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

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13 Running title: Fire adaptation in conifers

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19 **Summary**

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

39

40 **Key-words**

41 ancestral state reconstruction, Carboniferous, conifer, fire adaptation, fossil, Permian,
42 progymnosperm, plant–climate interactions, resprouting, seasonality, serotiny

43 **Introduction**

44 Wildfire is an important part of Earth-system processes (Bowman *et al.* 2009). Recent
45 research has identified an important role for fire in shaping the evolution of land plants
46 throughout ‘deep’ time (Bond & Scott 2010) and was a major driver of trait innovations
47 among terrestrial plants during the Upper Cretaceous/Paleocene (Crisp *et al.* 2011; He *et al.*
48 2011, 2012; Lamont *et al.* 2013). The terrestrial flora evolved from small nonvascular plants
49 into woody shrubs and trees with an herbaceous understorey that allowed large wildfires to
50 occur by 395 million years ago (Ma), while charcoal appears in the fossil record from the late
51 Silurian, 420 Ma (Scott & Glasspool 2006). Abundance of charcoal in deposits from the
52 Silurian through to the end of the Permian indicates that the frequency of Paleozoic fire was
53 essentially a function of atmospheric oxygen concentrations (Scott & Glasspool 2006;
54 Glasspool & Scott 2010). With atmospheric oxygen levels rising to 30% in the late Permian,
55 fire frequency progressively increased in many ecosystems (Belcher *et al.* 2013). Thus, fire
56 became widespread during the Lower Mississippian to Pennsylvanian Epochs, 350–300 Ma
57 (Falcon-Lang 2000; Scott 2000).

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

66 environmental evidence on climate and fire in the Carboniferous-Permian, with particular
67 reference to serotiny.

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

87 Many species in the coniferous families Pinaceae (Schwilk & Ackerley 2001; He *et al.* 2012)
88 and Cupressaceae (Ladd *et al.* 2013), and many other families in southern hemisphere
89 Mediterranean-climate shrublands (Lamont *et al.* 1991), store their seeds in woody

90 cones/fruits for a prolonged period, known as serotiny, and only release their seeds in
91 response to fire (pyriscence). Serotiny restricts seedling recruitment to the immediate postfire
92 environment when the opportunities for regeneration are optimal (Lamont *et al.* 1993;
93 Causley *et al.* in press). Removal of the dense canopy by fire also promotes wind dispersal of
94 the released seeds (Lamont 1985; He *et al.* 2004; Merwin *et al.* 2012). Serotiny is generally
95 accepted as an adaptive response to fire (Lamont *et al.* 1991; Lamont & Enright 2000;
96 Midgley & Bond 2013). The origin of serotiny in modern-day Pinaceae has been dated at *c.*
97 89 Ma (He *et al.* 2012). Charred remains of apparently serotinous pine cones have been
98 described from early Cretaceous wildfires (Allen 1998). To confirm a species as serotinous
99 from its fossil cone or fruit is difficult because they are invariably preserved in the open
100 condition, so that it is not possible to determine at what time after maturation dehiscence
101 occurred. The reliable clues for the presence of serotiny are a) woodiness of the supporting
102 structures (e.g. woody rachis) and compact seed bearing structures (e.g. crowded scale
103 complexes in the case of conifers) as an indication of the duration and effectiveness of seed
104 protection, b) winged seeds as an adaptation to wind dispersal in the open postfire
105 environment (Lamont 1985; Hughes *et al.* 1994), and c) whether the vegetation was fire-
106 prone at that time as the necessary seed-release mechanism and ultimate agent of natural
107 selection (Lamont *et al.* 1991).

108 The serotinous structure (fruit, cone or fertile zone) needs to be sufficiently long-lived to
109 store viable seeds for some years and so must become woody. Further, for seeds to survive
110 storage on the plant over several to many years requires their protection against granivores
111 and pathogens. Equally, cones/fruits must provide effective protection against the intense
112 heat from fires. Serotinous seeds should have wings or hairs, or be very small, as adaptations
113 for dispersal by wind (Hughes *et al.* 1994). Air bouyancy of seeds up to 255 mg in weight
114 (larger seeds are not serotinous but bird or squirrel-dispersed) among pines is a function of

115 wing area (Greene & Johnson 1993). Wind dispersal is enhanced in the immediate postfire
116 environment because fire frees the canopy and ground of many obstacles to the passage of
117 wind and seeds (Lamont 1985). In addition, heat generated by the blackened soil surface
118 following fire creates up-drafts and small whirlwinds that may disperse seeds to great
119 distances (He *et al.* 2004). The fitness advantages of serotiny can only be expressed under a
120 crown fire regime that ensures general seed release through heat-opening of the cones/fruits
121 and creates optimal conditions for recruitment (Lamont *et al.* 1991; He *et al.* 2012).

122 In certain circumstances, severe environmental stress (e.g. drought) can lead to patchy pre-
123 senescence death of adult plants, and subsequent erratic seed release through simple
124 desiccation processes. Indeed, Axelrod (1980) considered that summer drought was a more
125 probable agent selecting for serotiny among Californian pines, since he believed that fire was
126 an anthropogenic factor so was too recent to have any evolutionary impact. This ignores the
127 following fire-adapted traits: 1) cones in a few species of *Pinus* and *Cupressus* only open in
128 the presence of fire (Lamont *et al.* 1991), 2) most seeds in strongly serotinous cones of *Pinus*
129 species survive when exposed to temperatures up to 700°C for a few seconds in contrast to
130 unprotected seeds (Beaufait 1960, Linhart 1978), 3) opening of cones of *P. banksiana*
131 requires a minimum temperature of 93°C (Beaufait 1960) as heat melts the resins that bind
132 the apophyses of the cone scales (Ahlgren, 1974), 4) Crossley (1956) interpreted increase in
133 degree of serotiny with age in *P. contorta* as an adaptive response to decreasing opportunity
134 for seedling establishment as the canopy closed over, leaving fire as the key to opening up the
135 vegetation for seedling recruitment. In support, recent research has revealed a strong
136 evolutionary correlation between serotiny and crown fire (He *et al.* 2011, 2012; Lamont *et al.*
137 2013). Drought-induced seed release rarely leads to significant recruitment compared with
138 fire-induced release in modern ecosystems (Lamont *et al.* 1991; He *et al.* 2011, Causley *et al.*
139 in press). Besides, seasonal drought and incidence of fire are inextricably related (Pausas &

140 Keeley 2009): it is unknown for vegetation subject to bouts of dryness not also to be fire-
141 prone.

142 The time of origin of conifers has been estimated at 300–350 Ma in the Carboniferous
143 (Clarke *et al.* 2011; Crisp & Cook 2011; Magallon *et al.* 2013) when fire was widespread
144 (Belcher *et al.* 2010, 2013), supported by a peak in charcoal abundance and record high
145 atmospheric oxygen levels (25–30%, compared with the present 21%, Berner 2009) that
146 would greatly promote combustibility. In fact, at 25% oxygen, plant matter with 72%
147 moisture will burn and at 30% oxygen, even vegetation covered in free water will burn
148 (Watson & Lovelock 2013). This means that even bouts of dryness were irrelevant to the
149 occurrence of fire at that time. Lightning strikes are also likely a function of atmospheric
150 carbon dioxide that was 2–3 times current levels and so ignition sources were abundant
151 (Prince & Rind 1994).

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

162

163 **Materials and methods**

164

165 CONCEPTUAL MODEL OF ESSENTIAL REQUIREMENTS FOR THE EXPRESSION 166 OF SEROTINY

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

180 The conceptual model for serotiny was tested against modern-day ecosystems with four
181 typical data sets, containing species from the world's major fire-prone ecosystems: Pinaceae,
182 with over 210 species, and serotinous species dominating some forests subject to frequent
183 burning in the Northern Hemisphere (He *et al.* 2012); *Banksia sensu stricto* (Proteaceae), with
184 94 species and subspecies most of which are serotinous, and widespread in fire-prone
185 Australia (He *et al.* 2011); *Protea*, a major genus in fire-prone southern Africa (Lamont *et al.*
186 2013); plant species in two 40 × 40 m plots in the fire-prone and species rich southwest

187 Australian flora (Enright *et al.* 2007). Serotiny is particularly well-represented in
188 southwestern Australia with over half of the 1200 currently recognised serotinous species
189 occurring there (Lamont & Enright 2000).

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

$$197 \quad A_1 \times A_2 \times A_3 \times A_4 = S \quad (\text{Equation 1})$$

198 Where: $A_1 = 1$ for the presence of secondary xylem in the rachis as supportive structure,
199 otherwise $A_1 = 0$; $A_2 = 1$ for the presence of protective structure covering seeds, otherwise A_1
200 $= 0$; $A_3 = 1$ for winged, hairy or extremely small seeds, otherwise $A_3 = 0$; $A_4 = 1$ for crown
201 fire regime, otherwise $A_4 = 0$; When $S = 1$, the species should be serotinous; when $S = 0$, the
202 species should be non-serotinous. The fit of the model for each species was evaluated by
203 whether its serotinous state agreed with the outcome of Equation 1. The overall fit of the
204 model for each group of species was evaluated by Chi-square test, and was accepted when P
205 < 0.01 .

206

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

209 Seed cone structure, both supportive and protective, and seed appendages (winged versus
210 non-winged) are conservative across genera (except in *Pinus* that has winged and non-winged
211 seeds, Tomback & Linhart 1990) in extant coniferous families (see data and references in
212 Table S2). Thus, a phylogeny of conifers was constructed at the genus level. Taxon sampling
213 included all genera currently recognized in all gymnosperm families, and 23 species of
214 angiosperm and non-vascular plants as outgroup. The chloroplast DNA sequences for *rbcL*
215 and *matK* in all species were obtained from the NCBI database (Supplementary Information:
216 Table S2). We used BEAST v2.1.0 to estimate phylogeny and divergence times under a strict
217 clock model (Drummond *et al.* 2006). We used a Yule prior for rates of cladogenesis and ran
218 analyses of 10 million generations sampling every 1000 generations, with a 2.5 million
219 generations burn-in. We set 12 calibration points based on well-known fossil records that
220 have been critically evaluated in other studies (Clarke *et al.* 2011; Crisp & Cook 2011;
221 Magallon *et al.* 2013). The emergence of land plants was set at 460–472 Ma (Edward *et al.*
222 2014). The majority of the priors were set to lognormal as this distribution allows assignment
223 of the highest point probability for the node age that must be older than the oldest fossil (Ho
224 & Philips 2009).

225 Data for seed cone structure and seed appendages in each genus were collated from the
226 literature. ‘Presence of supportive structure’ was defined as cones taking >1 year to mature
227 and showing evidence of secondary xylem in rachis. ‘Presence of protective structure’ was
228 defined as existence of compact cone with bracts/scale covering seeds. Rothwell *et al.* (2011)
229 concluded that the overall evidence from both extant and fossil conifers strongly supports the
230 hypothesis of strong homology among seed cones. We assumed that these traits have
231 remained unchanged since the origin of that genus. In *Pinus*, 20 species have non-winged,
232 nut-like seeds (Tomback & Linhart 1990). The ancestral state of winged seeds in *Pinus* was
233 reconstructed using the likelihood approach in Mesquite (Maddison & Maddison 2007) and

234 accepted as the diagnostic state for *Pinus* when placed in the overall conifer phylogeny
235 (Supplementary Information: Fig. S1). Species in Cycadaceae and most Gnetidae have seeds
236 with a fleshy sarcotesta that is not homologous with the fleshy attachment (peduncle, aril) in
237 some coniferous families. Seed traits for the Cycadaceae and Gnetidae were therefore
238 assigned a different state from other conifers having seeds with a fleshy appendage. We used
239 MultiState in BayesTraits (Pagel & Meade 2006) to determine the ancestral states of
240 supportive structure, protective structure, and seed wingness. The MCMC method was used
241 to calculate the probability of the ancestral states at the stem of conifers in the gymnosperm
242 phylogeny generated from BEAST.

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

251

252 PALEOZOIC FIRE BEHAVIOUR

253 As serotiny is typically linked to crown-fire regimes, it was desirable to assess the likely fire
254 behaviour that the earliest conifers may have displayed if they were ignited. The Voltzian
255 conifers had a distinctive scale-leaf morphology. We performed flammability experiments to
256 test whether early scale-leaved conifers would have fuelled fires of sufficient intensity to heat
257 serotinous cones sufficiently to release their seeds. We tested the flammability of four

258 scale-leaved species: *Athrotaxis cupressoides* (Cupressaceae), *A. laxifolia* (Cupressaceae),
259 *Cryptomeria japonica* (Cupressaceae), *Dacrydium cupressinum* (Podocarpaceae), and four
260 needle-leaved species, *Abies recurvata* (Pinaceae), *Tsuga heterophylla* (Pinaceae), *Sequoia*
261 *sempervirens* (Cupressaceae), *Taxodium distichum* (Cupressaceae), that are more
262 characteristic of those that evolved later in the Mesozoic. The samples were dried in an oven
263 for 6 days at 50°C and then placed in a metal mesh basket (368 cm³ in volume) (Schemel *et*
264 *al.* 2008). We tested equal volumes of plant material in each case and tested three samples of
265 each plant type. We used oxygen depletion calorimetry to measure the heat release profiles of
266 each species (following ASTM E1354; Tewarson 2002). Each basket was placed in the cone
267 calorimeter and subjected to a heat flux of 30 kWm⁻² [within the typical range for
268 flammability testing (Tewarson 2002)]. A spark pilot ignition was positioned above the
269 sample and turned on at the same time as the heat source. On heating, the samples release
270 flammable gases that are ignited by the spark leading to flaming combustion of the samples.
271 The amount of energy released from each sample was measured as a function of time.

272

273 **Results**

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

281 *Banksia* ss included 94 species and subspecies that all have woody fruits with covered seeds
282 and winged seeds. There are 83 taxa in habitats with a crown fire regime, and all are
283 serotinous. Sixty-nine of the 87 *Protea* species are serotinous and occur in habitats
284 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
285 being non-serotinous ($S = 0$ expected). The remaining 17 species are non-serotinous and
286 occur in savanna grasslands that usually only experience surface fires. In the two 40×40 m
287 plots in SW Australia where the shrubland is subjected to crown fire, 153 species (75 genera
288 in 28 families) were recorded, with 43 species either having winged/hairy seeds or extremely
289 light seeds (< 1 mm) and 42 being serotinous. In all four datasets, Chi-Squared tests supported
290 a highly significant overall fit of the serotiny model with $P < 0.0001$ (Table 1).

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

298 The early conifers had a woody supportive rachis, and a compact cone with bracts/scales
299 covering the seeds that were winged, all with a posterior probability of $P > 0.90$, implying
300 that the three traits likely originated with the first appearance of conifers 332 Ma in the Mid-
301 Mississippian Epoch of the Carboniferous. Three extinct conifers and five families of
302 Voltziales with abundant fossil records from the Carboniferous through to the Cretaceous all
303 had fossils showing the existence of secondary xylem in the rachis and a compact-cone
304 structure. Adding these families to the coniferous family phylogeny further supported the

305 ancestral state of seed-bearing cones. Extinct taxa that appeared between the Carboniferous to
306 Permian, mainly Voltziales, showed evidence of seed wings, clearly suggesting that the early
307 conifers had winged seeds. Interestingly, there was little evidence of the presence of a seed
308 wing in fossil coniferous families existing in the Jurassic to early Cretaceous (Table 1, Fig. 3).
309 Ancestral-type, scale-leaved morphologies have higher peak heat release rates than a 'modern',
310 needle-leaved conifers (Fig. 4). The scale-leaf morphologies burnt with a rapid release of heat
311 that typically also quickly consumed the fuel, while the needle-leaf morphologies either
312 generated a burn of sustained duration with slow heat release or a low amount of heat release
313 compared with the scale-leaved conifers (Fig. 4). Rapid heat release rates are a consequence
314 of rapid oxidation of large volumes of pyrolysate gases released upon heating, which lead to
315 large flames. Flame height is known to be directly related to heat release rate (Quintiere 1998)
316 including in conifer litter fuels (e.g. see Table 1 in Morandini *et al.* 2013). Our experiments
317 therefore indicate that flame heights of the ancestral species would have been greater, and
318 heat from fires in dense litter mats would increase the chance of crown ignition and fire
319 spreading to the seed-bearing cones.

320

321 **Discussion**

322

323 REPRODUCTIVE STRUCTURES FAVOURING SEROTINY

324 The stem age of conifers was dated at 332 Ma (311–346 Ma, 95% HPD) in the Mississippian
325 Epoch of the Carboniferous. Bayesian MCMC ancestral-state reconstruction showed that the
326 common ancestor of modern conifers had 1) a woody rachis/stem that 2) supported a compact
327 cone/zone with bracts/scales covering the seeds which 3) were winged, all with a posterior

328 probability greater than 0.90, and together fulfilling the requirements for serotiny (Figs. 1,2).
329 Does the available fossil evidence support the presence of these structural features in the
330 Carboniferous? Seeds of most ‘transition’ conifers (e.g. Emporiaceae, Voltziales, dated at
331 305 Ma, Pennsylvanian, Upper Carboniferous) were winged and held in compact cones with
332 a woody axis bearing bark and megasporophylls with secondary xylem (Herandez-Castillo *et*
333 *al.* 2009). These authors concluded that “most ancient conifers already possessed a similar
334 reproductive biology...to that of extant conifers”, including seed dormancy (a necessary
335 feature of serotinous species aligned with cone woodiness). Fossil taxa of six of seven
336 families in the Voltziales showed the existence of secondary xylem in the rachis (or fertile
337 zone) and a compact cone with covered seeds (Table S2, Fig. 3). Interestingly, seed wings
338 were evident in fossil taxa present during the Carboniferous to Permian, while evidence for
339 seed wings is absent in fossil taxa present since the Triassic when fire activity has been
340 hypothesised as weaker then due to lower atmospheric oxygen concentrations (Glasspool &
341 Scott 2010).

342 There is also other fossil evidence that supports our findings that early conifers were
343 serotinous. Serbet *et al.* (2010) noted that the bracts of Voltzialean species were typically
344 held at an angle of 30° to the rachis and that seeds were attached to the lower half of the
345 short shoot (Looy & Stevensen 2014) – these would have provided protection against fire
346 heat of the type generated by scale-leaved conifer morphotypes. Also the fertile zones
347 (before terminal cones evolved) were much less leafy than the rest of the branch and would
348 not have burnt as intensely, perhaps exposed to heat but not flames. Whether this protection
349 was adequate is unclear, as no charred cones have been recorded in the literature at this
350 time. However, while many isolated fossil seeds have been documented in the
351 Pennsylvanian strata, none shows any fire damage, indicating that they were dispersed
352 postfire (or in the absence of fire) consistent with their serotinous nature. Further, our

353 simulation of Permo-Carboniferous fire suggests that scale-leaved conifers generated a
354 short but intense heat pulse (see below), implying that the time seed structures were
355 exposed to fire would be brief.

356

357 THE ROLE OF SEASONALITY, HIGH OXYGEN AND FIRE

358 The transition conifers typically occupied the drier, nutrient-impooverished, coastal plains
359 under seasonal climates that favoured woodland rather than forest (DiMichele *et al.* 2001),
360 conditions that would have promoted serotiny in the presence of fire (Lamont *et al.* 1991).
361 They were small, spreading trees with scale-like leaves, dwarfed in stature and leaf
362 morphology compared with the related pre-conifer, Cordaitales (Galtier *et al.* 1992, Mapes &
363 Rothwell 2003, Hernandez-Castillo *et al.* 2003, 2009b). Thus, hot, rapidly-burning surface-
364 initiated fires would have produced flames that could reach their crowns. Indeed,
365 Carboniferous laminated deposits record fire at intervals of about 35 years in proconifer
366 communities (Beerling *et al.* 1998; Falcon-Lang 2000). Nevertheless, the initial evolutionary
367 trigger for seed release might have been severe drought (in association with high atmospheric
368 temperatures in some cases, Zambito & Bennison 2013) that would kill the parent plant and
369 simple desiccation processes would result in seed release. But this would only create limited
370 opportunities for seedling recruitment beneath the dead parent plant (through gap creation).
371 High oxygen levels meant that the vegetation was combustible at any time of the year
372 (Watson & Lovelock 2013) so the timing of fire was tied to the incidence of lightning.
373 Certainly, there is evidence that the pro-conifer plants were alive at the time of fire (Scott &
374 Chaloner 1983). Not only would fire create much larger gaps than isolated plant deaths but,
375 in addition, greatly improve the availability of water, light and nutrients at ground level. Thus,
376 the selective pressure from fire would have far outstripped that from drought death. Since

377 strong seasonality in the presence of frequent lightning and high oxygen levels promote the
378 incidence and intensity of fire, fires would quickly become both the main source of
379 widespread parent death and the trigger for seed release. Thus, germination of seeds would
380 increasingly be tied to the wet season following fire (Looy & Stevensen 2014) and promote
381 structural shifts towards serotiny that ensured both protection of seeds from the elements,
382 including fire heat, and the delay of seed release until creation of the optimal seed bed for
383 germination and seedling establishment. In addition, the general removal of above-ground
384 plant mass by fire would promote postfire winds, and therefore seed dispersal by wind
385 (Lamont 1985). Thus, Rothwell *et al.* (2005) concluded that “the unexpected species richness
386 of Voltzialean conifers...resulted from the evolutionary ecology of unstable habitats”.
387 Increasing bouts of aridity and recurrent fire were the basis of this instability.

388 It is interesting to relate this interpretation to the six evolutionary scenarios proposed by
389 Keeley *et al.* (2011) for adaptations versus exaptations. For serotiny to be an adaptation to
390 drought but exaptation to fire would require 1) drought to precede fire as an evolutionary
391 force (Hopper 2009), and 2) adaptive traits to drought and fire to be identical. Neither
392 requirement is satisfied here. Fire and drought are only decoupled in semi-arid/arid systems
393 and rock outcrops/monadnocks where the vegetation is too scattered to propagate fire. In
394 woodlands, savannas and shrublands (as recorded in the Carboniferous/Permian), periods of
395 drought create dry fuel adequate to support widespread fire. Fire therefore does not follow
396 drought at some future time but is concurrent with it. In the high oxygen/high lightning
397 environment of the Carboniferous/Permian, even saturated live fuel would ignite (Watson &
398 Lovelock 2013), raising the possibility that fire even preceded drought as the prime selective
399 force favouring the evolution of serotiny. The ecology of seeds adapted to drought and fire
400 are different: a) seeds released in response to fire (pyriscence) have to be insulated from fire
401 heat but drought-released seeds (necriscence) do not (Lamont 1991); b) drought-released

402 seeds encounter a hostile seed bed: there may be a litter layer to penetrate before mineral soil
403 is reached, much of the surrounding vegetation remains alive to actively compete for
404 resources with the germinants, seeds are only released when the parent dies under the most
405 extreme of droughts so that seeds need to germinate and establish in that same extreme
406 drought year (Keeley *et al.* 2011), fire-released seeds have to contend with none of these
407 things (Cauley *et al.* in press); c) fire-released seeds get covered in soil and charred litter,
408 drought-released seeds do not (Lamont *et al.* 1993). In fact, Carboniferous conifers are
409 believed to have produced dense, highly flammable, litter mats (Looy 2013) that we show are
410 capable of burning at high intensity (Fig. 4). Thus, we view serotiny evolving through the
411 Paleozoic as essentially a response to intensifying fire (Scenario 5 of Keeley *et al.* 2011).
412 Nevertheless, we acknowledge the fluctuating fire regimes through the subsequent
413 Mesozoic/Cenozoic that would lead to selection in other directions (Lamont *et al.* 2013) so
414 that an overall sinuous response curve (their scenario 4b) is more accurate.

415

416 THE IMPORTANCE OF FIRE BEHAVIOUR

417 Serotiny is only expected to evolve in ecosystems with intense, crown-fire regimes. Our
418 analysis suggests that serotinous cones may have first appeared during the earliest period of
419 major fire episodes recorded (350–250 Ma). Estimates show that oxygen levels had risen to
420 as high as 30% at this time (Berner 2009; Lenton 2013) and increased oxygen levels greatly
421 enhance fire (Belcher *et al.* 2010). At such levels of oxygen, ignition potential is doubled
422 compared with ambient levels (Watson & Lovelock 2013) and fire spread rates is 1.5 times
423 faster (Lenton 2013). Fire behavior has also been shown to link to leaf traits (de Magalhaes
424 & Schwilk 2012). The earliest Voltzialean conifers had a distinctive, scale-leaved
425 morphology. Our flammability experiments on conifers of analogous morphology indicate

426 that the scale-leaved branches of early conifers were capable of carrying highly intense fires
427 either in litter or within the canopy itself. This suggests that fires in early conifer-dominated
428 ecosystems were likely quick-burning 'flashy' fires. Whether a surface fire will reach the
429 tree crown or ignite the crown relies strongly on fire intensity (Davies 2013). Because the
430 scale-leaved morphologies supported rapid energy release and therefore burned with high
431 peak fire intensities, thus generating long flame lengths, this would be more likely to
432 enhance drying and crown scorch, promoting ignition of live canopy fuels. Therefore, such
433 fires would likely have delivered a high heat flux to the serotinous cones in the crown,
434 promoting seed release and ultimately successful seedling recruitment.

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

443

444 OTHER ANCIENT FIRE-RELATED TRAITS

445 Plants in modern-day, fire-prone ecosystems possess a suite of adaptive traits that includes
446 serotiny, postfire resprouting, thick bark, branch-shedding, germination in response to heat
447 and smoke, and fire-stimulated flowering (Keeley *et al.* 2011; Lamont *et al.* 2011a,b). Apart
448 from serotiny, other fire-adapted traits in the early conifers may also have evolved as a
449 response to recurrent fire in the Carboniferous. Shedding dead branches from the crown is a

450 fire adaptation as it reduces plant flammability (Keeley & Zedler 1998; Schwilk & Ackerly
451 2001). Orderly branch abscission and healing has been reported in Voltzialean conifers,
452 which has been interpreted as an adaptation to wildfire (Looy 2013; Falcon-Lang 2014).
453 Robinson (1989) noted palaeobotanical evidence for the high frequency of fire-resistant
454 plants then, e.g. Carboniferous swamp floras invested heavily in the production of thick bark
455 and belowground storage tissues that may have functioned to protect meristematic tissues
456 from fire and thus enable resprouting to occur. Thick bark (~15 mm, sufficient to insulate
457 against heat during mild fires) was recorded in the pro-conifer, *Protopitys buchiana*, at an
458 inferred age of 359–347 Ma (Early Mississippian) in northeast Queensland, Australia
459 (Decombeix 2013). Resprouting is increasingly being shown to be an adaptive response to
460 fire (Lamont *et al.* 2011) and is relatively common among modern conifers. There are at least
461 94 coniferous species in 41 genera among all six extant families that have resprouting
462 capability after disturbance (Supplementary Information, Table S3). The widespread
463 taxonomic distribution of resprouting ability among modern conifers implies it must have had
464 an early origin in the conifer phylogeny, consistent with the selective pressure from recurrent
465 fires in the Paleozoic Era.

466

467 FLUCTUATING LEVELS OF SEROTINY THROUGH TIME

468 Biogeochemical models suggest that atmospheric oxygen levels may have been lower than
469 that of the present day at 250–240 Ma (Bergman *et al.* 2004; Berner 2009) and is supported
470 by an apparent gap in the charcoal record at this time (Belcher *et al.* 2010). The probability
471 of fires is also estimated to have been lower than the present day at ~240–150 Ma based on
472 ignition probability and the ability of fires to spread under the estimated levels of atmospheric
473 oxygen at this time (Belcher *et al.* 2010). Charred fossils do occur throughout the mid-late

474 Triassic and Jurassic but typically in lower abundances than in rocks from other geological
475 periods (Glasspool & Scott 2010).

476

477 When the required selective agent (i.e. crown fire) was rare, conifers in the Jurassic through
478 to the Lower Cretaceous were less likely to have remained strongly serotinous. However,
479 Leslie (2011) noted that species in the Araucariaceae, Cupressaceae and Pinaceae developed
480 increasingly robust, tightly packed cones with woody bracts/scales from the Jurassic. This
481 may indicate the rising importance of seed protection due to the evolution and radiation of
482 bird and mammal granivores in the Cenozoic (Leslie 2011). Seed wings that promote
483 dispersal away from the parent, so avoiding being taken by seed predators if they remained in
484 the cone, are also adaptive in fire-free environments. At the same time, heavier and arillate
485 (non-winged) seeds evolved as a response to the diversification of seed dispersal vectors
486 among birds and mammals, absent in the Paleozoic. As a consequence, both supportive and
487 protective structures of seed-bearing cones and winged seeds continued to enhance fitness
488 under a new selective regime where fire was less important.

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

498 Seed cones of species in the Araucariaceae usually have a woody rachis with a compact cone
499 covering the seeds and winged seeds, though the cones shatter at maturity. It is likely that the
500 ancestral araucarias lost their serotinous condition when their habitats become relatively fire-
501 free during extremely wet periods (e.g. Paleogene) or they contracted into fire-free habitats
502 where they currently remain. Araucarias are usually emergent in their vegetation types and
503 more likely to escape crown fires in the lower strata while wind dispersal of seeds would still
504 be advantageous. Nevertheless, some araucarias in Chile, Australia and New Caledonia may
505 be killed by fire when release of mature seeds still stored in cones is promoted through cone
506 death (Burns 1993; Enright 1995; Perry & Enright 2002), which can be viewed as a form of
507 proserotiny and points to the potential for evolution in either direction.

508

509 Robson *et al.* (2015) have described the fluctuating, but overall, marked drop in fire-derived
510 inertinite records for mires in Germany during the early Eocene (40–55 M) compared with
511 the Paleocene during one of the wettest periods known. This again raises the question of the
512 fate of fire-dependent species at such times. They considered a major explanation was that
513 oxygen levels had for the first time fallen to current levels so that fires were now controlled
514 by climate, including greater seasonality elsewhere. Other models (e.g. COPSE, Belcher *et al.*
515 2013) show oxygen levels were still above ambient (e.g. 24%) while carbon dioxide levels,
516 temperatures and burn probabilities were still much higher than currently (He *et al.* 2012).
517 Such inertinite levels have remained low ever since (He *et al.* 2012) despite the fact that most
518 conifer forests are currently fire-prone, highlighting the inherent taphonomic bias against the
519 fossil deposits in recording dryland fire at such wet sites. Further, there is corroborating
520 evidence of fire-prone floras and species existing or even evolving among pines (Stockey,
521 1984: subgenus *Pinus* for which fire-adapted traits are diagnostic, He *et al.* 2012) and other

522 plant groups elsewhere (Itzstein-Davey, 2004; Lamont & Downes, 2011) during the early-
523 mid Eocene. There is strong evidence that many parts of the world received only moderate
524 and highly seasonal rainfall at that time (Macphail 2007) and there is no reason to believe that
525 the vast area currently covered by pine forests was any different. Keeley (2012) has
526 postulated that conifers migrated to drier, and hence more fire-prone, uplands at that time.
527 We conclude that conifers, and other fire-adapted clades, were still subjected to fire during
528 the early Eocene, and that serotiny would have remained adaptive. Overall, the evolution of
529 woody cones and winged seeds in conifers has been shaped by multiple forces peaking at
530 different times throughout the history of these traits (Keeley *et al.* 2011).

531

532 CONCLUSIONS

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

544

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553

554 **Data accessibility**

555 Data for Figure 2 and Figure 3 are available in Supplementary Information Table S1 and
556 Table S2.

557

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781 **SUPPORTING INFORMATION**

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

783 **Table S1** Species, Genbank numbers and trait data

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

786 **Table S3** List of species with resprouting capacity among gymnosperms

787 **Figure S1** Reconstructing the ancestral state of seed wingness in *Pinus*

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791 missing files) should be addressed to the authors.

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

794 **Figure captions**

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

796 Fig. 2. Ancestral state reconstruction of existence of woody rachis, compact cone covering

797 seeds and seed wingness among conifers. Arau: Araucariaceae; Tax: Taxaceae; Angio:

798 Angiosperm; Non-V: non-vascular plants, G: Gnetales; *: Sciadopityaceae. Green bar: 95%

799 highest posterior density; red line: lineages with woody rachis, compact cone and winged

800 seeds; blue line: lineages lacking woody rachis, compact cone and/or winged seeds. Black

801 line: not considered.

802 Fig. 3. Ancestral state reconstruction of woodiness of seed cone and seed wingness in conifer

803 families including extinct families (in italics). Red line: lineages with woody rachis, compact

804 cone and winged seeds; blue line: lineages absent with woody rachis, compact cone covering

805 seeds and winged seeds; black line: not considered. Question mark indicates ancient state

806 was equivocal.

807 Fig. 4. Heat release rate (HRR) curves for extant conifers with analogous/non-analogous

808 Palaeozoic leaf morphology. X axes are 700 seconds; Y axes are 10 kW. The area under the

809 curve represents the total amount of heat released and relates to the amount of burnable fuel.

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

Data set	Total taxa (serotinous taxa)	Model fits	Model not fit	Chi-square test (<i>P</i>)
Pinaceae	202 (25)	197	5	<0.0001
<i>Banksia</i>	93 (83)	93	0	<0.0001
<i>Protea</i>	87 (69)	86	1	<0.0001
Shrublands	153 (42)	152	1	<0.0001

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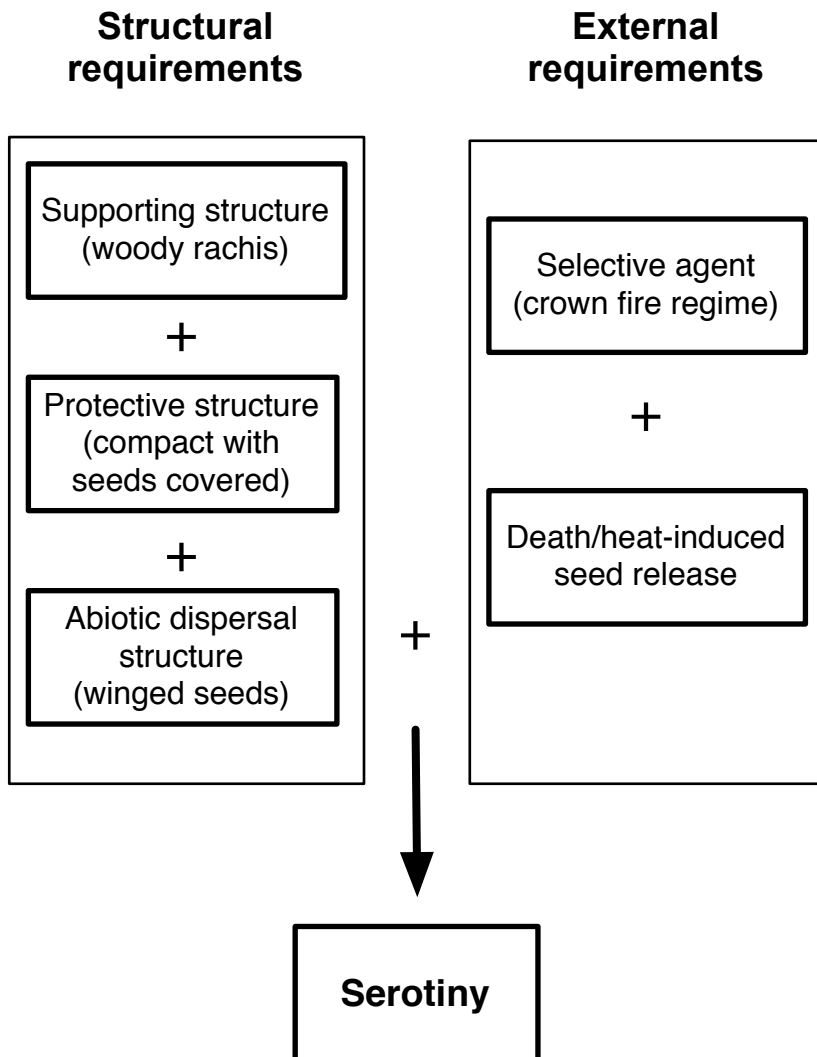
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831 Figure 1

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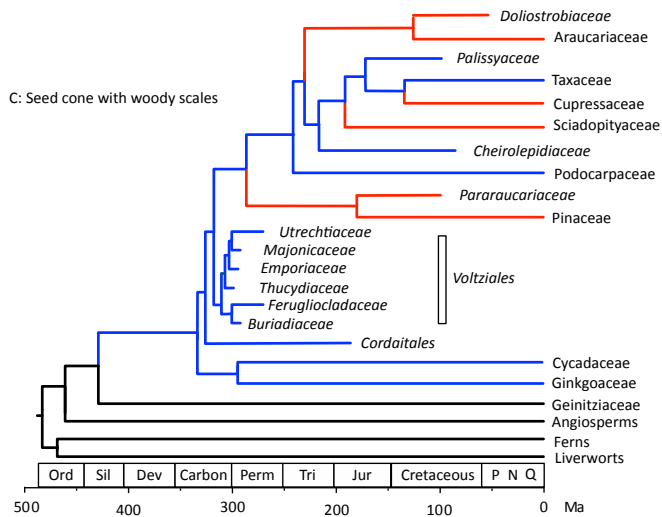
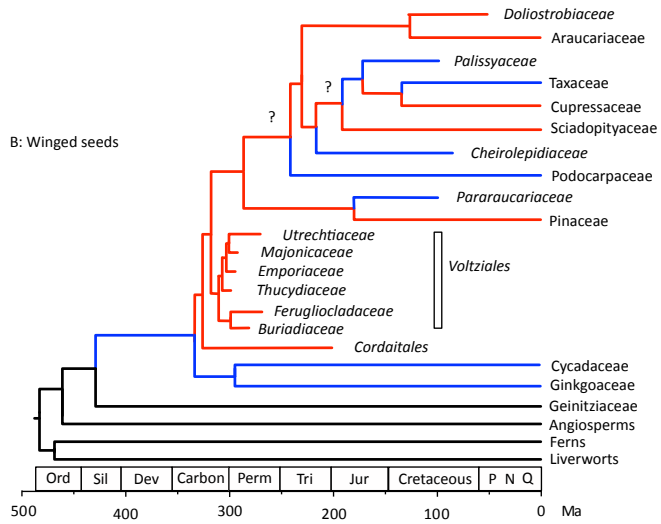
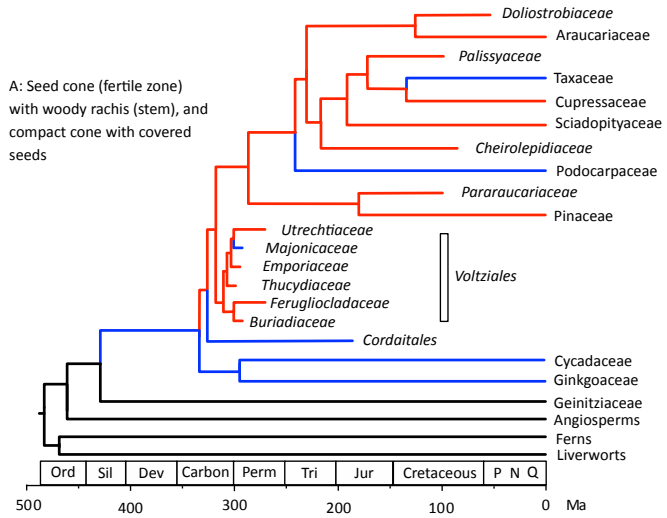
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839 Figure 2

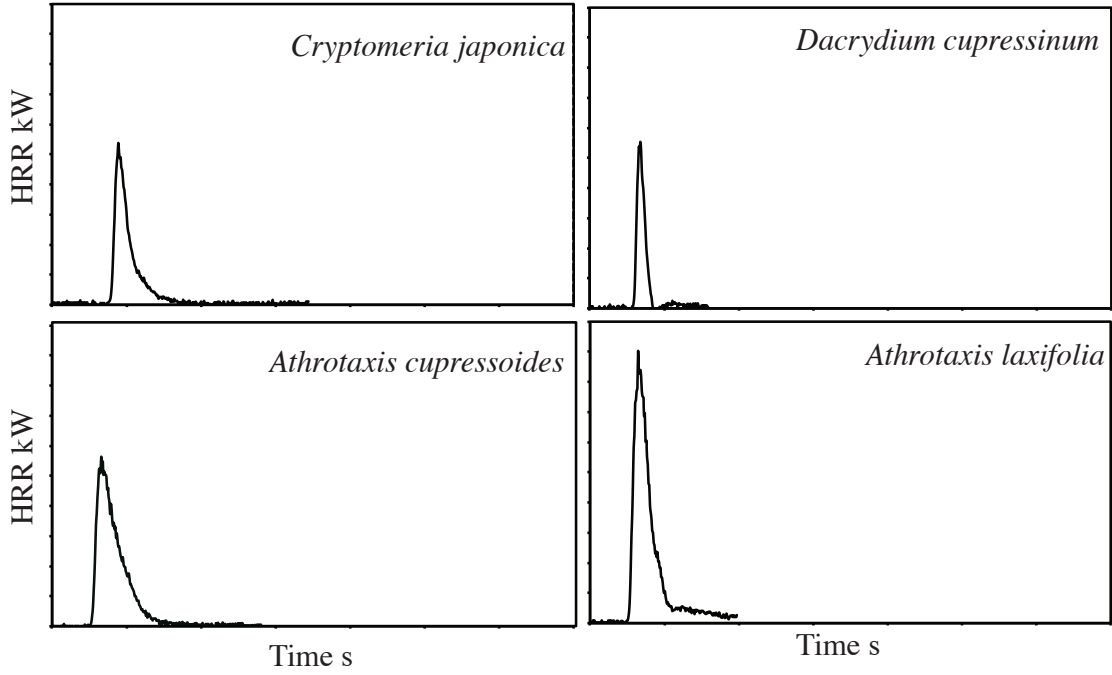


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842 Figure 3

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Scale-leaved Permo-Carboniferous conifers



Needle-leaved Jurassic type conifer

