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A 350-million-year legacy of fire adaptation among conifers

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Running title: Fire adaptation in conifers

Summary

1. Current phylogenetic evidence shows that fire began shaping the evolution of land plants 125 Ma, although the fossil charcoal record indicates that fire has a much longer history (>350 Ma). Serotiny (on-plant seed storage) is generally accepted as an adaptation to fire among woody plants.
2. We developed a conceptual model of the requirements for the evolution of serotiny, and propose that serotiny is only expressed in the presence of a woody rachis as supporting structure, compact scales covering seeds as protective structure, seed wing as dispersal structure, and crown fire as the agent of selection and mechanism for seed release. This model is strongly supported by empirical data for modern ecosystems.
3. We reconstructed the evolutionary history of intrinsic structural states required for the expression of serotiny in conifers, and show that these were diagnostic for early ('transitional') conifers from 332 Ma (late-Carboniferous).
4. We assessed the likely flammable characteristics of early conifers and found that scale-leaved conifers burn rapidly and with high intensity, supporting the idea that crown fire regimes may have dominated early conifer ecosystems.
5. Synthesis: Coupled with strong evidence for frequent fire throughout the Permian-Carboniferous and fossil evidence for other fire-related traits, we conclude that many early conifers were serotinous in response to intense crown fires, indicating that fire may have had a major impact on the evolution of plant traits as far back as 350 Ma.

Key words

ancestral state reconstruction, Carboniferous, conifer, fire adaptation, fossil, Permian, progymnosperm, resprouting, seasonality, serotiny

Introduction

Wildfire is an important part of Earth-system processes (Bowman *et al.* 2009). Recent research has identified an important role for fire in shaping the evolution of land plants throughout 'deep' time (Bond & Scott 2010) and was a major driver of trait innovations among terrestrial plants during the Upper Cretaceous/Paleocene (Crisp *et al.* 2011; He *et al.* 2011, 2012; Lamont *et al*. 2013). Earth's terrestrial flora evolved from small nonvascular plants into woody shrubs and trees with an herbaceous understorey that allowed large wildfires to occur by 395 million years ago (Ma), while charcoal appears in the fossil record from the late Silurian, 420 Ma (Scott & Glasspool 2006). Abundance of charcoal in deposits from the Silurian through to the end of the Permian indicated that the frequency of Paleozoic fire was essentially a function of atmospheric oxygen concentrations (Scott & Glasspool 2006; Glasspool & Scott 2010). With atmospheric oxygen levels rising to 30% in the late Permian, fire frequency progressively increased in many ecosystems (Belcher *et al*. 2013). Thus, fire became widespread during the Lower Mississippian to Pennsylvanian Epochs, 350–300 Ma (Falcon-Lang 2000; Scott 2000).

Conifers dominate current Northern Hemisphere ecosystems subject to recurrent fire. Many species within modern conifer families exhibit diverse fire–adapted traits, such as on-plant, seed-storing (serotinous) cones, thick bark and shedding of old branches (Schwilk & Ackerley 2001; He *et al*. 2012). Robinson (1989) was the first to propose that "the apparent propensity to fire tolerance that runs through gymnosperm [conifer] taxa may be a carryover from the role of fire in natural selection during the period in which gymnosperms originated". Our objective was to provide phylogenetic support for the postulated link between fire-related traits among extant and ancestral conifers and compare it against fossil and paleoenvironmental evidence on climate and fire in the Carboniferous-Permian, with particular reference to serotiny.

Despite the global importance of fire in accounting for the diversity of plant traits in modern-day ecosystems, the tracing of fire adaptations beyond the Lower Cretaceous has been a major challenge, because: a) many fire-related traits are not obviously, nor uniquely, linked to fire (Midgley & Bond 2013); b) adaptive traits are rarely preserved in the fossil record, particularly those related to temporal processes, such as the timing of seed release; and c) the agent of natural selection responsible for the trait (i.e. fire) has varied greatly in strength over such a long period of time. Recent studies have successfully used time-based phylogenies to provide insights on the role of fire in plant evolution (Bytieber *et al.* 2011; Crisp *et al.* 2011; He *et al.* 2011, 2012; Lamont *et al.* 2013a,b). The current approach of tracing the origin of functional traits by mapping the trait onto a time-based phylogeny assumes constant presence of the selective agent (i.e. fire) on the target trait. However, given that fire activity across geological timescales is closely linked to the abundance of oxygen in the atmosphere, there have been periods when fire has been greatly enhanced such as in the Permo-Carboniferous and Cretaceous periods (Glasspool & Scott 2010) and periods when fire has been retarded (Robson *et al.* 2015). For example, an extended period at 250–240 Ma, during which oxygen levels are estimated to have been too low to sustain extensive fire, is confirmed by an apparent gap in the record of charcoal in the Early Triassic and the incidence of fire may also have been lower than today throughout much of the Triassic and Jurassic Periods (Belcher *et al.* 2010).

Many species in the coniferous families Pinaceae (Schwilk & Ackerley 2001; He *et al.* 2012) and Cupressaceae (Ladd *et al.* 2013), and many other families in southern hemisphere Mediterranean-climate shrublands (Lamont *et al.* 1991), store their seeds in woody cones/fruits for a prolonged period and only release their seeds in response to fire, known as serotiny. Serotiny restricts seedling recruitment to the immediate postfire environment when the opportunities for regeneration are optimal (Lamont *et al.* 1993; Causley *et al.* in press). Removal of the dense canopy by fire also promotes wind dispersal of the released seeds (Lamont 1985; He *et al*. 2004; Merwin *et al*. 2012). Serotiny is generally accepted as an adaptive response to fire (Lamont *et al*. 1991; Lamont & Enright 2000; Midgley & Bond 2013). The origin of serotiny in modern-day Pinaceae has been dated at *c.* 89 Ma (He *et al.* 2012). Charred remains of apparently serotinous pine cones have been described from early Cretaceous wildfires (Allen 1998). To confirm a species as serotinous from its fossil cone or fruit is difficult because they are invariably preserved in the open condition, so that it is not possible to determine at what time after maturation dehiscence occurred. The reliable clues for the presence of serotiny are a) the extent of woodiness of the supporting structures (e.g. woody rachis) and compact seed bearing structures (e.g. crowded scale complexes in the case of conifers) as an indication of the duration and effectiveness of seed protection, b) whether the seeds are winged, as an adaptation to wind dispersal in the open postfire environment (Lamont 1985; Hughes *et al.* 1994), and c) whether the vegetation was fire-prone at that time as the necessary seed-release mechanism and ultimate agent of natural selection (Lamont *et al.* 1991).

The serotinous structure (fruit, cone or fertile zone) needs to be sufficiently long-lived to store viable seeds for some years and so must become woody. Further, for seeds to survive storage on the plant over several to many years requires their protection against granivores and pathogens. Equally, cones/fruits must provide effective protection against the intense heat from fires. Serotinous seeds should have wings or hairs, or be very small, as adaptations for dispersal by wind (Hughes *et al.* 1994). Greene & Johnstone (1993) showed that bouyancy of seeds up to 225 mg (larger seeds were not serotinous but bird or squirreldispersed) among pines were an exponential function of seed area. Wind dispersal is enhanced in the immediate postfire environment because fire frees the canopy and ground of many obstacles to the passage of wind and seeds (Lamont 1985). In addition, heat generated by the blackened soil surface following fire creates up-drafts and small whirlwinds that may disperse seeds to great distances (He *et al*. 2004). The fitness advantages of serotiny can only be expressed under a crown fire regime that ensures general seed release through heat-opening of the cones/fruits and creates optimal conditions for recruitment (Lamont *et al.* 1991; He *et al.* 2012).

In certain circumstances, severe environmental stress (e.g. drought) can lead to patchy pre-senescence death of adult plants, and subsequent erratic seed release through simple desiccation processes. Indeed, Axelrod (1980) considered that summer drought was a more probable agent selecting for serotiny among Californian pines, since he believed that fire was an anthropogenic factor so was too recent to have any evolutionary impact. This ignores the following fire-adapted traits (all but one cited papers were available in 1980): 1) cones in a few species of *Pinus* and *Cupressus* only open in the presence of fire (Lamont *et al*. 1991), 2) most seeds in strongly serotinous cones of *Pinus* species survive when exposed to temperatures up to 700°C for a few seconds in contrast to unprotected seeds (Beaufait 1960, Linhart 1978), 3) opening of cones of *P. banksiana* requires a minimum temperature of 93°C (Beaufait 1960) as heat melts the resins that bind the apophyses of the cone scales (Ahlgren, 1974), 4) Crossley (1956) interpreted increase in degree of serotiny with age in *P. contorta* as an adaptive response to decreasing opportunity for seedling establishment as the canopy closed over, leaving fire as the key to opening up the vegetation for seedling recruitment. In support, recent research has revealed a strong evolutionary correlation between serotiny and crown fire (He *et al.* 2011, 2012; Lamont *et al.* 2013). Drought-induced seed release rarely leads to significant recruitment compared with fire-induced release in modern ecosystems

(Lamont *et al.* 1991; He *et al.* 2011, Causley *et al.* in press). Besides, seasonal drought and incidence of fire are inextricably related (Pausas & Keeley 2009): it is unknown for vegetation subject to bouts of dryness not also to be fire-prone.

The time of origin of conifers has been estimated at 300–350 Ma in the Carboniferous (Clarke *et al.* 2011; Crisp & Cook 2011; Magallon *et al.* 2013) when fire was widespread (Belcher *et al.* 2010, 2013), supported by a peak in charcoal abundance and record high atmospheric oxygen levels (25–30%, relative to the present 21%, Berner 2009) that would greatly promote combustibility. In fact, at 25% oxygen, plant matter with 72% moisture will burn and at 30% oxygen, even vegetation covered in free water will burn (Watson & Lovelock 2013). This means that even bouts of dryness were irrelevant to the occurrence of fire at that time. Lightning strikes are a function of atmospheric carbon dioxide that was 2–3 times current levels and so ignition sources were abundant (Belchier *et al.* 2013).

Here, we propose that the appearance of serotiny as an adaptive trait facilitated the origin and evolution of conifers in the Carboniferous at a time of frequent fire. To test this, we first constructed a conceptual model to evaluate the essential requirements for the expression of serotiny in modern-day ecosystems, and then reconstructed these essential structural requirements through time to infer the existence of serotiny among the ancestors of modern conifers by mapping them onto a large, well-sampled molecular phylogeny/chronogram for conifers, including fossil taxa as terminal taxa. Finally, we tested whether Permian-Carboniferous-associated scale-leaved conifers could support intense fires and therefore provide the fuel for crown fires in these ecosystems, in order to couple the evolution of serotiny with a high risk of crown fire at this time.

Materials and methods

CONCEPTUAL MODEL OF ESSENTIAL REQUIREMENTS FOR THE EXPRESSION OF SEROTINY

There are at least three structural requirements for a species to be serotinous: a) a strong supportive structure to ensure that the serotinous fruits/cone (or fertile zone) survive for at least 12 months – indicated by the presence of secondary xylem in the rachis (or stem if it only has a fertile zone in the case of some ancestral conifers); b) sturdy protective structures to insulate stored seeds against the 'elements' (direct sunlight, low humidity, rain, fire), provided by bracts and scale-complexes that wrap around the seeds in compact (as distinct from open) cones in conifers; c) buoyant structures, usually wings, attached to the seeds that facilitate wind dispersal in the postfire environment (important at a time before the origin of animal-dispersal vectors). There are also environmental conditions to be met: a) serotiny is only able to enhance fitness in the presence of the selective agent, i.e. recurrent crown fire; and b) tissue-death (desiccation/heat)-induced seed release. We constructed a conceptual model (Fig. 1) and hypothesised that a species is most likely to be serotinous only if these four requirements are met simultaneously.

The conceptual model for serotiny was tested against modern-day ecosystems with four typical data sets, containing species from the world’s major fire-prone ecosystems: Pinaceae, with over 210 species, and serotinous species dominating some forests subject to frequent burning in the Northern Hemisphere (He *et al*. 2012); *Banksia sensu stricto* (Proteaceae), with 94 species and subspecies of which most are serotinous, and widespread in fire-prone Australia (He *et al.* 2011); *Protea*, a major genus in fire-prone southern Africa (Lamont *et al.* 2013); plant species in two 40 × 40 m plots in the fire-prone and species rich southwest Australian flora (Enright *et al.* 2007). Serotiny is particularly well-represented in southwestern Australia with over half of the 1200 currently recognised serotinous species occurring there (Lamont & Enright 2000).

For each species, we collated data for: a) supportive structure – presence/absence of secondary xylem in the rachis of the seed-bearing structure; b) protective structure – presence/absence of structure covering seeds during development (e.g. closed follicles/fruits in angiosperms; compact cone with bracts/scales covering seeds in conifers); c) wind dispersal capacity – winged or hairy, or extremely small (<1 mm long) versus non-winged, smooth or large (>1 mm); d) habitat with recurrent crown fire – presence versus absence. For each species, the model was fitted as:

$$A_1 \times A_2 \times A_3 \times A_4 = S \quad \text{(Equation 1)}$$

Where: $A_1 = 1$ for the presence of secondary xylem in the rachis as supportive structure, otherwise $A_1 = 0$; $A_2 = 1$ for the presence of protective structure covering seeds, otherwise $A_1 = 0$; $A_3 = 1$ for winged, hairy or extremely small seeds, otherwise $A_3 = 0$; $A_4 = 1$ for crown fire regime, otherwise $A_4 = 0$; When $S = 1$, the species should be serotinous; when $S = 0$, the species should be non-serotinous. The fit of the model for each species was evaluated by whether its serotinous state agreed with the outcome of Equation 1. The overall fit of the model for each group of species was evaluated by Chi-square test, and was accepted when $P < 0.01$.

RECONSTRUCTING THE ANCESTRAL STATE OF SEED-BEARING CONES AND SEED APPENDAGES IN CONIFERS

Seed cone structure, both supportive and protective, and seed appendages (winged versus non-winged) are conservative across genera (except in *Pinus* that has winged and non-winged seeds, Tomback & Linhart 1990) in extant coniferous families (see data and references in Table S2). Thus, a phylogeny of conifers was constructed at the genus level. Taxon sampling included all genera currently recognized in all gymnosperm families, and 23 species of angiosperm and non-vascular plants as outgroup. The chloroplast DNA sequences for *rbc*L and *mat*K in all species were obtained from the NCBI database (Supplementary Information: Table S2). We used BEAST v2.1.0 to estimate phylogeny and divergence times under a strict clock model (Drummond *et al.* 2006). We used a Yule prior for rates of cladogenesis and ran analyses of 10 million generations sampling every 1000 generations, with a 2.5 million generations burn-in. We set 12 calibration points based on well-known fossil records that have been critically evaluated in other studies (Clarke *et al.* 2011; Crisp & Cook 2011; Magallon *et al.* 2013). The emergence of land plants was set at 460–472 Ma (Edward *et al.* 2014). The majority of the priors were set to lognormal as this distribution allows assignment of the highest point probability for the node age that must be older than the oldest fossil (Ho & Philips 2009). .

Data for seed cone structure and seed appendages in each genus were collated from the literature. ‘Presence of supportive structure’ was defined as cones taking >1 year to mature and showing evidence of secondary xylem in rachis. ‘Presence of protective structure’ was defined as existence of compact cone with bracts/scale covering seeds. Rothwell *et al.* (2011) concluded that the overall evidence from both extant and fossil conifers strongly supports the hypothesis of strong homology among seed cones. We assumed that these traits have remained unchanged since the origin of that genus. In *Pinus*, 20 species have non-winged, nut-like seeds (Tomback & Linhart 1990). The ancestral state of winged seeds in *Pinus* was reconstructed using the likelihood approach in Mesquite (Maddison & Maddison 2007) and accepted as the diagnostic state for *Pinus* when placed in the overall conifer phylogeny (Supplementary Information: Fig. S1). Species in Cycadaceae and most Gnetidae have seeds with a fleshy sarcotesta that is not homologous with the fleshy attachment (peduncle, aril) in some coniferous families. Seed traits for the Cycadaceae and Gnetidae were therefore assigned a different state from other conifers having seeds with a fleshy appendage. We used MultiState in BayesTraits (Pagel & Meade 2006) to determine the ancestral states of supportive structure, protective structure, and seed wingness. The MCMC method was used to calculate the probability of the ancestral states at the stem of conifers in the gymnosperm phylogeny generated from BEAST.

Finally, by collapsing all genera into each family, a simplified coniferous family phylogeny was created. Three extinct coniferous families, five families of the pro-conifer Voltziales and the pre-conifer Cordaitales that have clear evidence of their seed cone structure and seed appendages were added to the simplified conifer family phylogeny (Supplementary Information: Table S3). Hypothetical phylogenetic relations of the extinct families were added to the extant families following Farjon (2008). Ancestral states of supportive structure, protective structure, and seed appendages were reconstructed using the likelihood approach in Mesquite (Maddison & Maddison 2007).

PALEOZOIC FIRE BEHAVIOUR

As serotiny is typically linked to crown-fire regimes, it was desirable to assess the likely fire behaviour that the earliest conifers may have displayed if they were ignited. The Voltzian conifers had a distinctive scale-leaf morphology. We performed flammability experiments to test whether early scale-leaved conifers would have fuelled fires of sufficient intensity to heat serotinuous cones sufficiently to release their seeds. We tested the flammability of four scale-leaved species: *Athrotaxis cupressoides* (Cupressaceae), *A. laxifolia* (Cupressaceae), *Cryptomeria japonica* (Cupressaceae), *Dacrydium cupressinum* (Podocarpaceae), and four needle-leaved species, *Abies recurvata* (Pinaceae), *Tsuga heterophylla* (Pinaceae), *Sequioa sempervirens* (Cupressaceae), *Taxodium distichum* (Cupressaceae), that are more characteristic of those that evolved later in the Mesozoic. The samples were dried in an oven for 6 days at 50°C and then placed in a metal mesh basket (368 cm^3 in volume) (Schemel *et al.* 2008). We tested equal volumes of plant material in each case and tested three samples of each plant type. We used oxygen depletion calorimetry to measure the heat release profiles of each species (following ASTM E1354; Tewarson 2002). Each basket was placed in the cone calorimeter and subjected to a heat flux of 30 kWm^{-2} [within the typical range for flammability testing (Tewarson 2002)]. A spark pilot ignition was positioned above the sample and turned on at the same time as the heat source. On heating, the samples release flammable gases that are ignited by the spark leading to flaming combustion of the samples. The amount of energy released from each sample was measured as a function of time, while flame height is known to be to be a function of heat release rate (Quintiere 1998).

Results

All 202 Pinaceous species for which we were able to obtain morphological data have supportive and protective structures in seed-bearing cones, while 182 species of these have winged seeds. Thirty species occur in habitats with a crown fire regime, with 25 of them serotinous. Overall, 197 species fit the serotiny model (Equation 1 supported), with only five species having $A_1 \times A_2 \times A_3 \times A_4 = 1$ but actually being non-serotinous (expected S = 0). Further checking showed that the habitats of those five species are dominated by a surface fire regime, while crown fires are rare.

Banksia ss included 94 species and subspecies that all have woody fruits with covered seeds and winged seeds. There are 83 taxa in habitats with a crown fire regime, and all are serotinous. Sixty-nine of the 87 *Protea* species are serotinous and occur in habitats characterised by a crown fire regime, with only one species at $A_1 \times A_2 \times A_3 \times A_4 = 1$ but actually being non-serotinous (S = 0 expected). The remaining 17 species are non-serotinous and occur in savanna grasslands that usually only experience surface fires. In the two 40 × 40 m plots in SW Australia where the shrubland is subjected to crown fire, 153 species (75 genera in 28 families) were recorded, with 43 species either having winged/hairy seeds or extremely light seeds (<1 mm) and 42 being serotinous. In all four datasets, Chi-Squared tests supported a highly significant overall fit of the serotiny model with $P < 0.0001$ (Table 1).

The timing of divergence and overall topology of the phylogeny including 66 conifer genera were consistent with previous reports on conifer phylogenies (Clarke *et al.* 2011; Crisp & Cook 2011; Magallon *et al.* 2013). Bayesian MCMC estimated that conifers and the Cycadaceae diverged 332 Ma [95% highest posterior density (HPD): 311–346 Ma], and the divergence was supported by the highest possible posterior probability ($P = 1.0$). Trait reconstruction revealed the ancestral traits of the seed-bearing cones and seed appendages in conifers (Fig. 2).

The early conifers had a woody supportive rachis, and a compact cone with bracts/scales covering the seeds that were winged, all with a posterior probability of $P > 0.90$, implying that the three traits likely originated with the first appearance of conifers 332 Ma in the Mid-Mississippian Epoch of the Carboniferous. Three extinct conifers and five families of Voltziales with abundant fossil records from the Carboniferous through to the Cretaceous all had fossils showing the existence of secondary xylem in the rachis and a compact-cone structure. Adding these families to the coniferous family phylogeny further supported the ancestral state of seed-bearing cones. Fossil taxa that appeared between the Carboniferous to Permian, mainly Voltziales, showed evidence of seed wings, clearly suggesting that the early conifers had winged seeds. Interestingly, there was little evidence of the presence of a seed wing in fossil coniferous families existing in the Jurassic to early Cretaceous (Table 1, Fig. 3).

Ancestral-type, scale-leaved morphologies have higher peak heat release rates than a 'modern', needle-leaved conifers (Fig. 4). The scale-leaf morphologies burnt with a rapid release of heat that typically also quickly consumed the fuel, while the needle-leaf morphologies either generated a burn of sustained duration with slow heat release or a low amount of heat release compared with the scale-leaved conifers (Fig. 4). This means that flame heights of the ancestral species would have been greater, and heat from fires in dense litter mats would increase the chance of crown ignition and fire spreading to the seed-bearing cones.

Discussion

REPRODUCTIVE STRUCTURES FAVOURING SEROTINY

The stem age of conifers was dated at 332 Ma (311–346 Ma, 95% HPD) in the Mississippian Epoch of the Carboniferous. Bayesian MCMC ancestral-state reconstruction showed that the common ancestor of modern conifers had 1) a woody rachis/stem that 2) supported a compact cone/zone with bracts/scales covering the seeds which 3) were winged, all with a posterior probability greater than 0.90, and meeting the requirements for the existence of serotiny (Fig. 2). Does the available fossil evidence support the presence of these structural features in the Carboniferous? Seeds of most ‘transition’ conifers (e.g. Emporiaceae, Voltziales, dated at 305 Ma, Pennsylvanian, Upper Carboniferous) were winged and held in compact cones with a woody axis bearing bark and megasporophylls with secondary xylem (Herandez-Castillo *et al.* 2009). These authors concluded that "most ancient conifers already possessed a similar reproductive biology…to that of extant conifers", including seed dormancy (a necessary feature of serotinous species aligned with cone woodiness). Fossil taxa of six of seven families in the Voltziales showed the existence of secondary xylem in the rachis (or fertile zone) and a compact cone with covered seeds (Table S2, Fig. 3). Interestingly, seed wings were evident in fossil taxa appearing during the Carboniferous to Permian, while evidence for seed wings is absent in fossil taxa appearing since the Triassic when fire activity has been hypothesised as weaker due to lower atmospheric oxygen concentrations then (Glasspool & Scott 2010).

Is there other fossil evidence that supports our findings that early conifers were serotinous? Serbet *et al.* (2010) noted that the bracts of Voltzialean species were typically held at an angle of 30° to the rachis and that seeds were attached to the lower half of the short shoot (Looy & Stevensen 2014) – these would have provided protection against fire heat of the type generated by scale-leaved conifer morphotypes. Also the fertile zones (before terminal cones evolved) were much less leafy than the rest of the branch and would not have burnt as intensely, perhaps exposed to heat but not flames. Whether this protection was adequate is unclear, as no charred cones have been recorded in the literature at this time. However, while many isolated fossil seeds have been documented in the Pennsylvanian strata, none shows any fire damage, indicating that they were dispersed postfire (or in the absence of fire) consistent with their serotinous nature. Further, our simulation of Permo-Carboniferous fire suggests that scale-leaved conifers generated a short but intense heat pulse (see below), implying that the time seed structures were exposed to fire would be brief.

THE ROLE OF SEASONALITY, HIGH OXYGEN AND FIRE

The transition conifers typically occupied the drier, nutrient-impoverished, coastal plains under seasonal climates that limited plant growth, favouring woodland rather than forest (DiMichele *et al.* 2001), conditions that would have promoted serotiny in the presence of fire (Lamont *et al.* 1991). They were small, spreading trees with scale-like leaves, dwarfed in stature and leaf morphology compared with the related pre-conifer, Cordaitales (Galtier *et al.* 1992, Mapes & Rothwell 2003, Hermandez-Castillo *et al.* 2003, 2009b) that would have allowed the hot, rapidly-burning surface-initiated fires to reach their crowns. Indeed, Carboniferous laminated deposits record fire at intervals of about 35 years in proconifer communities (Beerling *et al.* 1998; Falcon-Lang 2000). Nevertheless, the initial evolutionary trigger for seed release might have been severe drought (in association with high atmospheric temperatures in some cases, Zambito & Bennison 2013) that would kill the parent plant and simple desiccation processes would result in seed release. But this would only create limited opportunities for seedling recruitment beneath the dead parent plant (through gap creation).

Equally, the high oxygen levels meant that the vegetation was combustible at any time of the year (Watson & Lovelock 2013) so the timing of fire was tied to the incidence of lightning (e.g. during the wet season). Certainly, there is evidence that the pro-conifer plants were alive at the time of fire (Scott & Chaloner 1983). Not only would fire create much larger gaps than isolated plant deaths but, in addition, greatly improve the availability of water, light and nutrients at ground level. Thus, the selective pressure from fire would far have outstripped that from drought death. Since strong seasonality in the presence of frequent lightning and high oxygen levels promote the incidence and intensity of fire, fires would quickly become both the main source of widespread parent death and the trigger for seed release. Thus, germination of seeds would increasingly be tied to the wet season following fire (Looy & Stevensen 2014) and promote structural shifts towards serotiny that ensured both protection of seeds from the elements, including fire heat, and the delay of seed release until creation of the optimal seed bed for germination and seedling establishment. In addition, the general removal of above-ground plant mass by fire would promote postfire winds, and therefore seed dispersal by wind (Lamont 1985). Thus, Rothwell *et al.* (2005) concluded that "the unexpected species richness of Voltzialean conifers…resulted from the evolutionary ecology of unstable habitats". Increasing bouts of aridity and recurrent fire were the basis of this instability.

It is interesting to relate this interpretation to the six evolutionary scenarios proposed by Keeley *et al.* (2011) for adaptations versus exaptations. For serotiny to be an adaptation to drought but exaptation to fire would require 1) drought to precede fire as an evolutionary force (Hopper 2009), and 2) adaptive traits to drought and fire to be identical. Neither requirement is satisfied here. Fire and drought are only decoupled in semi-arid/arid systems and rock outcrops/monadnocks where the vegetation is too scattered to propagate fire. In woodlands, savannas and shrublands (as recorded in the Carboniferous/Permian), periods of drought create dry fuel adequate to support widespread fire, fire does not follow drought at some future time but is concurrent with it. In the high oxygen/high lightning environment of the Carboniferous/Permian, even saturated live fuel would ignite (Watson & Lovelock 2013), raising the possibility that fire even preceded drought as the prime selective force favouring the evolution of serotiny. The ecology of seeds adapted to drought and fire are different: a) seeds released in response to fire (pyriscence) have to be insulated from fire heat, drought-released seeds (necriscence) do not (Lamont 1991); b) drought-released seeds encounter a hostile seed bed: there may be a litter layer to penetrate before mineral soil is reached, much of the surrounding vegetation remains alive to actively compete for resources with the germinants, seeds are only released when the parent dies under the most extreme of droughts so that seeds need to germinate and establish in that same extreme drought year (Keeley *et al.* 2011), fire-released seeds have to contend with none of these things (Cauley *et al.* in press); c) fire-released seeds get covered in soil and charred litter, drought-released seeds do not (Lamont *et al.* 1993). In fact, Carboniferous conifers are believed to have produced dense, highly flammable, litter mats (Looy 2013) that we show are capable of burning at high intensity (Fig. 4). Thus, we view serotiny evolving through the Paleozoic as essentially a response to intensifying fire (Scenario 5 of Keeley *et al* 2011). Nevertheless, we acknowledge the fluctuating fire regimes through the subsequent Mesozoic/Cenozoic that would lead to selection in other directions (Lamont *et al.* 2013) so that an overall sinuous response curve (their scenario 4b) is more accurate.

THE IMPORTANCE OF FIRE BEHAVIOR

Serotiny is only expected to evolve in ecosystems with intense, crown-fire regimes. Our analysis suggests that serotinous cones may have first appeared during the earliest period of major fire episodes (350–250 Ma) on the planet. Estimates show that oxygen levels had risen to as high as 30% at this time (Berner 2009; Lenton 2013) and increased oxygen levels greatly enhance fire (Belcher *et al.* 2010). At such levels of oxygen, ignition potential is doubled compared with ambient levels (Watson & Lovelock 2013) and fire spread rates is 1.5 times faster (Lenton 2013). Fire behavior has also been shown to link to leaf traits (de Magalhaes & Schwilk 2012). The earliest Voltzialean conifers had a distinctive, scale-leaved morphology. Our flammability experiments on conifers of analogous morphology indicate that the scale-leaved branches of early conifers were capable of carrying highly intense fires either in litter or within the canopy itself. This suggests that fires in early conifer-dominated ecosystems were likely quick-burning 'flashy' fires. Whether a surface fire will reach the tree crown or ignite the crown relies strongly on fire intensity (Davies

2013). Because the scale-leaved morphologies supported rapid energy release and therefore burned with high peak fire intensities, thus generating long flame lengths, this would be more likely to enhance drying and crown scorch, promoting ignition of live canopy fuels. Therefore, such fires would likely have delivered a high heat flux to the serotinous cones in the crown, promoting seed release and ultimately successful seedling recruitment.

It is therefore clear that fire activity was much enhanced during the period in which we estimate serotiny to have arisen, both due to super ambient oxygen and leaf traits supportive of intense fires. Serotinous cones only release their seeds *en masse* when exposed to direct heat from a wildfire (Lamont *et al.* 1991). Therefore, the finding that serotiny was present among Carboniferous conifers implies that Carboniferous forests were able to fuel fires of sufficient intensity, either through extreme surface fires that desiccated and strongly heated the crown or that such fires transitioned into canopy fires (as shown for pines much later in the Cretaceous) promoted by their small stature.

OTHER ANCIENT FIRE-RELATED TRAITS

Plants in modern-day, fire-prone ecosystems possess a suite of adaptive traits that includes serotiny, postfire resprouting, thick bark, branch-shedding, germination in response to heat and smoke, and fire-stimulated flowering (Keeley *et al.* 2011; Lamont *et al.* 2011a,b). Apart from serotiny, other fire-adapted traits in the early conifers may also have evolved as a response to recurrent fire in the Carboniferous. Shedding dead branches from the crown is a fire adaptation as it reduces plant flammability (Keeley & Zedler 1998; Schwilk & Ackerly 2001). Orderly branch abscission and healing has been reported in Voltzialean conifers, which has been interpreted as an adaptation to wildfire (Looy 2013; Falcon-Lang 2014). Robinson (1989) noted palaeobotanical evidence for the high frequency of fire-resistant plants then, e.g. Carboniferous swamp floras invested heavily in the production of thick bark and belowground storage tissues that may have functioned to protect meristematic tissues from fire and thus enable resprouting to occur. Thick bark (~15 mm, sufficient to insulate against heat during mild fires) was recorded in the pro-conifer, *Protopitys buchiana*, at an inferred age of 359–347 Ma (Early Mississippian) in northeast Queensland, Australia (Decombeix 2013). Resprouting is increasingly being shown to be an adaptive response to fire (Lamont *et al.* 2011) and is relatively common among modern conifers. There are at least 94 coniferous species in 41 genera among all six extant families that have resprouting capability after disturbance (Supplementary Information, Table S3). The widespread taxonomic distribution of resprouting ability among modern conifers implies it must have had an early origin in the conifer phylogeny, consistent with the selective pressure from recurrent fires in the Paleozoic Era.

FLUCTUATING LEVELS OF SEROTINY THROUGH TIME

Biogeochemical models suggest that atmospheric oxygen levels may have been lower than that of the present day at 250–240 Ma (Bergman *et al.* 2004; Berner 2009) and is supported by an apparent gap in the charcoal record at this time (Belcher *et al.* 2010). The probability of fires is also estimated to have been lower than the present day at ~240–150 Ma based on ignition probability and the ability of fires to spread under the estimated levels of atmospheric oxygen at this time (Belcher *et al.* 2010). Charred fossils do occur throughout the mid-late Triassic and Jurassic but typically in lower abundances than in rocks from other geological periods (Glasspool & Scott 2010).

When the required selective agent (i.e. crown fire) was rare, conifers in the Jurassic through to the Lower Cretaceous were less likely to have been strongly serotinous. However, Leslie (2011) noted that species in the Araucariaceae, Cupressaceae and Pinaceae continued to develop robust, tightly packed cones with woody bracts/scales from the Jurassic. This may indicate the increasing importance of seed protection during the Jurassic to Cretaceous due to the radiation of bird and mammal granivores in the Cenozoic (Leslie 2011). Seed wings that promote dispersal, so avoiding being taken by seed predators, are also adaptive in fire-free environments, though non-winged seeds evolved as a response to diversification of seed dispersal vectors due to the evolution of birds and mammals, absent in the Paleozoic. As a consequence, both supportive and protective structures of seed-bearing cones and winged seeds continued to enhance fitness under a new selective regime where fire was less important.

Our own analysis (Fig. 3C) indicates that woody scales appeared later than non-woody protective scales but earlier than the Jurassic, for example, at 280 and 230 Ma. Such woody cones give even better insulation against fire heat (see Introduction) and there were post-Carboniferous periods (300–250, 125–75 Ma) of high oxygen and inertinite deposition levels suggestive of frequent and intense fires (He *et al.* 2012) where such cones would have remained fire-adaptive on these grounds alone. Recent research has shown that serotiny was delayed in Pinaceae until 89 Ma in when fire again became more frequent and of greater intensity, switching from surface to crown fires, fuelled by elevated atmospheric oxygen levels in the middle Cretaceous (Belcher *et al.* 2010, He *et al.* 2012).

Seed cones of species in the Araucariaceae usually have a woody rachis with a compact cone covering the seeds and winged seeds, though all extant species are non-serotinous: the cones shatter at maturity. It is likely that the ancestral araucarias lost their serotinous condition when their habitats become relatively fire-free during extremely wet periods (e.g. Paleogene) or they contracted into fire-free habitats where they currently remain. Araucarias are usually emergent in their vegetation types and more likely to escape crown fires in the lower strata while wind dispersal of seeds would still be advantageous.

Robson *et al.* (2015) have described the fluctuating, but overall, marked drop in fire-derived inertinite records for mires in Germany during the early Eocene (40–55 M) compared with the Paleocene during one of the wettest periods known. This again raises the question of the fate of fire-dependent species at such times. They considered a major explanation was that oxygen levels had for the first time fallen to current levels so that fires were now controlled by climate, including greater seasonality elsewhere. Other models (e.g. COPSE, Belcher *et al.* 2013) show oxygen levels were still above ambient (e.g. 24%, COPSE) while carbon dioxide levels, temperatures and burn probabilities were still much higher than currently (He *et al.* 2012). Such inertinite levels have remained low ever since (He *et al.* 2012) despite the fact that most conifer forests are currently fire-prone, highlighting the inherent taphonomic bias against the fossil deposits in recording dryland fire at such wet sites. Further, there is corroborating evidence of fire-prone floras and species existing or even evolving among pines (Stockey, 1984: subgenus *Pinus* for which fire-adapted traits are diagnostic, He *et al.* 2012) and other plant groups elsewhere (Itzstein-Davey, 2004; Lamont & Downes, 2011) during the early-mid Eocene. There is strong evidence that many 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. Keeley (2012) has postulated that conifers migrated to drier, and hence more fire-prone, uplands at that time. We conclude that conifers, and other fire-adapted clades, were still subjected to fire during the early Eocene, and that serotiny would have remained adaptive. Overall, the evolution of woody cones and winged seeds in conifers has been shaped by multiple forces peaking at different times throughout the history of these traits (Keeley *et al.* 2011).

CONCLUSIONS

Taken together, our conceptual model of serotiny, ancient trait-based reconstructions, flammability experiments, palaeoclimate reconstruction and extensive analysis of the fossil record support the existence of serotinous traits among early conifers. We conclude that serotiny was but one of an array of fire-adapted traits that enhanced fitness of plants in fire-prone environments in the Carboniferous. These can be matched to the strong evidence of frequent fire (high atmospheric oxygen levels, abundant charcoal in the fossil record) at the time and likely intense fires based on the flammability of scale-leaved conifers. Although the history of fire may have varied subsequently as a result of further fluctuations in atmospheric oxygen, there can be little doubt that fire has had a major impact on plant form and ecosystem function for at least 350 million years, a legacy that has continued into many modern-day ecosystems.

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Supplementary materials

Table S1 Species, Genbank numbers and trait data

Table S2 Traits of fossil and extant conifers and related orders and families and their geological history.

Table S3 List of species with resprouting capacity among gymnosperms

Fig S1 Reconstructing the ancestral state of seed wingness in *Pinus*

Table 1. Model fitting of requirements for the presence of serotiny among four data sets.

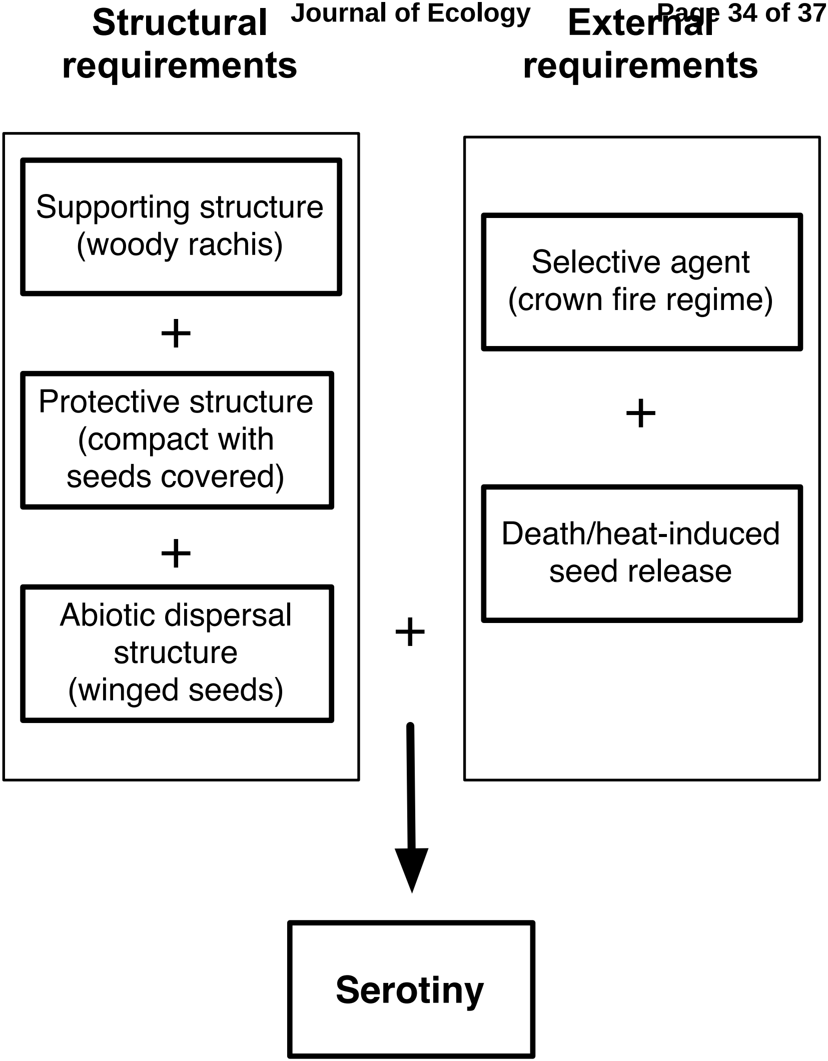
Figure captions

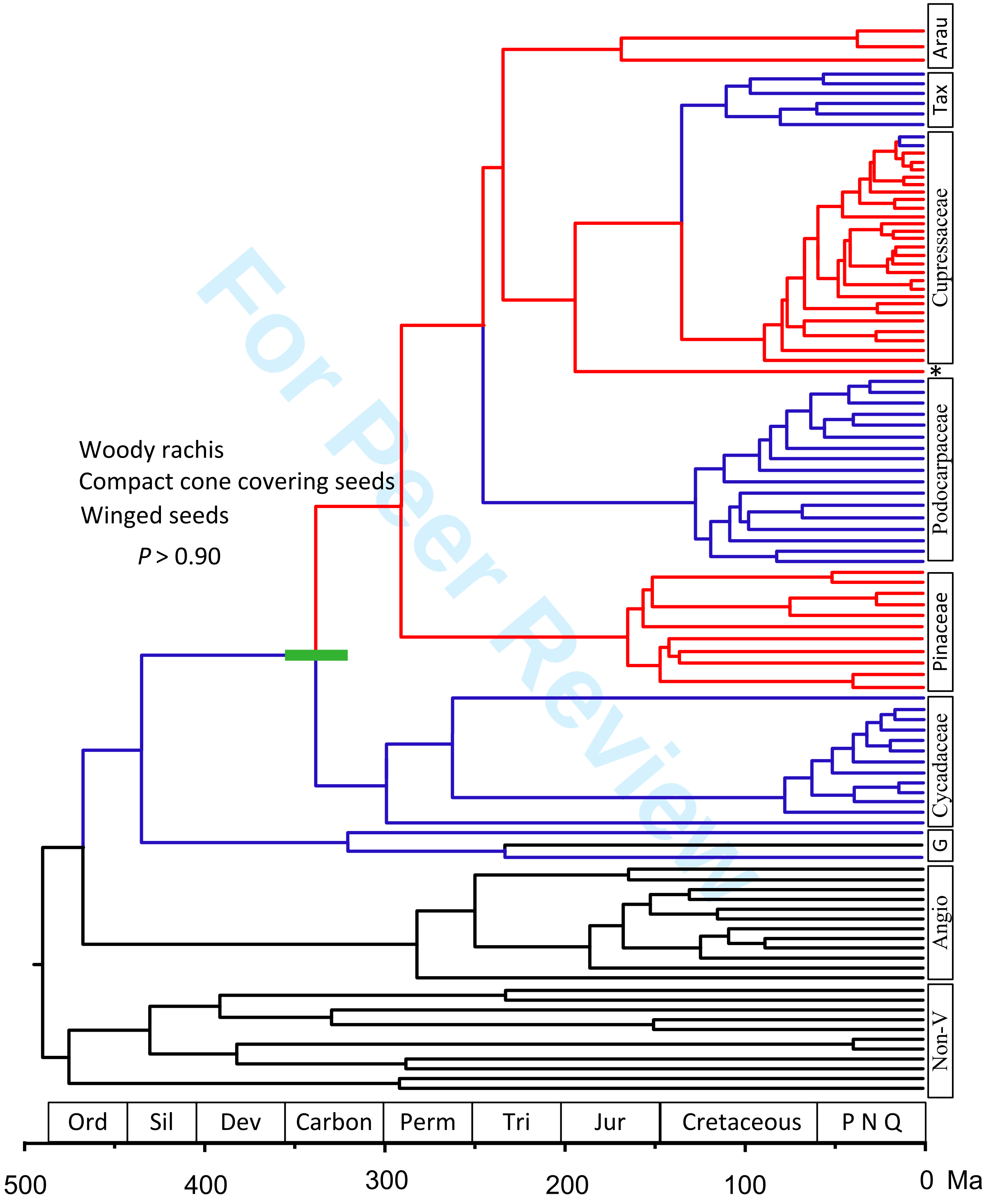
Fig. 1. Conceptual model of essential requirements for the expression of serotiny

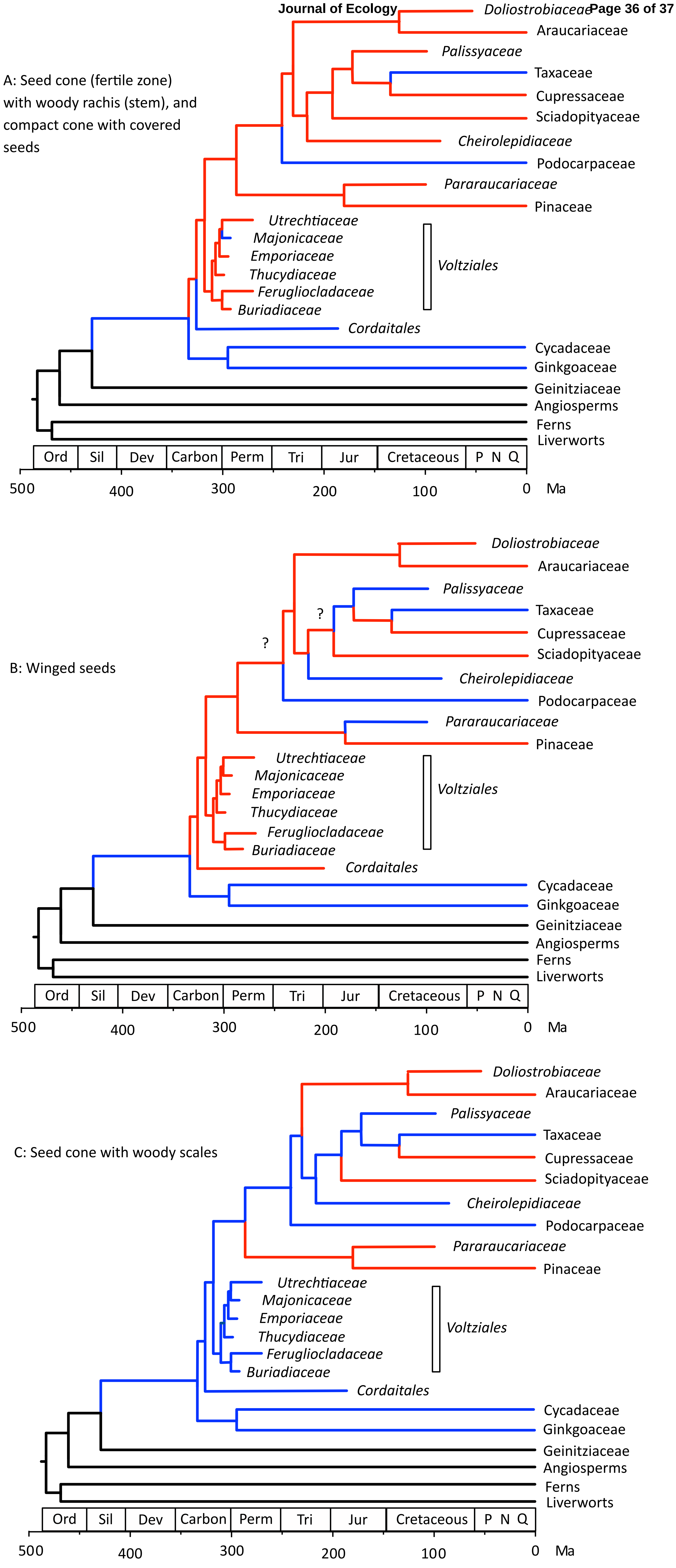
Fig. 2. Ancestral state reconstruction of existence of woody rachis, compact cone covering seeds and seed wingness among conifers. Arau: Araucariaceae; Tax: Taxaceae; Angio: Angiosperm; Non-V: non-vascular plants, G: Gnetidae; *: Sciadopityaceae. Green bar: 95% highest posterior density; red line: lineages with woody rachis, compact cone and winged seeds; blue line: lineages lacking woody rachis, compact cone and/or winged seeds. Black line: not considered.

Fig. 3. Ancestral state reconstruction of woodiness of seed cone and seed wingness in conifer families including extinct families (in italics). Red line: lineages with woody rachis, compact cone and winged seeds; blue line: lineages absent with woody rachis, compact cone covering seeds and winged seeds; black line: not considered. Question mark indicates ancient state was equivocal.

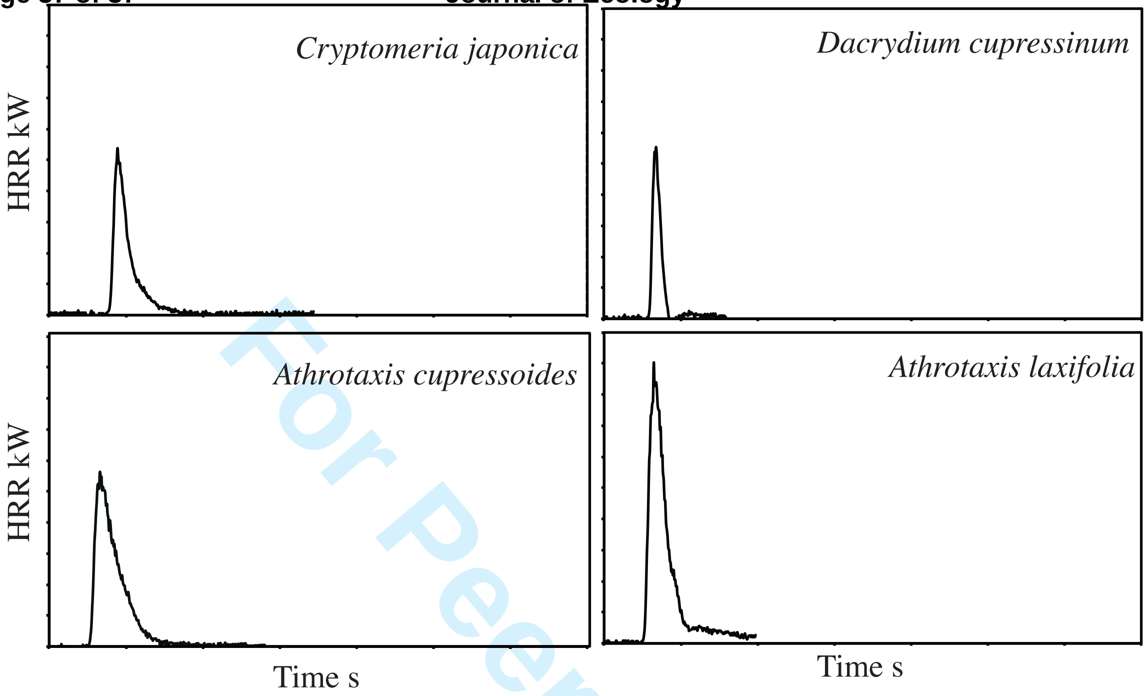
Fig. 4. Heat release rate (HRR) curves for extant conifers with analogous/non-analogous Palaeozoic leaf morphology. X axes are 700 seconds; Y axes are 10 kW. The area under the curve represents the total amount of heat released and relates to the amount of burnable fuel.


Structural requirements
External requirements
Supporting structure (woody rachis)
+
Protective structure (compact with seeds covered)
+
Abiotic dispersal structure (winged seeds)
Selective agent (crown fire regime)
+
Death/heat-induced seed release
+
Serotiny


Arau
Tax
Cupressaceae
*
Podocarpaceae
Pinaceae
Cycadaceae
G
Angio
Non-V
Woody rachis
Compact cone covering seeds
Winged seeds
P > 0.90
Ord
Sil
Dev
Carbon
Perm
Tri
Jur
Cretaceous
P N Q
500
400
300
200
100
0 Ma


A: Seed cone (fertile zone) with woody rachis (stem), and compact cone with covered seeds
Doliostrobiaceae
Araucariaceae
Palissyaceae
Taxaceae
Cupressaceae
Sciadopityaceae
Cheirolepidiaceae
Podocarpaceae
Pararaucariaceae
Pinaceae
Utrechtiaceae
Majonicaceae
Emporiaceae
Thucydiaceae
Ferugliocladaceae
Buriadiaceae
Voltziales
Cordaitales
Cycadaceae
Ginkgoaceae
Geinitziaceae
Angiosperms
Ferns
Liverworts
Ord
Sil
Dev
Carbon
Perm
Tri
Jur
Cretaceous
P
N
Q
500
400
300
200
100
0
Ma
B: Winged seeds
?
?
Doliostrobiaceae
Araucariaceae
Palissyaceae
Taxaceae
Cupressaceae
Sciadopityaceae
Cheirolepidiaceae
Podocarpaceae
Pararaucariaceae
Pinaceae
Utrechtiaceae
Majonicaceae
Emporiaceae
Thucydiaceae
Ferugliocladaceae
Buriadiaceae
Voltziales
Cordaitales
Cycadaceae
Ginkgoaceae
Geinitziaceae
Angiosperms
Ferns
Liverworts
Ord
Sil
Dev
Carbon
Perm
Tri
Jur
Cretaceous
P
N
Q
500
400
300
200
100
0
Ma
C: Seed cone with woody scales
Doliostrobiaceae
Araucariaceae
Palissyaceae
Taxaceae
Cupressaceae
Sciadopityaceae
Cheirolepidiaceae
Podocarpaceae
Pararaucariaceae
Pinaceae
Utrechtiaceae
Majonicaceae
Emporiaceae
Thucydiaceae
Ferugliocladaceae
Buriadiaceae
Voltziales
Cordaitales
Cycadaceae
Ginkgoaceae
Geinitziaceae
Angiosperms
Ferns
Liverworts
Ord
Sil
Dev
Carbon
Perm
Tri
Jur
Cretaceous
P
N
Q
500
400
300
200
100
0
Ma

Scale-leaved Permo-Carboniferous conifers



Needle-leaved Jurassic type conifer