR = (1/t) \times (N_{i+t} - N_i)/N_i$, where the number of lineages (N) at the start i and end $i + t$ of the time interval t was applied to the five periods/epochs in which *Banksia* has been recorded. i was set at the time the trait first appeared in the period if it was not present at the start of the period. The geological boundaries were set according to the International Commission on Stratigraphy (available from <http://www.stratigraphy.org>), and the start time in the Palaeocene was set at the time that *Banksia* first appeared. The net proliferation rate of the species was calculated in a similar way, but with the preferred logarithmic correction applied.

Correlated evolution of traits

Correlated evolution between pairs of discrete binary traits was analysed in *BayesDiscrete* (Pagel & Meade, 2007). *BayesDiscrete* tests for the correlated evolution of two binary traits by comparing the fit (log-likelihood) of two continuous-time Markov models. One of these is a model in which the two traits evolve independently on the tree (*independent* model). The other model allows the traits to evolve in a correlated fashion (*dependent* model). RJ-MCMC was adopted following Pagel & Meade (2007). A few test runs were implemented to choose a *ratedev* value that produced an acceptable rate of 0.20–0.40. Using the chosen *ratedev* value and a *hyperprior* seeding an exponential distribution from a uniform 0–30 distribution, a reversible jump *dependent* model was first tested. The analysis was repeated by confining the RJ chain to the *independent* model. In each model run, 5×10^6 iterations were implemented and the results were sampled every 10^3 iterations. The overall results were summarized as the harmonic mean. The log-Bayes factor (LBF) is twice the difference between the two harmonic means derived from the *dependent* and *independent* models of evolution. The LBF is nominally distributed as a χ^2 with degrees of freedom equal to the difference in the number of parameters between the two models, which is four: the *independent* model requires two parameters for each of two traits and the *dependent* model has eight parameters (Pagel

& Meade, 2007). A difference between these two harmonic means of > 9.49 indicates strong support ($P < 0.05$) for correlation between the two traits.

Results

Phylogenetic reconstruction and chronogram

The phylogenetic reconstruction of 85 *Banksia* species/subspecies/varieties and five representative *Dryandra* species using Bayesian MCMC procedures shows consistency with the original phylogeny reported in Mast & Givnish (2002), and the earlier phylogeny in Mast (1998), but with better resolution of more recent divergences. *Dryandra* was maintained as a terminal, outlying monophyletic group. Among 89 nodes in the maximum clade credibility tree that was summarized from 1000 possible phylogenies generated by the Bayesian MCMC procedure, 61 nodes were supported by $> 80\%$ of trees, whereas only nine nodes were supported by $< 40\%$.

The chronogram shows that the two major lineages of the crown group diverged 44.5 Ma (95% highest posterior density (HPD), 36.6–51.9 Ma) and radiated from 30 Ma through the Miocene and Pliocene. The molecular dating also suggests that the *Banksia/Macadamia–Musgravea* lineages separated 60.8 Ma (95% HPD, 55.5–66.8 Ma). *Dryandra*, forming an outer branch of *Banksia sensu stricto*, radiated from 20.0 Ma (95% HPD, 12.8–25.2 Ma). The majority of eastern Australian species (plus *B. dentata*, the only tropical *Banksia*, and distributed across northern Australia) formed a clade that separated from western congeners 25.6 Ma (95% HPD, 18.1–34.5 Ma). An additional three eastern species, *B. ornata*, *B. serrata* and *B. aemula*, diverged from western banksias 7.6 Ma (95% HPD, 3.8–12.9 Ma).

Ancestral traits and proliferation rates

Whether death or resprouting was the ancestral whole-plant response to fire in *Banksia* could only be resolved for 55 of the 89 nodes. It was not possible to elucidate the ancestral fire response for the root of *Banksia*, roots of the two major lineages and for the earlier divergence of *Banksia* lineages before 20 Ma (Miocene) by which time both responses were common. Trait reconstruction successfully revealed four ancestral traits in *Banksia* (Figs 1–4): nonclonal ($P = 0.68 \pm 0.23$), serotiny ($P = 0.94 \pm 0.12$), retention of dead florets around the follicles ($P = 0.69 \pm 0.20$) and shedding of dead leaves ($P = 0.86 \pm 0.12$), implying that these traits originated with the first appearance of *Banksia* 60.8 Ma (Palaeocene). Taxa with these ancestral states proliferated in the Miocene, but the rate of proliferation slowed in the Pliocene and Quaternary (Table 1). The alternative states for these traits first appeared 26–16 Ma and

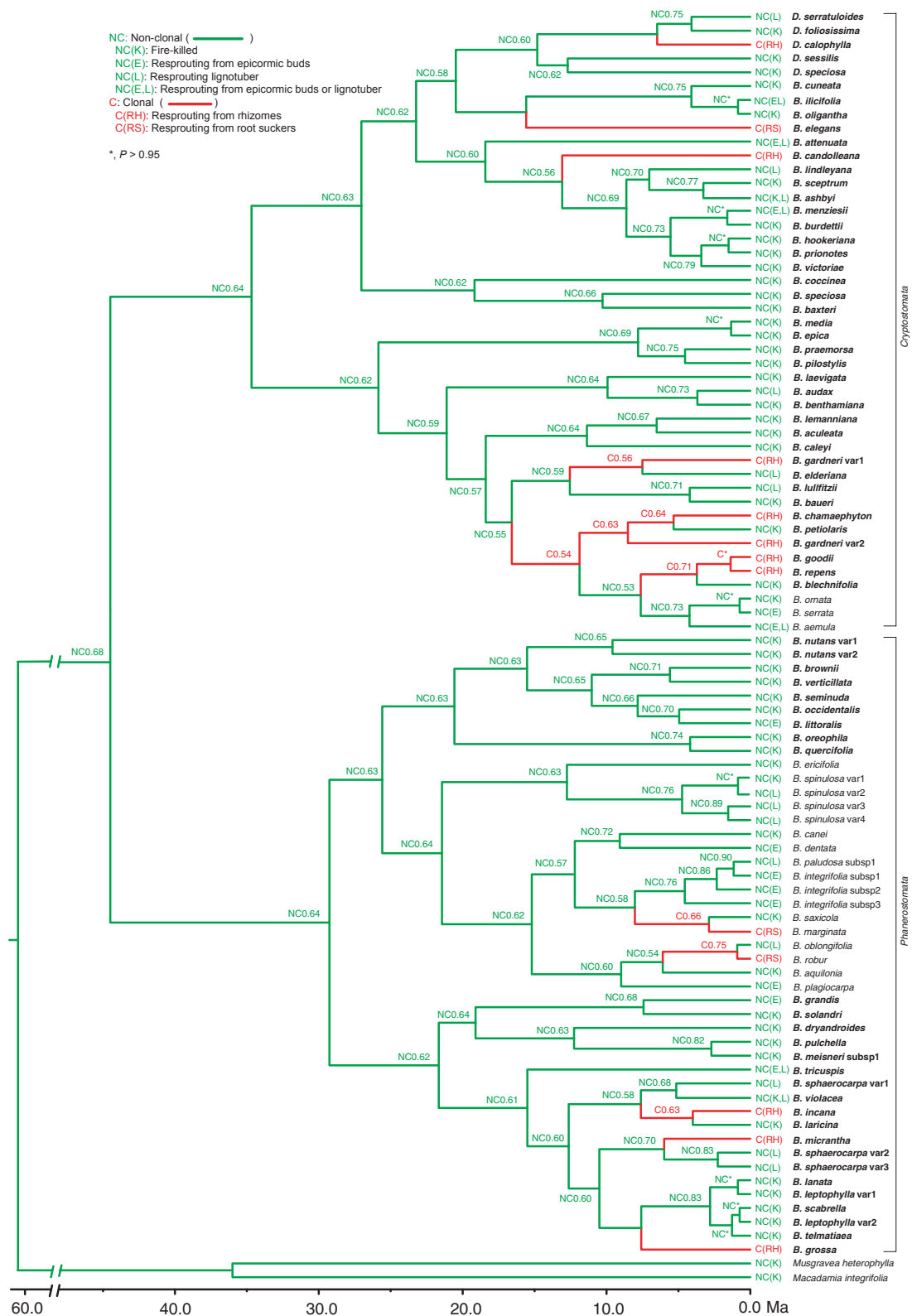


Fig. 1 Reconstruction of the ancestral trait for growth form in *Banksia*/*Dryandra*. Numbers following the abbreviations are the average posterior probabilities if below 0.95. The line remains the same colour as the ancestral state at the root if the ancestral trait was indeterminable, and with no indication of posterior probabilities. Southwest Australian species in bold. Full variety and subspecies' names are listed in Supporting Information Notes S1. Ma, million years ago.

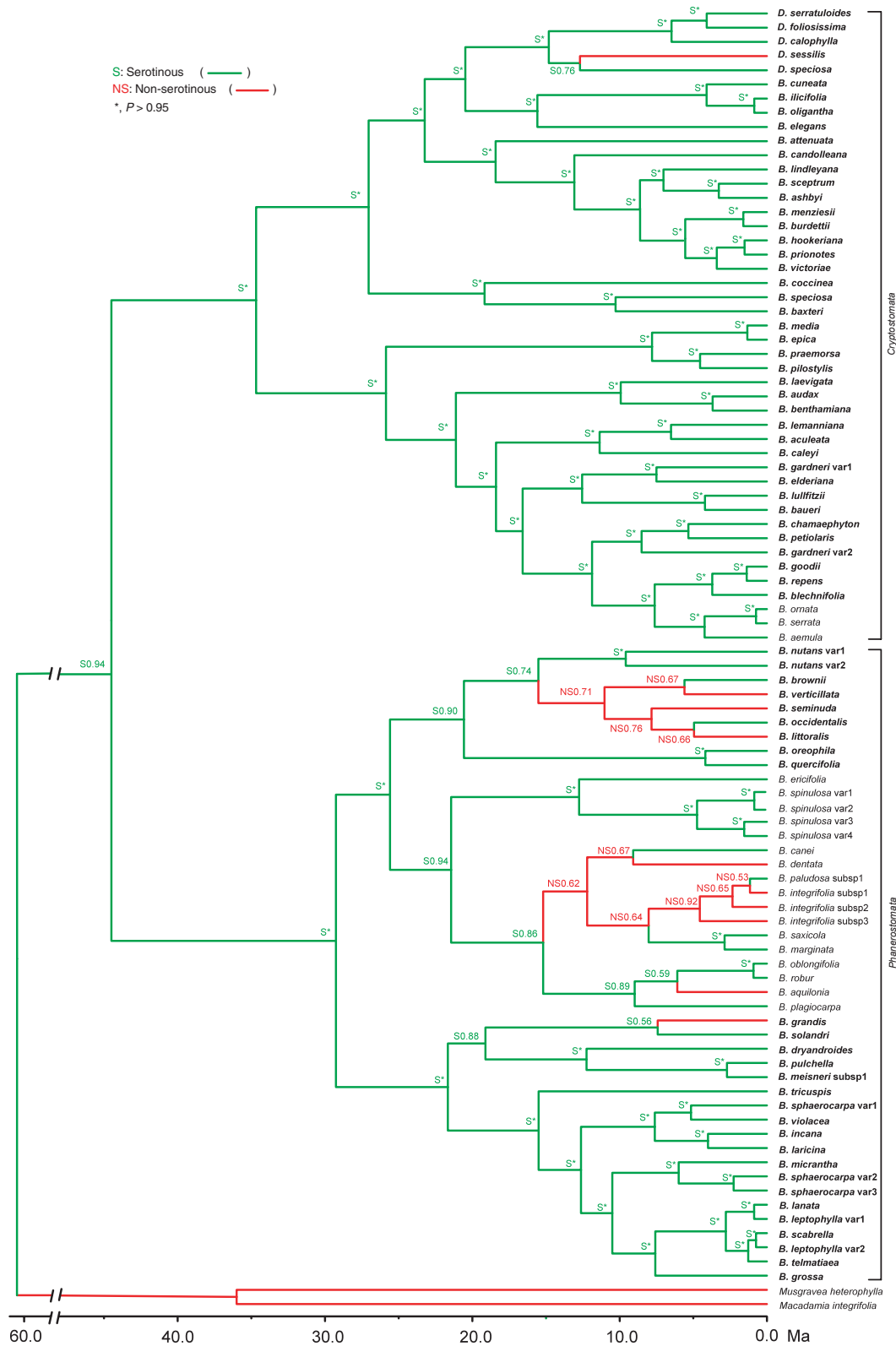


Fig. 2 Reconstruction of the ancestral trait for serotiny in *Banksia*/*Dryandra*. Numbers following the abbreviations are the average posterior probabilities if below 0.95. The line remains the same colour as the ancestral state at the root if the ancestral trait was indeterminable, and with no indication of posterior probabilities. Southwest Australian species in bold. Full variety and subspecies' names are listed in Supporting Information Notes S1. Ma, million years ago.

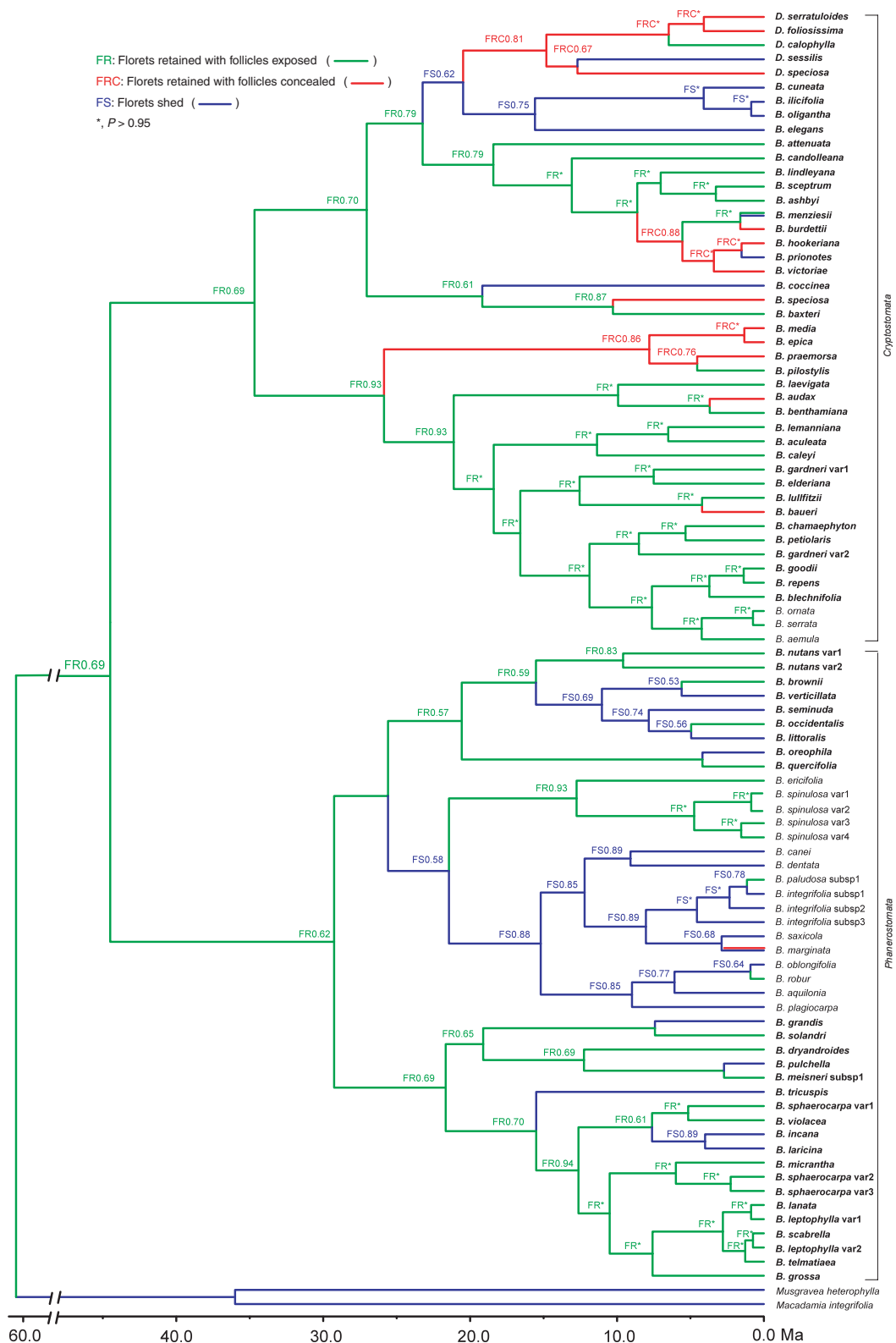


Fig. 3 Reconstruction of the ancestral trait for the retention of dead florets in *Banksia*/*Dryandra*. The dual colour line indicates the existence of both trait states in the crown species. Numbers following the abbreviations are the average posterior probabilities if below 0.95. The line remains the same colour as the ancestral state at the root if the ancestral trait was indeterminable, and with no indication of posterior probabilities. Southwest Australian species in bold. Full variety and subspecies' names are listed in Supporting Information Notes S1. Ma, million years ago.

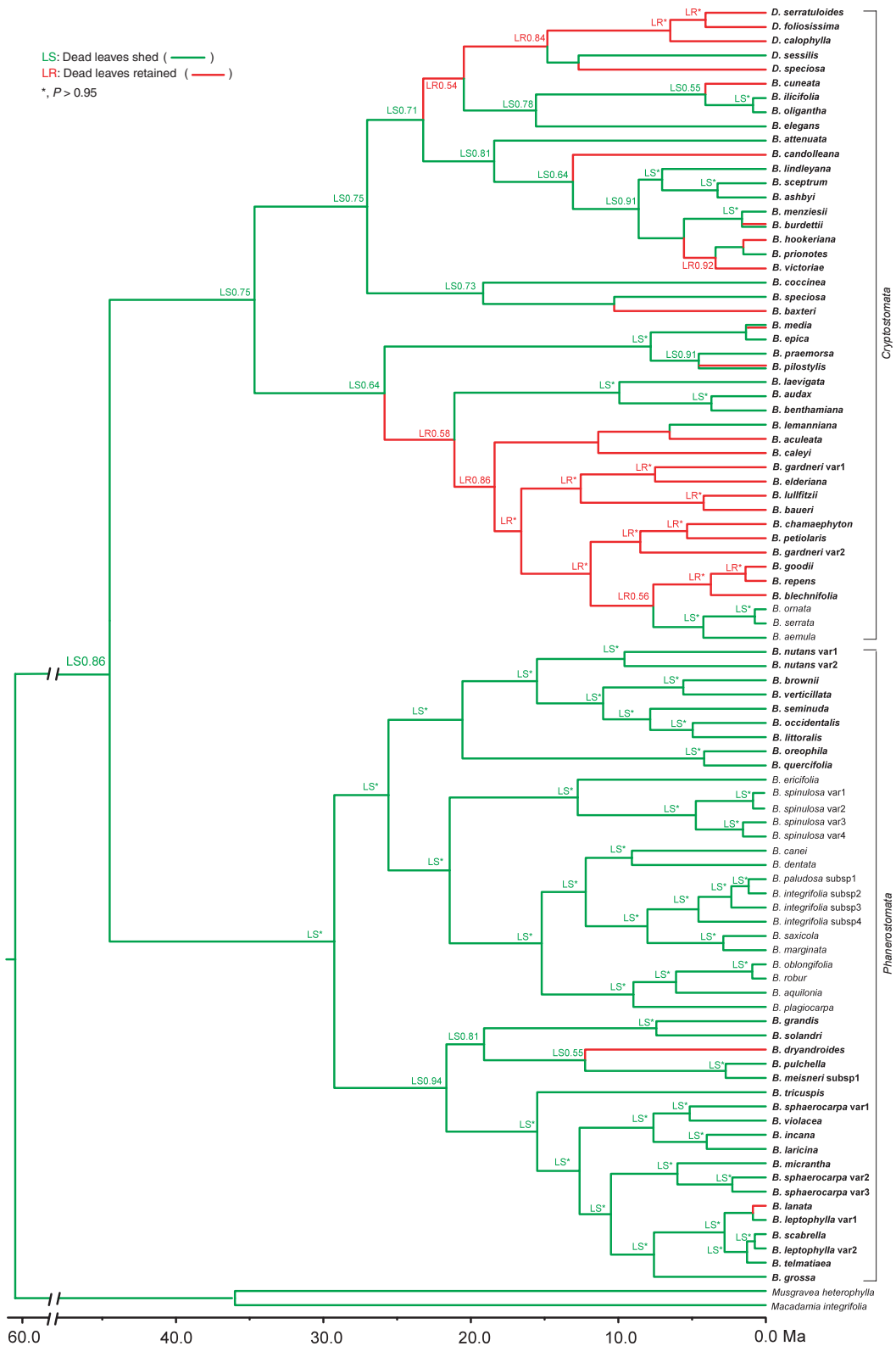


Fig. 4 Reconstruction of the ancestral trait for the retention of dead leaves in *Banksia*/*Dryandra*. The dual colour line indicates the existence of both trait states in the crown species. Numbers following the abbreviations are the average posterior probabilities if below 0.95. The line remains the same colour as the ancestral state at the root if the ancestral trait was indeterminable, and with no indication of posterior probabilities. Southwest Australian species in bold. Full variety and subspecies' names are listed in Supporting Information Notes S1. Ma, million years ago.

taxa with these derived traits proliferated in the Miocene–Pliocene.

It was possible to assign clonality or nonclonality to 86 of the 89 nodes in the maximum clade credibility tree for *Banksia* (plus five *Dryandra* species) (Fig. 1). Nonclonality was the ancestral trait for the *Banksia* root node, and the roots of the two major clades ($P_s = 0.64$), indicating that the first species were nonclonal. Clonality (reproduction via rhizomes or root suckers) first occurred at 16 Ma or, possibly, 20 Ma (the ancestral species of *B. elegans* has equal probability of being clonal or nonclonal). Since its origin, clonality has evolved many times independently across the *Banksia* phylogeny, proliferating explosively in the Miocene, and slowing during the Pliocene and Quaternary (Table 1).

Seventy-eight of 89 nodes were unambiguously assigned as serotinous, with P values for 69 of these of > 0.95 (Fig. 2). Serotiny was the ancestral trait for the *Banksia* root node, and the roots of the two major clades ($P_s > 0.95$), indicating that the first species were serotinous. Taxa exhibiting serotiny proliferated during the Miocene with the rapid radiation of *Banksia* in this period, but the proliferation of taxa with these traits slowed during the Pliocene and Quaternary (Table 1). Although taxa exhibiting serotiny proliferated in the widespread clade, *Cryptostomata* (*sensu* Mast & Givnish, 2002), nonserotinous taxa evolved in eastern and northern Australia in the clade *Phanerostomata* (*sensu* Mast & Givnish, 2002) at *c.* 15.6 Ma. The proliferation rate of taxa that were nonserotinous was lower than that for serotinous taxa during the Miocene and Quaternary, but higher during the Pliocene (Table 1).

Dead floret retention is the ancestral trait for the root node of *Banksia* and the two major clades within the genus (Fig. 3), indicating that florets were retained around the follicles from the first appearance of the genus. Dead florets (bracts in the case of *Dryandra*, a general feature of the genus) started to conceal completely the persistent fruits by

25.9 Ma, but taxa with this trait only proliferated during the last 5 My (Table 1). *Banksia* started to shed its old florets as it radiated eastwards. Floret shedding as the ancestral trait of the lineage leading to the major cluster of eastern species has a posterior probability of 0.62 ± 0.21 .

Dead leaf shedding is the ancestral state for the root node in *Banksia* and also the two major clades (Fig. 4). Dead leaf retention first appeared at 25.9 Ma in several lineages within the *Cryptostomata*, and taxa with this trait proliferated in the Miocene (Table 1). Dead leaf shedding remained as the ancestral state in all nodes and crown species within *Phanerostomata*, whereas dead leaf retention evolved independently in the crown species, *B. dryandroides* and *B. lanata*, both fire-killed. The ancestral states switched frequently in *Cryptostomata*. Although the ancestral state of the closest lineage was dead leaf shedding, dead leaf retention was ancestral for *Dryandra* and proliferated rapidly (it is a general trait for the genus).

Trait transition rates

Model testing showed that, for fire response, dead leaf retention and floret retention, the two rate coefficients of trait transition did not differ significantly (Table 2), implying that the number of gains in one trait and gains in the alternative trait were equal during the evolution of *Banksia*. Fire response showed higher rates of turnover than the other pairs of traits that were an order of magnitude lower. Serotiny and growth form had different rate coefficients between the alternative states, implying directional evolution favouring serotiny over nonserotiny, and clonality over nonclonality (Table 2).

Correlated evolution of traits

The LBF test showed that serotiny strongly correlated with floret retention during the evolution of *Banksia* (LBF =

Table 1 Time of origin (T_o) and age of oldest species (T_s), species diversification rates (SDR) and trait proliferation rates (all other trait entries) for 85 extant *Banksia* taxa (plus five *Dryandra* spp.) through geological time

Attribute	Trait	T_o	T_s	Palaeocene/Eocene	Oligocene	Miocene	Pliocene	Quaternary
		Ma	Ma	61–35	34–24	23–5.4	5.3–1.9	1.8–0
SDR		60.8	19.2	0.074	0.152	0.347	0.095	0.102
Growth form	Nonclonal	60.8	19.2	0.074	0.121	0.299	0.110	0.086
	Clonal	16.8	15.6	–	–	0.408	0.063	0.048
Serotiny	Serotinous	60.8	19.2	0.074	0.121	0.341	0.108	0.098
	Nonserotinous	15.5	12.7	–	–	0.284	0.139	0.062
Dead florets	Retained	60.8	19.2	0.074	0.061	0.364	0.135	0.101
	Retained, conceal fruits	25.9	12.7	–	0.091	0.170	0.508	0.101
	Shed	23.6	9.1	–	–	0.739	0.159	0.126
Dead leaves	Shed	60.8	19.2	0.074	0.061	0.398	0.111	0.109
	Retained	25.9	8.5	–	0.091	0.341	0.099	0.117

–, trait not yet present.

Table 2 Trait transition rate in *Banksia*

Attribute	Trait transition	Rate
Fire response	RS → K	0.957 ± 0.667
	K → RS	0.957 ± 0.667
Growth form	C → NC	0.008 ± 0.004
	NC → C	0.012 ± 0.004
Serotiny	S → NS	0.012 ± 0.005
	NS → S	0.019 ± 0.035
Dead leaves	LR → LS	0.025 ± 0.062
	LS → LR	0.023 ± 0.021
Dead florets	FR → FS	0.072 ± 0.088
	FS → FR	0.075 ± 0.126

Values are means ± SD. The unit is the number of changes per lineage per My. Bold indicates a significant difference between the two rates ($P < 0.05$). C, clonal (resprouting via rhizomes or root suckers); FR, dead florets retained; FS, dead florets shed; K, fire-killed; LR, dead leaves retained; LS, dead leaves shed; NC, nonclonal (resprouting via epicormic or lignotuberous buds, or killed by fire); NS, non-serotinous; RS, resprouting; S, serotinous.

15.5, $P < 0.01$), but not with dead leaf retention (LBF = 4.7, $P > 0.05$). Dead leaf retention correlated weakly with the fire-killed regeneration response (LBF = 10.4, $P < 0.05$) and strongly with floret retention (LBF = 13.6, $P < 0.01$). Clonality was not correlated with any other trait ($P > 0.05$).

Discussion

Resprouting after fire

Resprouting after shoot damage is a widespread trait in all fire-prone (and many nonfire-prone) environments, and clearly is an ancient trait among plants generally (Pausas & Keeley, 2009). Although it was not possible to reconstruct the ancestral state at the root and early nodes in the *Banksia* phylogeny, we report the highest rate of switching between resprouting and nonsprouting among all pairs of traits examined. This agrees with earlier findings that whole-plant fire response is a highly labile trait in some genera (despite the fact that it is actually a syndrome of correlated traits; Lamont & Wiens, 2003) and has evolved independently and repeatedly in many lineages (Bond & Midgley, 2001).

Although some argue that resprouting after fire is an exaptation to fire that evolved in response to many other types of selection pressures, such as herbivory (Hopper, 2009), our work suggests that fire has at least had a major influence on the evolution of resprouting types in *Banksia*. This also highlights that resprouting should not be treated as a single trait in trying to understand its evolution, as resprouting via rhizomes and root suckers, in particular, is clearly an advanced growth form in *Banksia*. Banksias evolved towards clonality from 16 Ma (possibly 20 Ma) during the Miocene. Clonal species have the lowest stature of all

growth forms in *Banksia*, and so are most vulnerable to ignition, yet their meristematic tissues are insulated by soil, a poorer conductor of heat than is bark. Thus, they are rarely killed by fire (Lamont, 1989; Drechsler *et al.*, 1999; Burrows, 2002). Seeds make a negligible contribution to postfire population size, avoiding the risk of recruitment failure completely and conserving nutrients for vegetative growth, and ramet formation promoted by fire may be as effective, if not more so, than postfire seedling recruitment in population recovery (Lamont & Barrett, 1988; Enright & Lamont, 1989; Witkowski & Lamont, 1997). Lamont & Wiens (2003) have even argued that there is no mutational penalty either. The extreme longevity of clonal banksias (e.g. 1000 yr for *B. candolleana*, T. He and B. Lamont, unpublished data; 500 yr for *B. goodii*, Drechsler *et al.*, 1999) attests to their success in surviving recurrent fire and drought, and also buffering changes in fire regimes that may have been marked in the Miocene (Hopper, 1979).

Evolution of serotiny and flammability

Serotiny has long been regarded as a fire-adapted trait (Lamont *et al.*, 1991). The key to fitness is general seed release by fire in a time in which conditions are most conducive to germination and seedling recruitment, i.e. in the wet season immediately following fire, rather than storage as such, which can be seen as a general protective mechanism in a seed-limited environment. Drought might be considered as an alternative follicle-opening cue, although seeds would fall onto a less-ideal seedbed. In this regard, we observed 15% of follicles open 2 yr after severe drought in 2006–07 in a population of *Banksia hookeriana* (serotinous, and retaining its dead leaves and florets) with 120 plants (97% dead), and the six seedlings had all died by 2009. By contrast, a nearby population that was burnt in 2007, and thus experienced the same drought, had 100% follicles open among 117 plants (100% dead) with 117 seedlings (3% dead). As here, and with other serotinous banksias, there is little follicle opening following plant death caused by drought (Lamont & Barker, 1988; Lamont *et al.*, 1991; Lamont, 1996), leaving fire by far the more effective cue in seed release and seedling recruitment.

Further support for fire rather than simply branch death as the critical cue for fruit opening is provided by the results here. We show that the retention of dead florets has been intimately associated with serotiny since the origin of *Banksia* in the Palaeocene. Combustion of the mantle of dead florets ensures that the critical temperature for melting the resin sealing the valves is reached (Lamont & Cowling, 1984). Further, these species have a higher temperature requirement for follicle opening, corresponding to their greater degree of serotiny (Enright & Lamont, 1988). In fact, the florets obstruct seed release in unburnt cones, especially where the mantle is so dense it conceals the follicles –

shown here to have evolved over the last 20 My and only proliferating during the last 5 My – suggesting the ‘fine-tuning’ of both traits to the increasing fire proneness of southwest Australia in particular.

Alternative interpretations of dead floret retention seem to be implausible: shading the follicles from direct sunlight to prevent premature opening only serves to highlight the importance of delaying this until the ideal recruitment conditions are created by fire. If complete concealment of the fruits from granivores, such as the cockatoo *Calyptorhynchus latirostris* (Lamont *et al.*, 2007), was critical for fitness, this should not have been delayed by 40 My, as these birds arrived long before the evolution of *Banksia* (Barker *et al.*, 2004). The florets make a negligible contribution to flammability in terms of combustible carbon content of the above-ground plant (Witkowski & Lamont, 1996), if the aim was to ‘kill thy neighbour’ (Bond & Midgley, 1995), that gives the species a fitness advantage. If serotiny/seed release can best be considered as a fire-dependent trait and dead floret retention a fire-enhancing trait, and they originated and evolved together, it seems reasonable to conclude that *Banksia* evolved in a fire-prone environment. The rainforest sister groups of *Banksia*, here taken as *Macadamia* and *Musgravea*, lack both of these traits. A drought-prone environment is neither adequate nor conducive to the same pairing of traits (later evolution showed that they are optional and independent traits). Patches of sclerophyll vegetation occurred (probably in southwest Australia; Hopper & Gioia, 2004) among the rainforest clothing Australia at 61 Ma that could ignite at intervals shorter than the lifespans of the ancestral banksias (Enright *et al.*, 1998), and these traits enabled them to invade and flourish there.

Is there also adaptive significance in the recent loss of serotiny and floret shedding among some species? Not only does floret shedding precede the loss of serotiny by 15 My (necessary in the absence of fire as noted above), but the latter began only 10 Ma. This is in special habitats in which plants may escape fire and where interfire recruitment is likely (Lamont & Connell, 1996): *B. dentata* is confined to savanna/grasslands (that arose world-wide 8 Ma; Edwards *et al.*, 2010) where the almost annually occurring ground fires would fail to reach the cones of this tree, thus being ineffective as a seed release mechanism; *B. verticillata* (rock outcrops) and *B. littoralis* (swamp margins) are not reliably fire prone; *B. integrifolia* and *B. grandis* may be tall trees whose cones again may escape the heat from noncrown fires.

Surprisingly, the retention of dead leaves first appeared 35 My later than serotiny and floret retention, and is restricted to southwest Australia. Further, the retention of dead leaves has not coevolved with serotiny, in contrast with previous observations on pines (Schwilk & Ackerly, 2001). Dead leaf and branch retention have been suggested as fire-enhancing traits (Mutch, 1970). It has been hypothesized

that dead biomass is more likely to increase combustion and ensure a complete burn of plants (Schwilk & Ackerly, 2001). Dead leaf retention in *Banksia* is strongly correlated with dead floret retention and also with the fire-killed response. Cowan & Ackerly (2010) reported that fire-killed shrubs in Californian chaparral had higher fractions of dead branches. We suggest that the retention of dead leaves ensures ignition of the florets and maximum but brief heating of serotinous cones in fire-killed banksias, where seed release/seedling recruitment is critical for postfire fitness. In this regard, our results do not support the expected correlation between high flammability and resprouting ability recently proposed by Gagnon *et al.* (2010). The minimization of heating of the surface soil via dead leaf retention is irrelevant to fire-killed species that store their seeds in their crown, as we have here.

The retention of dead leaves could also have another advantage for fire-killed banksias. Seedlings of fire-killed species have high growth rates (Pate *et al.*, 1990) that imply great nutrient demand. Mineral nutrients stored in the retained dead leaves in fire-killed species (Witkowski & Lamont, 1996) are released after fire and will benefit offspring that establish under their dead crown, especially nutrients such as calcium that are not readily translocated into the seeds (Lamont & Groom, 2002). It has been noted that most fire-killed banksias are restricted to nutrient-impooverished sands, whereas resprouting species occur on more fertile lateritic soils (Lamont & Connell, 1996).

Fire as a selective force in the origin and evolution of plant traits

Our results show that the fire-dependent trait, on-plant seed storage, and the fire-enhancing trait, dead floret retention, have coevolved in *Banksia* since the first appearance of the genus 60.8 Ma, implying that fire was already an effective agent of selection then. Together, these two traits ensure maximum seed release when the conditions for germination and seedling recruitment, as created by fire, are optimal. The results of our analyses provide support for two hypotheses: (1) there has been a long association of (particular) land plants with fire (Pausas & Keeley, 2009), certainly well before the onset of seasonal aridity 25 Ma in parts of Australia (Hopper & Gioia, 2004); and (2) some plant groups have evolved a set of traits consistent with adaptation to particular fire regimes (Simon *et al.*, 2009), including towards (or sometimes away from) more effective fire-related traits, such as dead leaf retention, clonality and a denser mantle of dead florets. We have shown that the mapping of certain traits, here putative fire-related traits, onto a chronophylogeny can be used to test evolutionary theory, providing powerful insights into the identity and time of origin of ancestral traits and the selective forces that have driven their evolution. Our findings open the way for new

hypotheses on the role of fire in the origin and evolution of plant traits.

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Notes S1 Names of varieties and subspecies in the figures.

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