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Research

Fire-adapted traits of *Pinus* arose in the fiery Cretaceous

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Summary

- The mapping of functional traits onto chronograms is an emerging approach for the identification of how agents of natural selection have shaped the evolution of organisms. Recent research has reported fire-dependent traits appearing among flowering plants from 60 million yr ago (Ma). Although there are many records of fossil charcoal in the Cretaceous (65–145 Ma), evidence of fire-dependent traits evolving in that period is lacking.
- We link the evolutionary trajectories for five fire-adapted traits in Pinaceae with paleoatmospheric conditions over the last 250 million yr to determine the time at which fire originated as a selective force in trait evolution among seed plants.
- Fire-protective thick bark originated in *Pinus c.* 126 Ma in association with low-intensity surface fires. More intense crown fires emerged *c.* 89 Ma coincident with thicker bark and branch shedding, or serotiny with branch retention as an alternative strategy. These innovations appeared at the same time as the Earth's paleoatmosphere experienced elevated oxygen levels that led to high burn probabilities during the mid-Cretaceous.
- The fiery environments of the Cretaceous strongly influenced trait evolution in *Pinus*. Our evidence for a strong correlation between the evolution of fire-response strategies and changes in fire regime 90–125 Ma greatly backdates the key role that fire has played in the evolution of seed plants.

Introduction

Increasing evidence from paleontological records and phylogenetic analyses suggests that fire is an ancient process that has influenced the history of life on Earth as far back as the origin of land plants (Bowman *et al.*, 2009; Pausas & Keeley, 2009). Many plants have acquired adaptive traits that enable them to persist and reproduce in fire-prone environments (Gómez-González *et al.*, 2011; Keeley *et al.*, 2011; Pausas *et al.*, 2012). The fossil charcoal record dating from over 400 million yr ago (Ma) is a valuable source of information on the historical occurrence of fire (Scott, 2000, 2010; Glasspool *et al.*, 2004). However, the evolutionary impact of fire on plant evolution is difficult to trace because of inherent habitat biases in the fossil record and because fire-related traits are rarely evident among fossils (Midgley & Bond, 2011). Therefore, dating the origin and evolution of fire-adapted traits from time-based molecular phylogenies can provide new insights into the role of fire in plant evolution, especially the identification of the time at which fire started to act as an agent of natural selection.

Recent research has reported that fire-dependent traits appeared at least 60 Ma (early Paleocene) in Australia (Crisp *et al.*, 2011; He *et al.*, 2011; Lamont *et al.*, 2011) and at least 20 Ma (Oligocene) in South Africa (Bytebier *et al.*, 2011; Lamont & Downes, 2011). So far, no evidence of fire-dependent traits evolving in the Cretaceous (65–145 Ma) has been obtained, a time at which abundant fossil charcoal/inertinite has been recorded (Scott, 2000, 2010; Glasspool & Scott, 2010) and during which concentrations of atmospheric oxygen were markedly higher than today (23–29% compared with 21%; Bergman *et al.*, 2004; Belcher *et al.*, 2010; Glasspool & Scott, 2010). The Cretaceous was also a period in which conifer forests were widespread (Spicer & Herman, 2001; Harland *et al.*, 2007), and it has been hypothesized that frequent Cretaceous fires opened up these forests and facilitated the expansion of early angiosperms (Berendse & Scheffer, 2009; Bond & Scott, 2010).

Species in the Pinaceae currently occur across a wide range of habitats throughout the temperate, Mediterranean, subtropical, boreal, montane and subalpine forests of North and Central America, Asia and Europe. Many of these ecosystems are strongly influenced by fire, and their component species possess a range of fire-dependent functional traits (Keeley & Zedler, 1998; Rodríguez-Trejo & Fulé, 2003). Some of these traits have been mapped on the pine phylogeny and have been shown to be correlated

(Schwilk & Ackerly, 2001), but have yet to be given a time basis (Pausas & Schwilk, 2012). The Pinaceae is also an ancient family with fossil and molecular evidence pointing to an early Triassic origin (Miller, 1999; Clarke *et al.*, 2011). In addition, there is fossil evidence that conifer forests burnt frequently during the Cretaceous (Batten, 1998; Collinson *et al.*, 2000; Falcon-Lang *et al.*, 2001).

Therefore, the known paleohistory of Pinaceae provides a unique opportunity to investigate the impact of fire on the evolution of plants from 'deep time' to the present. In this study, we link evolutionary trajectories for five fire-adapted traits in the family Pinaceae to paleoatmospheric conditions over the last 250 million yr (My) to determine the time at which fire originated as a selective force on trait evolution in this family, and test the hypothesis that its fire-driven evolution began in the Cretaceous (Berendse & Scheffer, 2009; Bond & Scott, 2010).

Materials and Methods

Phylogenetic reconstruction and fossil calibration

Many species in *Pinus* are fire dependent, but few species in other genera of the Pinaceae possess fire-related functional traits (Keeley & Zedler, 1998). We therefore first constructed a phylogeny for 101 species of *Pinus*, one or two species of the 10 other recognized genera and three outgroup species (*Araucaria araucana*, *Podocarpus elatus*, *Sciadopitys verticillata*) by retrieving their DNA sequences (*mat*K and *rbc*L; Gernandt *et al.*, 2005) from GenBank (http://www.ncbi.nlm.nih.gov/genbank/). Phylogenies for all other multi-species genera (except for *Keteleeria*, which only has three species) were reconstructed by retrieving DNA sequences from Genbank, and outgroup species were chosen as in the original literature (Supporting Information Notes S1).

The phylogeny with branch lengths proportional to time was derived using Bayesian inference with fossil calibrations. When radiometric dates were unavailable, fossil ages were assigned on the basis of a recent geological timescale and the midpoint of the age was used. Gernandt *et al.* (2008) and Willyard *et al.* (2007) performed extensive analyses of the fossil record for Pinaceae and suggested the optimal calibration regime for phylogenetic dating. In constructing the phylogeny with branch lengths proportional to time (chronogram) of *Pinus*, we assumed that *Pityostrobus berinissartensis* (Alvin, 1957) is the sister group to *Pinus* and calibrated the node corresponding to the most recent common ancestor of *Pinus*, *Picea* and *Cathaya* at 123 Ma (Gernandt *et al.*, 2008). Within *Pinus*, Gernandt *et al.* (2008) delineated two subgenera (*Pinus* and *Strobus*) using chloroplast DNA (*mat*K and *rbc*L), which is also supported by morphological, biogeographical and biochemical character analyses (Kaundun & Lebreton, 2010). A subgeneric divergent calibration at 85 Ma was therefore used (Willyard *et al.*, 2007). A minimum age calibration was also set at the node for the most recent common ancestor of *Pseudolarix*, *Tsuga* and *Nothotsuga* at 156 Ma, given the radiometric date of 156 ± 0.76 Ma (mid-Oxfordian) for the oldest *Pseudolarix* fossil, *P. erensis* (Keller & Hendrix, 1997). Based on the macrofossil record for individual genera, a minimum age was set: *Abies*, 45.5 Ma (Erwin & Schorn, 2005); *Larix*, 45.0 Ma (Schorn, 1994); *Picea*, 45.5 Ma (Erwin & Schorn, 2005); *Tsuga*, 44.9 Ma (Erwin & Schorn, 2005); *Pseudotsuga*, 32 Ma (Schorn, 1994); *Cedrus*, 54.8 Ma (Blokhina, 1998).

The chronograms were derived using Bayesian inference in BEAST ver. 1.6.1 (Drummond & Rambaut, 2007); the input file was generated in BEAUTi (Drummond & Rambaut, 2007). We used a Yule process to describe speciation, and an uncorrelated log-normal model to describe the relaxed molecular clock. For each calibration point, a normal prior distribution, with a standard deviation of 5% to account for temporal uncertainty, was used in a Markov Chain Monte Carlo (MCMC) procedure, as the normal prior distribution best allows for uncertainty in the calibration estimates. Posterior distributions of parameter values and the tree topology were estimated by MCMC sampling. Five replicate MCMC runs were performed for each genus phylogeny, with the tree and parameter values sampled every 5×10^3 generations over a total of 1×10^8 steps. MCMC diagnostics were evaluated in Tracer v1.5 (Drummond & Rambaut, 2007). The consequent tree file containing 1000 plausible phylogenies with branch lengths (divergence times) was used for the reconstruction of the ancestral state as described below. Posterior samples from the five independent runs were combined and the trees and parameter values were summarized. A maximum clade credibility tree was identified using TreeAnnotator (Drummond & Rambaut, 2007). The topologies of all subsequently dated phylogenies resembled the original phylogeny, but with improved resolution as a result of Bayesian inference not used in the original reconstruction. For illustrative purposes, a supertree of the Pinaceae family was constructed by grafting the maximum clade credibility tree of individual genera onto the *Pinus* phylogeny (maximum clade credibility tree) that included all genera in the backbone.

Trait data and fire regime

We define two fire regimes for fire-prone pine forests: a *surface* fire regime, in which fire burns the ground stratum and litter but does not reach the crown stratum (canopy), and a *crown* fire regime, in which fire also burns the canopy of the dominant vegetation (Gill & Allan, 2008). Surface and crown fires not only have different effects on ecosystem processes, but also exert different selective pressures on the evolution of plant traits (Keeley & Zedler, 1998). We define any landscape as nonfire prone if the mean fire interval is > 150 yr as that is considered to be the lower estimate of longevity for many pines (Knowles & Grant, 1983).

Five functional traits (bark thickness, serotiny, branch shedding, presence of grass stage and resprouting capacity) are widely recognized as fire-adapted traits (Keeley & Zedler, 1998; Schwilk & Ackerly, 2001; Rodríguez-Trejo & Fulé, 2003; Myers & Rodríguez-Trejo, 2009) and under strong genetic control (Schoenike, 1976; Nelson *et al.*, 2003).

Bark thickness Thick bark in *Pinus* has been demonstrated to insulate the cambium from heat generated by surface fires (Jackson *et al.*, 1999) and thus has a significant effect on plant survival after fire, especially from surface fires (Jackson *et al.*, 1999; Brando *et al.*, 2012). Bark thicker than 15 mm is sufficient to insulate the cambium from low-intensity surface fires (temperature of 400°C for up to 3 min; Vines, 1968; Wright & Bailey, 1982; van Mantgem & Schwartz, 2003). Thicker bark (> 30 mm) provides protection from intense surface fires (temperatures over 800°C for > 10 min; van Mantgem & Schwartz, 2003). Alternative explanations for the ecological function of thick bark have been proposed in pines, but all lack generality. Thick bark could be an adaptation to protect trees from bark beetles, but there is evidence that they infest most conifers indiscriminately over a wide range of bark thicknesses (Wood, 1982). Bark has been reported to thicken in *Taxodium distichum* when flooded (Yamamoto, 1992), but most extant pine species prefer dry habitats (except for *P. krempfii* which grows in rainforest). Thick bark could be a protection from extreme cold, but, among 39 pine taxa in tropical areas, 30 have thick bark (Myers & Rodríguez-Trejo, 2009), implying that other functions usually apply to thick bark. Bark thickness is related to tree age (Jackson *et al.*, 1999), so that, whenever possible, we compiled bark thickness data at breast height for adult trees.

Serotiny Serotiny in pines (apart from *Pinus*, only *Picea mariana* and, perhaps, some populations of *Larix gmelinii* are serotinous in Pinaceae) is marked by the retention of mature cones that open promptly only after exposure to high temperatures generated by fire. This mechanism ensures prolific seed release after fire when conditions for seedling establishment are highly favorable, that is, when resources, such as light, moisture and nutrients, are maximal and competition with adults is minimized (Lamont *et al.*, 1991; Keeley *et al.*, 2011). Mass seed release may also allow the satiation of predators (Lamont *et al.*, 1991). Much research has shown that serotiny is tied to fire environments and, in the absence of fire, there is usually negligible recruitment, making clear the fitness benefits of serotiny in fire-prone systems, especially among fire-killed species (Lamont *et al.*, 1991; He *et al.*, 2011; Keeley *et al.*, 2011).

Branch shedding Some *Pinus* species have the ability to shed their lower branches once their foliage has died (self-pruning or branch shedding), thus creating a fuel gap between the forest floor and the crown. This gap limits the ability of surface fires to climb into the canopy, and thus branch shedding is typically linked to surface fire ecosystems and the absence of serotiny, whereas branch retention is linked to crown fire systems and the presence of serotiny (Keeley & Zedler, 1998; Schwilk & Ackerly, 2001). Dead branch retention has been shown to increase fire intensity in shrub systems (Schwilk, 2003).

Grass stage A curious life-history trait in *Pinus* is delayed trunk development ('grass stage'), whereby internode elongation is suppressed for the first 5–10 yr of development, with the resulting juveniles persisting in a bunchgrass growth form (Keeley & Zedler, 1998; Rodríguez-Trejo & Fulé, 2003). During this stage, needles are decumbent and accumulate as a mantle over the apex and insulate the apical bud against fire heat (Rodríguez-Trejo & Fulé, 2003).

Resprouting capacity Although rare in the family Pinaceae, resprouting among pines occurs on sites of low productivity and high fire frequency (Keeley & Zedler, 1998), and is considered to be a fire-adapted trait in many lineages, including gymnosperms (Pausas & Keeley, 2009; Lamont *et al.*, 2011).

Trait states for the above five fire-adapted traits and the fire regime within which each species lives were collated from the literature, personal observations and contact with local ecologists (Table S1).

Ancestral state reconstruction and trait evolution correlation through time

The times of origin of functional traits and their alternative states were identified by the reconstruction of ancestral traits within the studied taxa. We first reconstructed the ancestral trait state for each of the five fire-adapted traits for *Abies*, *Picea*, *Tsuga*, *Pseudotsuga*, *Cedrus* and *Larix*. The consequent ancestral traits of the above genera were then used in the ancestral trait reconstruction for Pinaceae, which included 101 species of *Pinus* and one or two apparent ancestral species in all other genera. For ancestral trait reconstruction, the agglomerative technique assumes that the current trait state for each species or lineage remains unchanged from the node until fusion with its sister lineage, when the probability of that trait is retested by simultaneously comparing all previous probabilities linked to that node (Hardy, 2006). Bayesian MCMC methods were used to derive posterior distributions of log-likelihoods and the probability values of traits at the nodes, and were implemented in *BayesTrait* (Pagel & Meade, 2007). For each dataset, the tree file containing 1000 plausible phylogenies with the branch length (node age) generated in BEAST and the trait files containing the discrete state of the trait for each species were used for further analysis.

A continuous-time Markov model, in which the trait state can evolve repeatedly between the two possible states at any branch of the phylogenetic tree (Pagel *et al.*, 2004), was used to construct the ancestral state at each internal node, and a reversible-jump (RJ-)MCMC was adopted (Pagel & Meade, 2007). A few Markov chains were run for each trait and the acceptance rate of newly proposed values of the rate parameters was monitored to determine a suitable value for the *ratedev* parameter that generated an acceptance rate of 0.20–0.40 (Pagel & Meade, 2007). A *hyperprior* seeding of an exponential distribution from a uniform 0–30 distribution was applied to the Markov chain runs. The Markov chains were run for 1×10^8 iterations with a burn-in of 5×10^4, and every 5×10^3 iteration was logged. The ancestral state was defined as the trait state with the higher average posterior probability determined by a *t*-test ($P < 0.05$).

Tests for correlated evolution between binary functional traits (or with their putative selective environment) were implemented by comparing the fit of two continuous-time Markov models: one that presumes that the two traits evolve independently on the tree (*independent* model), and one that allows the traits to evolve together (*dependent* model). The log-Bayes factor is nominally distributed as a chi-square with degrees of freedom equal to the difference in the number of parameters between the two models, and a log-Bayes factor > 9.49 indicates strong support ($P < 0.05$) for an evolutionary correlation between the two traits (or with a particular fire regime). In each model run, 5×10^6 iterations were implemented and the results were logged every 2×10^3 iterations. The overall results were summarized by the log-Bayes factor, which is twice the difference between the two harmonic means derived from the *dependent* and *independent* models of evolution (Pagel & Meade, 2007).

Reconstruction of biomass burning probability

Burn probabilities over the last 350 My were estimated using the FIREOX model of Belcher *et al.* (2010). The FIREOX model is a cellular automaton model, analogous to a lattice cellular automaton of a SIR-type epidemic (Karafyllidis & Thanailakis, 1997). FIREOX simulates the propagation of a smouldering fire (Stott, 2000; Drysdale, 2011) as a contact invasion process when ignited from a localized external source at a given atmospheric O_2 concentration. The model has two parameters, the probability of local fire spread (β) and the probability of local fire extinction (μ), and is driven by spread rate and burn duration information from experiments undertaken at different concentrations of atmospheric O_2 (Belcher *et al.*, 2010). The model generates burn probabilities for different values of β and μ, and refers to the amount of area burned in the grid of the cellular automata. The modeled relationship between burn probability and atmospheric O_2 concentration is then used to estimate the burn probability over the past 350 Ma according to modeled estimates of paleoatmospheric O_2 (in this case, the COPSE model of Bergman *et al.*, 2004).

Results

We constructed a dated molecular phylogeny for Pinaceae, showing that the evolutionary history of crown Pinaceae can be traced to 237 Ma (189–293 Ma; 95% highest posterior probability, HPD) in the mid-Triassic (Fig. 1). The *Pinus* clade, the largest genus in the Pinaceae, originated 126 Ma (105–147 Ma, 95% HPD), with a crown age at 89 Ma (80–96 Ma), when the subgenera *Pinus* and *Strobus* diverged (Fig. 1). Key fire-adapted traits are well represented in *Pinus* (Figs 1, 2). Thin bark, seed release at maturity and branch retention are ancestral (Figs 1, 2, S2). We first tested whether the proliferation of the main fire-related traits in *Pinus* is correlated with the two standard fire regimes, surface and crown fires. The selected traits were the presence of thick bark and canopy seed storage (serotiny). We found that thick bark (> 15 mm) is closely correlated through time with the surface fire regime in *Pinus* ($P < 0.01$), and the correlation is even stronger for very thick bark (> 30 mm) ($P < 0.001$, Fig. S1), supporting the postulated evolution of bark thickness as a response to surface fires. Serotiny is strongly adaptive in the presence of crown fires and is closely associated with the crown fire regime in pines ($P < 0.001$).

To determine the time of origin of these fire-adapted traits, we reconstructed the ancient state of thick bark (> 15 mm), very thick bark (> 30 mm) and serotiny on the dated Pinaceae

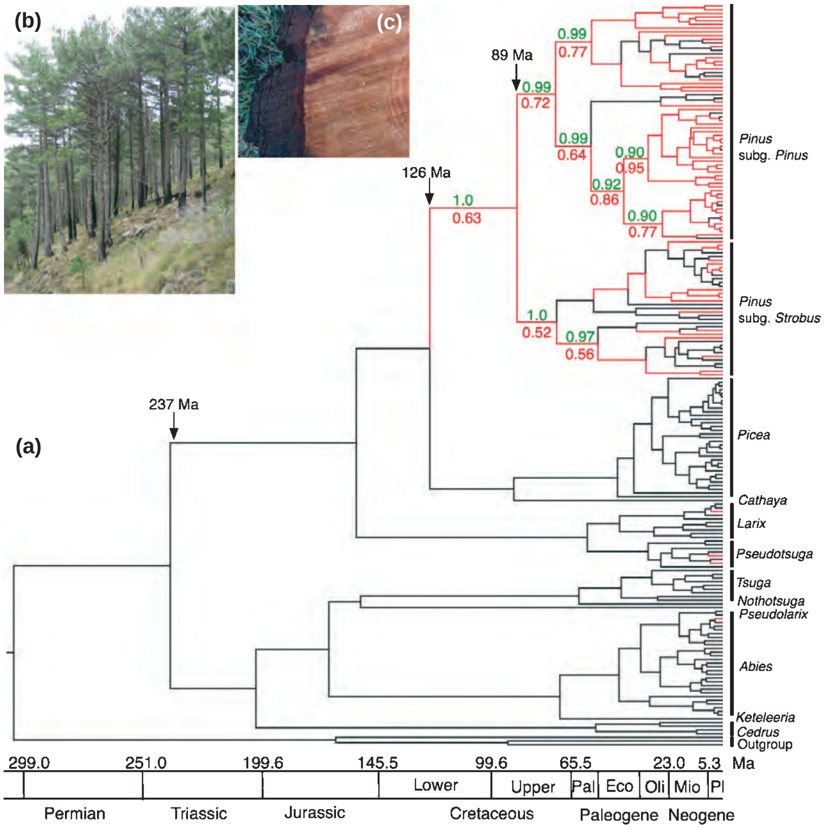

Fig. 1 Ancestral state reconstruction for thick bark (> 15 mm). (a) Green values are the posterior probability of phylogenetic certainty at the corresponding node. Red values are the posterior probability of the reconstructed state at the corresponding node. Red lines represent evolutionary pathways (extant species/internal node/stem) for thick bark (> 15 mm). Ma, million years ago. Pal, Paleocene; Eco, Eocene; Oli, Oligocene; Mio, Miocene; Pl, Pliocene. (b) Pine (*Pinus nigra*) forest following surface fire, with trees, *c.* 20 m tall, showing old branch shedding. (c) Cross-section of trunk of *Pinus* sp. showing bark, 45 mm thick at its maximum.

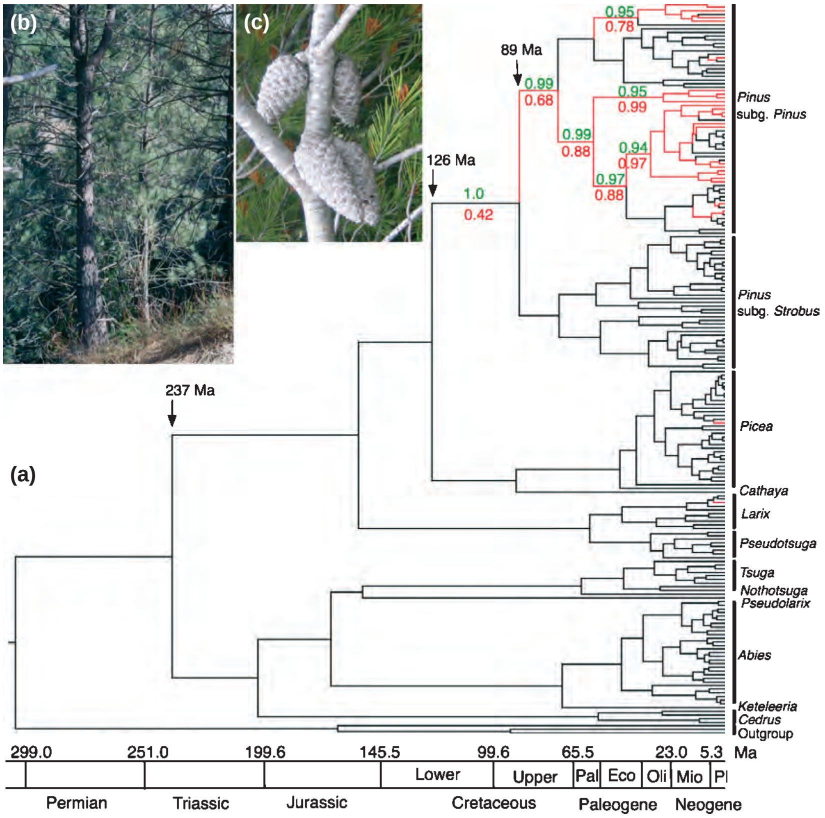

Fig. 2 Ancestral state reconstruction for canopy seed storage (serotiny). (a) Green values are the posterior probability of phylogenetic certainty at the corresponding node. Red values are the posterior probability of the reconstructed state at the corresponding node. Red lines represent evolutionary pathways (extant species/internal node/stem) for serotiny. Ma, million years ago. Pal, Paleocene; Eco, Eocene; Oli, Oligocene; Mio, Miocene; Pl, Pliocene. (b) Serotinous *Pinus attenuata*, showing retention of dead branches on its ~ 20 cm thick trunk. (c) Five-year-old serotinous cones, 12 cm long, of *Pinus halepensis*.

phylogeny. We found that thick bark originated up to 126 Ma (105–147 Ma) (stem age) in *Pinus* (posterior probability = 0.63, $P < 0.05$; Fig. 1), suggesting that surface fires existed then and were already shaping the direction of trait evolution in *Pinus*. Very thick bark (> 30 mm), coupled with branch shedding (correlated evolution: $P < 0.001$), appeared up to 89 Ma (80–96 Ma) (stem age) in subgenus *Pinus* (posterior probability = 0.67, $P < 0.05$; Fig. S1). Serotiny occurs in sections Pinaster, Contortae and Australes of subgenus *Pinus*. It was traced back to 89 Ma (posterior probability = 0.68, $P < 0.05$; Fig. 2) (stem age) and was correlated with lower branch retention ($P < 0.05$).

The other fire-related traits examined, fire-tolerant grass stage and ability to resprout, are rare and clearly derived among pines; their lineages were traceable to < 40 Ma (Fig. S3). These two traits are correlated with recurrent fire through time ($P < 0.01$). Thick bark and serotiny, but not grass stage and branch shedding, are also present in other genera of Pinaceae, but are rare and recent, and appear to represent convergent evolution among those species in fire-prone environments (Figs 1, 2, S1–S3).

Modeled (Bergman *et al.*, 2004) and proxy (Glasspool & Scott, 2010) estimates of atmospheric O_2 concentrations over the past 350 My show that exceptionally high concentrations of atmospheric O_2 (> 26%) occurred *c.* 100 Ma in the Cretaceous (Fig. 3). Such high O_2 levels are estimated to have led to increased burn probabilities in the Earth's forests, and inertinite showed a local peak at that time. Atmospheric CO_2 concentrations and temperatures were also elevated during this time (Fig. 3).

Discussion

Fire is known to have been a regular occurrence in Cretaceous conifer ecosystems, and the Pinaceae is well represented in the fossil charcoal record at this time (Batten, 1998; Collinson *et al.*, 2000; Falcon-Lang *et al.*, 2001; Glasspool & Scott, 2010), but ascertaining the evolutionary impact of fire on early seed plants has been elusive (Pausas & Keeley, 2009; Pausas & Schwilk 2012). Our analysis shows that, although the Pinaceae has a 237-My evolutionary history, fire-adapted traits only appeared in this family from 126 Ma. The development of thick bark coincided with increases in atmospheric O_2 and burn probabilities (Fig. 3). Initial low-intensity surface fires, having a minor effect on plant traits, were replaced by highly intense crown fires by 89 Ma (possibly as early as 96 Ma), and resulted in the evolution of two alternative suites of highly successful, fire-adapted strategies: very thick bark with branch shedding, and serotiny with branch retention. We conclude that the change to a more intense fire regime has been the main selective force in the evolution of these fire-related traits in pines.

The Cretaceous fossils assignable to subgenus *Pinus* (which best expresses all fire-adapted traits) are widespread throughout America and Eurasia at mid- to high latitudes (Myers & Rodríguez-Trejo, 2009), suggesting that recurrent fire might have existed on a broad scale at that time. The trait innovations of thick bark, serotiny and branch shedding appeared at the same time as the Earth's atmosphere experienced record high O_2 concentrations that would have led to greatly increased plant

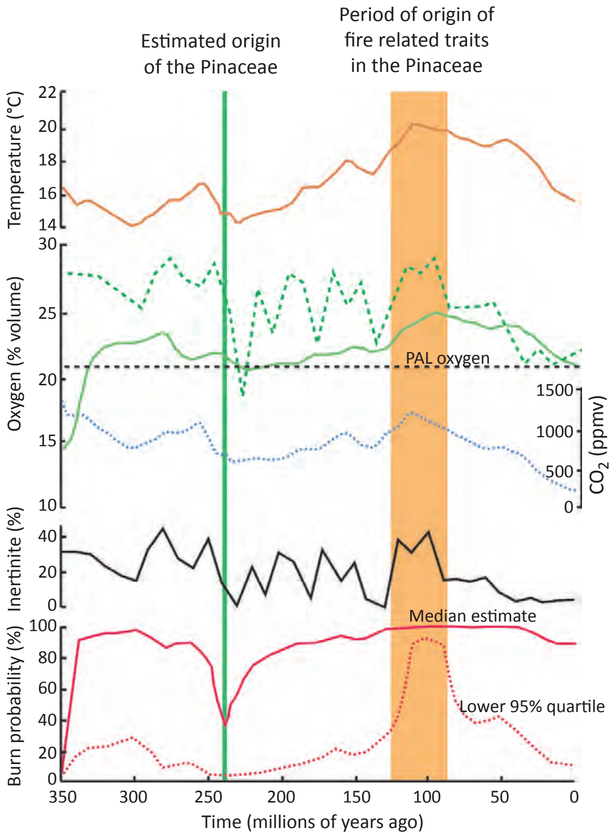

Fig. 3 Time of origin of fire-related traits in the Pinaceae set against the probability of burning estimated using the FIREOX model of Belcher *et al.* (2010) (red lines), abundance of inertinite (fossil charcoal) from Glasspool & Scott (2010) (black line), atmospheric oxygen concentrations as estimated by the COPSE model of Bergman *et al.* (2004) (solid green line) and from the oxygen proxy method of Glasspool & Scott (2010) (dashed green line) (black broken horizontal line corresponds to the ambient oxygen level at present), estimates of atmospheric carbon dioxide (dashed blue line) and mean average global temperatures (orange line), both from the COPSE model of Bergman *et al.* (2004). PAL, present atmospheric level.

flammability. We also note that many pinaceous fossils cannot be assigned to any modern groups (Gernandt *et al.*, 2008), suggesting extinction of those taxa unable to adapt to these novel fire regimes. For the nucleus of Pinaceae that did adapt to fires originating as far back as 126 Ma, speciation was both strong and widespread across the Northern Hemisphere until pines became, and remain, the dominant group there.

Fuel characteristics and weather are the two most important drivers of fire in modern fire-prone ecosystems (Archibald *et al.*, 2009; Krawchuk & Moritz, 2011; Pausas & Paula, 2012). However, the ignition and spread of fire are also strongly dependent on O_2 concentration in the atmosphere (Belcher & McElwain, 2008), such that burn probabilities are known to increase abruptly above 24% O_2 (Belcher *et al.*, 2010). Because atmospheric O_2 concentrations have fluctuated throughout Earth's history (Bergman *et al.*, 2004; Glasspool & Scott, 2010), O_2 has been a major factor controlling fire activity in the past (Scott, 2000, 2010; Pausas & Keeley, 2009; Belcher *et al.*, 2010). During the Cretaceous, high atmospheric O_2 concentrations at *c.* 125 Ma would have greatly increased the flammability of the Earth's ecosystems, as indicated by the elevated biomass burning probabilities (Fig. 3). Higher atmospheric CO_2 concentrations at a similar time would have led to an increase in primary productivity (LaMarche *et al.*, 1984) and, consequently, greater fuel loads. Huang *et al.* (2007) reviewed evidence from CO_2 enrichment experiments and dendrochronological studies, and concluded that atmospheric CO_2 fertilization increases photosynthetic rates, above- and below-ground biomass. As the carboxylating enzyme has a much greater affinity for CO_2 than O_2, and the increase in CO_2 was an order of magnitude greater than the increase in O_2 at any time, these trends would have been unaffected by the higher levels of O_2 during this period (Fig. 3). Combined with the globally warm temperatures during this period (Fig. 3; Spicer & Herman, 2001; Spicer, 2003) all these conditions probably made the Cretaceous one of the most flammable periods in the Earth's history.

Our results support not only the pivotal role of fire in the evolution of *Pinus*, but also provide a place for fire in the evolution of flowering plants (Bond & Scott, 2010). Angiosperms diversified from *c.* 135 Ma and rapidly increased their ecological importance from *c.* 100 Ma (Crane & Lidgard, 1989). The earliest angiosperms (Magnoliids and pre-Magnoliids) are believed to have been 'weedy', understory herbs, shrubs and small trees in 'dark, damp and disturbed' habitats (Feild & Arens, 2007). Although mostly of tropical origin, it is possible that these early angiosperms contributed to the initial low-intensity surface fire regime in the early Cretaceous conifer forests, together with cycads, ferns and seed-ferns. By 90–100 Ma, the monocots and early eudicots had arisen, diverged and migrated into conifer-dominated forests of the Northern Hemisphere (Stevens, 2012), including such fire-tolerant, fine-leaved rhizomatous and cormaceous clades as Liliales (stem Melanthiaceae at 109 Ma), Asparagales (Iridaceae at 103 Ma, Aparagaceae at 91 Ma), Commelinales (Haemodoraceae at 98 Ma; Lamont & Downes, 2011) and Poales (Cyperaceae at 88 Ma, Poaceae at 89 Ma; Janssen & Bremer, 2004), 'weedy' Ranunculaceae (> 100 Ma; Krassilov & Volynets, 2008) and shrubby Berberidaceae. Thus, the highly flammable understory angiosperms would have greatly expanded during the second wave of fire-related evolution among pines, and promoted equivalent fire-related responses among them as well (Bond & Scott, 2010). Thus, our evidence for a strong correlation between the evolution of fire-response strategies and increase in fire intensity at 90 and 125 Ma among pines greatly backdates and extends the key role played by fire in the evolution of seed plants more generally.

Bond & Scott (2010) noted the marked drop in fire-derived inertinite records for Northern Hemisphere mires during the early Eocene (40–55 Ma) and attributed this to the suppression of fire by the now-dominant angiosperm forests during one of the wettest periods known. This raises the question of the fate of fire-dependent species at that time (and thus the validity of our findings). First, atmospheric O_2 and CO_2 levels, temperatures and burn probabilities were still much higher than currently (Fig. 3); second, inertinite levels have remained low (Fig. 3), despite the fact that most pine forests are currently fire prone, highlighting the inherent bias against recording dryland fire at wet sites; third, there is corroborating evidence of fire-prone floras and species existing or arising among pines (Stockey, 1984: subgenus *Pinus* for which fire-adapted traits are diagnostic; Figs 1, 2, S1) and elsewhere (Itzstein-Davey, 2004; Lamont & Downes, 2011) during the early Eocene; fourth, some parts of the world received only moderate and highly seasonal rainfall at that time (Macphail, 2007), and there is no reason to believe that the vast area currently covered by pine forests was any different; fifth, Keeley (2012) has postulated that pines migrated to drier, and hence more fire-prone, uplands at that time. We conclude that *Pinus*, and other fire-adapted clades, were well placed to survive the Eocene.

By analyzing the evolutionary history of the Pinaceae, we have obtained compelling evidence that fire has been an effective agent of natural selection since *c.* 126 Ma. Whether fire was equally potent before this remains an open question. From our analysis, fire appears to have been ineffective among the Pinaceae for the first 100 My of its existence. Cycads are a much older clade and are universally fire tolerant with fire-stimulated coning (Lamont & Downes, 2011) – they would seem to be the ideal candidate for further research, but for the recent findings that extant cycads originated in the early (Nagalingum *et al.*, 2011) to mid- (Crisp & Cook, 2011) Cenozoic, with long unbranched stems, greatly reducing the probability of following fire-adapted traits into the Cretaceous, let alone beyond. *Ginkgo* is older than the crown cycads, but there is no evidence of fire in its history that might explain its ability to resprout when pruned. Modern tree and rhizomatous ferns may resprout after fire and extend back to the Cretaceous (no earlier than *Pinus*; McLoughlin, 2001; Schneider *et al.*, 2004), and ferns are often represented in fossil charcoal and may become abundant immediately postfire (Collinson *et al.*, 2000), but these instances are too poorly represented or documented among extant species to be able to impute their fire history using molecular methods. The tracing of fire adaptations beyond the Lower Cretaceous appears to be a major challenge for future research.

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References

Alvin KL. 1957. On the two cones *Pseudoaraucaria heeri* (Coemans) nov comb and *Pityostrobus villerotensis* nov sp from the Wealden of Belgium. *Memoirs of the Royal Belgian Institute of Natural Sciences* **135**: 1–27.

Archibald S, Roy DP, van Wilgen BW, Scholes RJ. 2009. What limits fire? An examination of drivers of burnt area in Southern Africa. *Global Change Biology* **15**: 613–630.

Batten DJ. 1998. Palaeoenvironmental implications of plant, insect and other organic-walled microfossils in the Weald Clay Formation (Lower Cretaceous) of southeast England. *Cretaceous Research* **19**: 279–315.

Belcher CM, McElwain JC. 2008. Limits for combustion in low O_2 redefine paleoatmospheric predictions for the Mesozoic. *Science* **321**: 1197–1200.

Belcher CM, Yearsley JM, Hadden RM, McElwain JC, Rein G. 2010. Baseline intrinsic flammability of Earth's ecosystems estimated from paleoatmospheric oxygen over the past 350 million years. *Proceedings of the National Academy of Sciences, USA* **107**: 22448–22453.

Berendse F, Scheffer M. 2009. The angiosperm radiation revisited, an ecological explanation for Darwin's 'abominable mystery'. *Ecology Letters* **12**: 865–872.

Bergman NM, Lenton TM, Watson AJ. 2004. COPSE, a new model of biogeochemical cycling over Phanerozoic time. *American Journal of Science* **304**: 397–437.

Blokhina NI. 1998. Fossil wood of *Cedrus* (Pinaceae) from the Paleogene of Kamchatka. *Palaeontology Journal* **32**: 532–538.

Bond WJ, Scott AC. 2010. Fire and the spread of flowering plants in the Cretaceous. *New Phytologist* **188**: 1137–1150.

Bowman DMJS, Balch JK, Artaxa P, Bond WJ, Carlson JM, Cochrane MA, D'Antonio CM, DeFries RS, Doyle JC, Harrison SP *et al.* 2009. Fire in the earth system. *Science* **324**: 481–484.

Brando PM, Nepstad DC, Balch JK, Bolker B, Christman MC, Coe M, Putz FE. 2012. Fire-induced tree mortality in a neotropical forest: the roles of bark traits, tree size, wood density, and fire behavior. *Global Change Biology* **18**: 630–641.

Bytebier B, Antonelli A, Bellstedt DU, Linder HP. 2011. Estimating the age of fire in the Cape flora of South Africa from an orchid phylogeny. *Proceedings of the Royal Society of London. Series B, Biological Sciences* **278**: 188–195.

Clarke JT, Warnock RCM, Donoghue PCJ. 2011. Establishing a time-scale for plant evolution. *New Phytologist* **192**: 266–301.

Collinson ME, Featherstone C, Cripps JA, Nichols GJ, Scott AC. 2000. Charcoal-rich plant debris accumulations in the lower Cretaceous of the Isle of Wight, England. *Acta Palaeobotanica Supplement* **2**: 93–105.

Crane PR, Lidgard SH. 1989. Angiosperm diversification and paleolatitudinal gradients in Cretaceous floristic diversity. *Science* **246**: 675–678.

Crisp MD, Burrows GE, Cook LG, Thornhill AH, Bowman DMJS. 2011. Flammable biomes dominated by eucalypts originated at the Cretaceous–Paleogene boundary. *Nature Communications* **2**: 193.

Crisp MD, Cook LG. 2011. Cenozoic extinctions account for the low diversity of extant gymnosperms compared with angiosperms. *New Phytologist* **192**: 997–1009.

Drummond AJ, Rambaut A. 2007. BEAST, Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology* **7**: 214.

Drysdale D. 2011. *An introduction to fire dynamics*. Chichester, UK: Wiley–Blackwell.

Erwin DM, Schorn HE. 2005. Revision of the conifers from the Eocene Thunder Mountain flora, Idaho, USA. *Review of Palaeobotany and Palynology* **137**: 125–145.

Falcon-Lang HJ, Kvacek J, Ulicny D. 2001. Fire-prone plant communities and palaeoclimate of a Late Cretaceous fluvial to estuarine environment, Pecínov quarry, Czech Republic. *Geological Magazine* **138**: 563–576.

Feild TS, Arens NC. 2007. The ecophysiology of early angiosperms. *Plant, Cell & Environment* **30**: 291–309.

Gernandt DS, Gaeda López G, Ortiz García SA, Liston A. 2005. Phylogeny and classification of *Pinus*. *Taxon* **54**: 29–42.

Gernandt DS, Magallon SA, Lopez GG, Zeron FO, Willyard A, Liston A. 2008. Use of simultaneous analyses to guide fossil-based calibrations of Pinaceae phylogeny. *International Journal of Plant Sciences* **169**: 1086–1099.

Gill AM, Allan G. 2008. Large fires, fire effects and the fire-regime concept. *International Journal of Wildland Fire* **17**: 688–695.

Glasspool IJ, Edwards D, Axe L. 2004. Charcoal in the Silurian as evidence for the earliest wildfires? *Geology* **32**: 381–383.

Glasspool IJ, Scott AC. 2010. Phanerozoic concentrations of atmospheric oxygen reconstructed from sedimentary charcoal. *Nature Geosciences* **3**: 627–630.

Gómez-González S, Torres-Díaz C, Bustos-Schindler C, Gianoli E. 2011. Anthropogenic fire drives the evolution of seed traits. *Proceedings of the National Academy of Sciences, USA* **108**: 18743–18747.

Hardy CR. 2006. Reconstructing ancestral ecologies, challenges and possible solutions? *Diversity and Distributions* **12**: 7–19.

Harland M, Francis JE, Brentnall SJ, Beerling DJ. 2007. Cretaceous (Albian–Aptian) conifer wood from Northern Hemisphere high latitudes: forest composition and palaeoclimate. *Review of Palaeobotany and Palynology* **143**: 167–196.

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

Huang JG, Bergeroa Y, Denneler B, Berninger F, Tradif J. 2007. Response of forest trees to increased CO_2. *Critical Reviews in Plant Science* **26**: 265–283.

Itzstein-Davey F. 2004. A spatial and temporal Eocene palaeoenvironmental study, focusing on the Proteaceae family, from Kambalda, Western Australia. *Review of Palaeobotany and Palynology* **131**: 159–180.

Jackson JF, Adams DC, Jackson UB. 1999. Allometry of constitutive defense, a model and a comparative test with tree bark and fire regime. *American Naturalist* **153**: 614–632.

Janssen T, Bremer K. 2004. The age of major monocot groups inferred from 800+ *rbcL* sequences. *Botanical Journal of the Linnean Society* **14**: 385–398.

Karafyllidis I, Thanailakis A. 1997. A model for predicting forest fire spreading using cellular automata. *Ecological Modelling* **99**: 87–97.

Kaundun SS, Lebreton P. 2010. Taxonomy and systematics of the genus *Pinus* based on morphological, biogeographical and biochemical characters. *Plant Systematics and Evolution* **284**: 1–15.

Keeley JE. 2012. Ecology and evolution of pine life histories. *Annals of Forest Science*, in press.

Keeley JE, Pausas JG, Rundel PW, Bond WJ, Bradstock RA. 2011. Fire as an evolutionary pressure shaping plant traits. *Trends in Plant Science* **16**: 406–411.

Keeley JE, Zedler PH. 1998. Evolution of life histories in *Pinus*. In: Richardson DM, ed. *Ecology and biogeography of* Pinus. Cambridge, UK: Cambridge University Press, 219–249.

Keller AM, Hendrix MS. 1997. Paleoclimatologic analysis of a Late Jurassic petrified forest, southeastern Mongolia. *Palaios* **12**: 282–291.

Knowles P, Grant MC. 1983. Age and size structure analyses of Ponderosa pine, Engelmann spruce, lodgepole pine and limber pine in Colorado. *Ecology* **64**: 1–9.

Krassilov V, Volynets Y. 2008. Weedy Albian angiosperms. *Acta Palaeobotanica* **48**: 151–169.

Krawchuk MA, Moritz MA. 2011. Constraints on global fire activity vary across a resource gradient. *Ecology* **92**: 121–132.

LaMarche VC, Graybill DA, Fritts HC, Rose MR. 1984. Increasing atmospheric carbon dioxide, tree ring evidence for growth enhancement in natural vegetation. *Science* **225**: 1019–1021.

Lamont BB, Downes KS. 2011. Fire-stimulated flowering among resprouters and geophytes in Australia and South Africa. *Plant Ecology* **212**: 2111–2125.

Lamont BB, Enright NJ, He T. 2011. Fitness and evolution of resprouters in relation to fire. *Plant Ecology* **212**: 1945–1957.

Lamont BB, Maitre DL, Cowling RM, Enright NJ. 1991. Canopy seed storage in woody plants. *Botanical Review* **57**: 277–317.

Macphail M. 2007. Australian palaeoclimates: Cretaceous to Tertiary – a review of palaeobotanical and related evidence to the year 2000. Perth, Australia: *CRC LEME Open File Report 151*.

van Mantgem P, Schwartz M. 2003. Bark heat resistance of small trees in Californian mixed conifer forests, testing some model assumptions. *Forest Ecology and Management* **178**: 341–352.

McLoughlin S. 2001. The breakup history of Gondwana and its impact on pre-Cenozoic floristic provincialism. *Australian Journal of Botany* **49**: 271–300.

Midgley J, Bond W. 2011. Pushing back in time, the role of fire in plant evolution. *New Phytologist* **191**: 5–7.

Miller CN. 1999. Implications of fossil conifers for the phylogenetic relationships of living families. *Botanical Review* **65**: 239–277.

Myers RL, Rodríguez-Trejo DA. 2009. Fire in tropical pine ecosystems. In: Cochrane MA, ed. *Tropical fire ecology, climate change, land use and ecosystem dynamics*. Heidelberg, Germany: Springer-Praxis, 557–605.

Nagalingum NS, Marshall CR, Quental TB, Rai HS, Little DP, Mathews S. 2011. Recent synchronous radiation of a living fossil. *Science* **334**: 796–799.

Nelson CD, Weng C, Kubisiak TL, Stine M, Brown CL. 2003. On the number of genes controlling the grass stage in longleaf pine. *Journal of Heredity* **94**: 392–398.

Pagel M, Meade A. 2007. Manual, *BayesTraits*. [WWW document] URL http://www.evolution.rdg.ac.uk [accessed on 28 September 2009].

Pagel M, Meade A, Barker D. 2004. Bayesian estimation of ancestral states on phylogenies. *Systematic Biology* **53**: 673–684.

Pausas JG, Alessio GA, Moreira B, Corcobado G. 2012. Fires enhance flammability in *Ulex parviflorus*. *New Phytologist* **193**: 18–23.

Pausas JG, Keeley JE. 2009. A burning story, the role of fire in the history of life. *BioScience* **59**: 593–601.

Pausas JG, Paula S. 2012. Fuel shapes the fire–climate relationship: evidence from Mediterranean ecosystems. *Global Ecology and Biogeography*, in press.

Pausas JG, Schwilk DW. 2012. Fire and plant evolution. *New Phytologist* **193**: 301–303.

Rodríguez-Trejo DA, Fulé PZ. 2003. Fire ecology of Mexican pines and a fire management proposal. *International Journal of Wildland Fire* **12**: 23–37.

Schneider H, Schuettpelz E, Pryer KM, Cranfill R, Magallón S, Lupia R. 2004. Ferns diversified in the shadow of angiosperms. *Nature* **428**: 553–557.

Schoenike RE. 1976. Geographical variations in jack pine (*Pinus banksiana*). *Minnesota Agriculture Experiment Station Technique Bulletin No* 304. St. Paul, Minnesota.

Schorn HE. 1994. A preliminary discussion of fossil larches (*Larix*, Pinaceae) from the Arctic. *Quaternary International* **22/23**: 173–183.

Schwilk DW. 2003. Flammability is niche construction: canopy architecture affects fire intensity. *American Naturalist* **162**: 725–733.

Schwilk DW, Ackerly DD. 2001. Flammability and serotiny as strategies, correlated evolution in pines. *Oikos* **94**: 326–336.

Scott AC. 2000. The pre-Quaternary history of fire. *Palaeogeography, Palaeoclimatology, Palaeoecology* **164**: 281–329.

Scott AC. 2010. Charcoal recognition, taphonomy and uses in palaeoenvironmental analysis. *Palaeogeography, Palaeoclimatology, Palaeoecology* **291**: 11–39.

Spicer RA. 2003. Changing climates and biota. In: Skelton P, ed. *The Cretaceous world*. Cambridge, UK: Cambridge University Press, 85–163.

Spicer RA, Herman AB. 2001. The Albian–Cenomanian flora of the Kukpowruk River, western North Slope, Alaska: stratigraphy, palaeofloristics, and plant communities. *Cretaceous Research* **22**: 1–40.

Stevens PF. 2012. Angiosperm phylogeny website. [WWW document] URL http://www.mobot.org/MOBOT/research/APweb/[accessed on 15 January 2012].

Stockey RA. 1984. Middle Eocene pines remains from British Columbia. *Botanic Gazette* **145**: 265–274.

Stott P. 2000. Combustion in tropical biomass fires: a critical review. *Progress in Physical Geography* **24**: 355–377.

Vines RG. 1968. Heat transfer through bark, and the resistance of trees to fire. *Australian Journal of Botany* **16**: 499–514.

Willyard A, Syring J, Gernandt DS, Liston A, Cronn R. 2007. Fossil calibration of molecular divergence infers a moderate mutation rate and recent radiations for *Pinus*. *Molecular Biology and Evolution* **24**: 90–101.

Wood DL. 1982. The role of pheromones, kairomones, and allomones in the host selection and colonization behavior of bark beetles. *Annual Review of Entomology* **27**: 411–446.

Wright HA, Bailey AW. 1982. *Fire ecology, United States and southern Canada*. New York, NY, USA: John Wiley and Sons.

Yamamoto F. 1992. Effects of depth of flooding on growth and anatomy of stems and knee roots of *Taxodium distichum*. *IAWA Bulletin* **13**: 93–104.

Supporting Information

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

Fig. S1 Ancestral state reconstruction of very thick bark (> 30 mm).

Fig. S2 Ancestral state reconstruction of capacity for branch shedding.

Fig. S3 Ancestral state reconstruction for the presence of a grass stage and resprouting ability.

Table S1 Species, trait data and fire regime for Pinaceae analyses

Notes S1 List of genera and references from which original molecular phylogenies were reported.

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