

**Trends box:**

- Ancestral trait reconstruction using dated molecular phylogenies is revolutionizing our understanding of fire-directed evolution among plants.
- Ancestral fire-prone lineages may also be identified on molecular phylogenies using reconstruction techniques.
- Exaptations and fire-mimicking (multi-agent) selection are alternative explanations of apparent fire-adapted traits in fire-prone regions that require selection via drought or non-unique components of fire to precede selection by fire.
- Review of fire-adapted trait origins among six plant phylogenies shows that fire-proneness of their habitats precedes, or rarely coincides with, initiation of the trait. Drought as a selective agent arrived later.
- Fire-mimicking selection and associated exaptations have no place in explaining the origin of fire-adapted traits.
- Smoke-stimulated germination among plants in non-fire-prone habitats is explainable via a dormant gene mechanism inherited from its fire-prone past that is not normally expressed and is not currently under selection.
- Evolution of clades with fire-adapted traits has occurred in concert with fire over at least the last 100 million years, with novel traits characterizing the Cretaceous and lineages with conserved traits escalating in the Cenozoic.

# 1 **Fire-proneness as prerequisite for the evolution of fire-adapted traits**

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## 9 **Keywords:**

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11 Exaptation, fire adaptation, flowering, germination, selection, serotiny, smoke

12

## 13 **Abstract**

14

15 Fire as a major evolutionary force has been disputed because it is considered to lack  
16 supporting evidence. If a trait has evolved in response to selection by fire then the plant's  
17 environment must have been fire-prone prior to the appearance of that trait. Using  
18 outcomes of trait assignments on molecular phylogenies for fire-stimulated flowering,  
19 seed-release and germination, in this Opinion article we show that fire-proneness  
20 precedes, or rarely coincides with, the evolution of these fire-adapted traits. In addition,  
21 fire remains central to understanding germination promoted by smoke among species  
22 occurring in non-fire-prone environments because of the historical association of their  
23 clade with fire. Fire-mimicking selection and associated exaptations have no place in  
24 understanding the evolution of fire-adapted traits as we find no support for any reversal  
25 in the fire-trait sequence through time.

26

## 27 **Fire and plant trait evolution**

28

29 Plant properties that are characteristically associated with fire-prone environments are  
30 called fire-related traits [1], whereas **fire-adapted traits** are not only associated with fire

1 or one of its byproducts (heat, smoke, combustion products, nutrient release, increased  
2 diurnal temperature fluctuations) but have also been shown to increase plant fitness as a  
3 consequence [2]. A species is regarded as **fire-prone** if the habitat in which it occurs  
4 ignites at least once during its lifespan: it is an environmental trait of the species of  
5 interest. The concept can be extended to lineages and clades if it can be shown that their  
6 members also have a history of exposure to fire. If a trait has evolved in response to  
7 selection by fire then it follows that the plant's environment must have been fire-prone  
8 *prior to* the appearance of that trait as the context in which selection occurs. An  
9 alternative is that the trait is an adaptive response to a limiting factor that is unrelated to  
10 fire but has elements in common with it, giving the erroneous impression that it is a fire-  
11 adapted trait. This can be viewed as the outcome of multi-agent selection, or, in the  
12 present context, **fire-mimicking selection** (Fig. 1). Examples include ethylene in smoke  
13 that may induce postfire flowering [3], but ethylene is also released by decomposing plant  
14 matter that could be an alternative source; and nitrate salts that promote germination  
15 that are released from litter and vegetation in response to ignition, but so too following  
16 soil disturbance and exposure of soil organic matter to sunlight after gap creation [4].

17 This is to be distinguished from **convergent evolution** where the same selective  
18 agent acts on different gene processes that control evolution of the same or equivalent  
19 trait in unrelated clades. Examples of convergent evolution include fire-stimulated  
20 germination in disparate clades that is promoted by different chemical components of  
21 smoke (e.g. karrikins, ethylene, glyceronitrile, nitrogen dioxide) that act independently on  
22 different parts of the genetic pathway that leads to postfire germination [5]. As a result of  
23 fire-mimicking selection, a trait may evolve in a non-fire-prone environment in response  
24 to a non-fire-related limiting factor but be expressed, and have equal fitness benefit in, the  
25 new fire-prone environment *without undergoing additional selection*. This conserved  
26 adapted response to a prior agent of selection is called an **exaptation**; it is a fire-exapted  
27 trait (Fig. 1). If fire-mimicking selection and associated exaptations apply then fire-  
28 proneness will *postdate* the appearance of the trait in geological time. Our objective in this  
29 Opinion article is to document which sequence of events has actually occurred  
30 historically: are fire-adapted traits true adaptations or merely exaptations?

31 Fire-related traits have sometimes been viewed as adaptations to drought but  
32 exaptations to fire in the belief that the former preceded the latter historically [2]. There is  
33 a parallel here with fire-mimicking selection, as the dryness and heat associated with

1 summer drought can be viewed as a mild form of the drying and heating effect of fire.  
2 However, the biological and fitness effects of fire and drought on biota can never be  
3 considered synonymous, as required for the identification of exaptations. Drought-prone  
4 environments are inextricably associated with fire in current ecosystems but not  
5 necessarily historically, when high atmospheric oxygen levels often dictated flammability  
6 of plant material [2]. While this makes determining which constraint is ancestral a difficult  
7 task, such a distinction is essential in the present context. Organisms may appear to  
8 tolerate intensities of constraints that they have not experienced in their past (**pre-**  
9 **adaptation**) but this might merely reflect ignorance about their evolutionary history.  
10 Besides, as soon as the trait appears it will be subject to selection by the more constraining  
11 agent, leading to further adaptation and making such labels as exaptation and pre-  
12 adaptation redundant.

13         Recently, Hopper et al. [6] criticized current fire ecology studies conducted within  
14 the 'adaptationist paradigm' noting that "we still lack the evidence...for a long history of  
15 fire as an evolutionary force at local to broad spatial scales". They consider that  
16 "hypotheses on fire regimes proposed by authors such as Keeley et al. [7], Mucina and  
17 Wardell-Johnson [8] and Groom and Lamont [9] remain hypothetical...", describing them as  
18 "just-so stories". While Carpenter et al. [10] record abundant charcoal during the late  
19 Cretaceous in Central Australia that supports the hypothesis, they too remain dubious  
20 about its evolutionary significance in the absence of tangible fossil evidence. For reasons  
21 unknown, others simply ignore the prevailing or past fire regime as a possible alternative  
22 explanation for the evolution of the fire-related traits they study and opt instead for a  
23 climatic [11] or biogeographical [12] explanation.

24         Thus, if fire is indeed a significant evolutionary force, then the first requirement is  
25 to show that fire-proneness precedes (or at least coincides with) the appearance of the  
26 purported fire-adapted trait. First, we consider what methodologies are available to  
27 determine sequences of these two events and how the approach has been verified. We  
28 then examine the evolution of three fire-adapted, reproductive traits: fire-stimulated  
29 flowering, seed release and germination, as revealed by a selection of molecular  
30 phylogenies described in the literature to determine if fire or the trait appeared first. We  
31 then collate the records for 134 reproductive trait/fire-prone pairs from over 40  
32 papers/studies to reveal overall patterns through time. Finally, we attempt to interpret

1 two notable examples of fire-related traits from the literature currently occurring in non-  
2 fire-prone environments in terms of a possible previous history of fire-proneness.

3

#### 4 **Methodology and corroboration**

5

6 An understanding of the evolution of fire-adapted traits requires knowledge of fire-  
7 proneness of the supporting vegetation and presence of the fire-adapted trait of interest  
8 over geological time. The existence of charcoal (fusain) is a proxy for fire but has often  
9 been overlooked in the fossil record where pollen identification is the usual focus of the  
10 study. In claiming to identify a fire scar for the first time among fossil trees, Byers et al.  
11 [13] was surprised that this had not been observed before and suggested that disciplinary  
12 ‘tunnel-vision’ was the reason. Where possible, we used fossil charcoal to indicate the  
13 antiquity of fire-proneness but it has four drawbacks even when researchers are looking  
14 for it: a) charcoal is rarely preserved in strata because fires occur remote from wetlands  
15 where preservation is favored, b) fossil records are just a ‘snapshot’ in time, c) there is no  
16 guarantee that the lineages of interest actually co-occurred with sources of charcoal  
17 recorded in the general area or even at the same site, and d) the mere presence of  
18 charcoal gives no indication of the associated fire regime nor whether it was sufficient to  
19 have an evolutionary impact. Identifying fire-related traits is even more problematic:  
20 these are rarely preserved in the fossil record, especially phenological traits as examined  
21 here. Thus, cones are invariably preserved in the ‘open’ condition [2] with no clue as to  
22 the circumstances causing death of the supporting stem leading to seed release (drought,  
23 senescence, physical damage, fire?). The interannual timing of flowering with respect to  
24 fire-stimulated flowering cannot be gauged from the pollen record as it is too coarse.  
25 Regarding soil-stored seeds, Baskin et al. [14] note: “we know of no study that shows seed  
26 coat anatomy of fossil seeds of families whose extant members have physical dormancy”.

27 In view of the limited fossil evidence for the presence of fire or fire-related traits, a  
28 major breakthrough in determining the origin of fire-adapted traits has occurred over the  
29 last five years. This involves the use of molecular phylogenies that have a time dimension  
30 added to all internal nodes of the phylogeny (chronogram) and for which the state of the  
31 fire-related traits (present/absent) of their component species or genera is known. The  
32 probability of a given trait existing at a given node in the phylogeny is determined by  
33 comparing with pre-existing probabilities elsewhere in its lineage and adjacent lineages

1 (by Bayesian Markov-Chain Monte-Carlo ancestral-state reconstruction methods) [2,  
2 15–18]. While it is accepted that the trait may vary along a given branch of the tree trait  
3 probabilities can only be applied to the nodes. Another limitation of the approach is that it  
4 assumes the extant species are genetically (and thus trait) representative of all species that  
5 have ever existed in the clade, i.e. there have been no extinctions of major lineages that  
6 might lead to bias in the assignments. Thus, traits are traced back in time until the  
7 probability of their existence is no longer significant and that date is treated as its origin.

8 Assignment of fire-proneness is based on whether or not each species in the  
9 phylogeny is subjected to recurrent fire within its lifespan based on knowledge of its  
10 ecology. Species that occur in, say, rainforest, succulent or alpine vegetation are allocated  
11 to the non-fire-prone category. Fire-proneness is thus treated as an independent  
12 environmental trait assigned to each taxon and it too can be traced back in time through  
13 the phylogeny until the probability that the lineage is fire-prone falls below a critical level  
14 when it is no longer considered fire-prone compared with extant fire-prone taxa [2,15–18].  
15 Alternatively, fire-proneness can be treated on a spatial basis that spreads across the  
16 phylogeny from one lineage to another (areagram as used in historical biogeography). See  
17 Fig. S1 for how knowledge of the fire-proneness of each component species of *Pinus* is used  
18 to determine the fire-prone history of the entire pine phylogeny using these techniques.  
19 Note that this approach gives a continuous record of the extent to which the lineage is  
20 exposed to fire through time (at least node by node) that is not possible with charcoal  
21 records. The correlation of fire-proneness over time with the fire-related trait may also be  
22 calculated and thus its level of co-dependence with fire [15–18]. These two traits, one  
23 biotic and the other abiotic, do not necessarily co-occur, e.g. lineages may be fire-prone but  
24 lack the targeted trait, i.e. the usual rules of a standard  $2 \times 2$  contingency analysis apply.  
25 Once the time dimension can be added to a trait, whose superior fitness in the presence of  
26 fire has been demonstrated, the requirements to label it as a fire adaptation are satisfied.

27 Recent attempts at identifying ancestral traits and fire conditions through the  
28 phylogeny using the above approaches have prompted palaeontologists to seek fossil  
29 evidence to test the predictions. For example, from their trait-assignment analysis,  
30 Lamont and He [17] concluded that the Proteoideae was exposed to fire by 88 million  
31 years ago (Ma) and possessed soil or on-plant stored seeds by 76 Ma. Thus, Carpenter et  
32 al. [10], reporting some four years later, examined fossils assignable to Proteoideae in  
33 central Australia for the period 76–70 Ma and showed that charcoal was indeed abundant

1 then. However, they were not able to identify macrofossil seeds or cones that might  
2 support the purported existence of seed storage. Similarly, He et al. [16] concluded that  
3 *Pinus* became fire-prone at an estimated 126 Ma (Fig. S1), and, from trait assignment,  
4 thick bark arose at about the same time. Falcon-Lang et al. [18], also in a report four years  
5 later, located the oldest pine fossil known (Nova Scotia, Canada), which they dated to  
6 133–140 Ma, and showed that it was often preserved as charcoal and that the abundant  
7 resin ducts would have made the species highly flammable. As there is some debate about  
8 interpreting what probability to assign internodes with Monte Carlo procedures, we note  
9 that only by treating the stem as having the same fire regime as assigned to the crown  
10 node did we get the actual fire condition of ancestral *Pinus* correct. Thus, in the two cases  
11 where it has been possible to assess the indirect methodology as outlined here, it has been  
12 fully supported.

13

#### 14 **Fire-stimulated flowering (FSF)**

15

16 Arising 60 Ma, geophytic orchids in South Africa can be expected to be fire-prone as that  
17 region has a long history of fire [20] and located on the ground deems them highly  
18 vulnerable to fire. On a world-scale, terrestrial orchids account for more species  
19 displaying FSF than in any other family [21]. Two lineages of the Cape orchid, *Disa*,  
20 initiated obligate FSF some 2–5 My after the origin of the genus, 20 Ma (Fig. 2b, [22]). *Disa*  
21 is enveloped phylogenetically by five other genera showing FSF and by three more-  
22 recently evolved genera that are also fire-prone but lack FSF, and more recently again by  
23 *Peristylis* that is not fire-prone and thus cannot display FSF. Separating from its non-fire-  
24 prone sisters/immediate ancestor 75 Ma, the family Xanthorrhoeaceae became fire-prone  
25 in the period 75–70 Ma while the Xanthorrhoeidae-Asphodeloideae in Australia and the  
26 Cape developed near-obligate FSF 70–65 Ma (Table S1, [24]). Thus, over 5 My since  
27 becoming fire-prone, these subfamilies passed from a  $P = 0.70$  that they lacked FSF to a  $P$   
28 = 0.70 that they possessed FSF.

29

#### 30 **Fire-stimulated seed release**

31

1 He et al. [16] estimated by trait assignment, and Falcon-Lang et al. [19] later confirmed  
2 directly from charcoal, that pines were probably fire-prone (at least to surface fires) from  
3 their very beginning in the Lower Cretaceous, 135 Ma (Fig. 2a) with  $P = 0.63$  that the  
4 lineage already possessed thick bark. In contrast, at 159 Ma,  $P = 0.22$  that the clade was  
5 fire-prone and  $P = 0.18$  that it possessed thick bark. *Pinus* developed even thicker bark  
6 over the next 46 My with 100% certainty that the crown was also fire-prone by 89 Ma. By  
7 the Upper Cretaceous, subgenus *Pinus* was characterized by *either* retention of intact  
8 cones (serotiny) and their supporting side branches (with consequent seed release in  
9 response to crown fire) *or* even thicker bark with shedding of side branches (with  
10 consequent deterrence of the fire 'ladder' effect) and no serotiny. By contrast, subgenus  
11 *Strobus* remained fitfully fire-prone and did not develop such fire-adapted traits. Not only  
12 is speciating into non-fire-prone vegetation a later development among pines, some  
13 lineages have also oscillated from one fire type to the other over their evolutionary  
14 history (Fig. S1).

15         Though the Proteaceae arose ~115 Ma it did not become fire-prone until 88 Ma via  
16 the subfamily Proteoideae (Fig. 2d, [17]). Seed storage could be detected by 81 Ma but  
17 whether in the soil or on the plant was not clear. By 73.5 Ma, the *Petrophile* (Australia)-  
18 *Aulax* (Cape) lineage had developed closed cones with fire-stimulated seed release.  
19 Serotiny appeared independently as the ancestral condition in *Leucadendron* [25] with  
20 smoke-stimulated germination a later development among some lineages (R. Newton,  
21 unpubl.). Woody cones or fruits, with fire-stimulated seed release, also appeared as the  
22 ancestral state among *Protea* (28 Ma), *Lambertia-Xylomelum* (46 Ma), *Banksia* (62 Ma)  
23 and *Hakea* (18 Ma) (Fig. 2d), with the last three lineages in subfamily Grevilleoideae and  
24 each independently becoming fire-prone directly from rainforest ancestors followed by  
25 evolution of serotiny along the ancestral stem. *Protea* is interesting for it occupied  
26 shrubland with a moderate fire frequency at 13.6 Ma and  $P = 0.91$  that it was serotinous  
27 but by 8.3 Ma one lineage had invaded subtropical grassland with frequent fire and  $P =$   
28 0.84 that it was no longer serotinous (seed storage is redundant if not maladaptive when  
29 annual fires are likely) [17]. Thus, over 5.2 My, the lineage passed from summer to late  
30 winter fires and from serotiny to non-serotiny.

31

## 32 **Fire-stimulated germination**

33



1 At the same time as serotiny unequivocally appeared for the first time in Proteaceae, so  
2 too did fire-stimulated germination, 15 My after becoming fire-prone (Fig. 2d). The fire-  
3 prone *Grevillea-Hakea* complex separated from its rainforest sister *Buckinghamia* ~35 Ma  
4 [25] but not until 18 Ma did *Hakea*, with canopy-stored seeds, separate from its sister  
5 lineage within *Grevillea*, that retained the ancestral trait of soil-stored seeds. Similarly, the  
6 entire family Haemodoraceae has been fire-prone since its inception 89.5 Ma but soil-  
7 stored seeds appeared 8.5 My later in the subfamily, Conostylidoideae [26]. Germination  
8 in this group is essentially smoke-stimulated [27,28], with the chemical inducer,  
9 glyceronitrile, becoming effective at least 42 Ma and, nested within that, the karrikin,  
10 KAR<sub>1</sub>, 18 Ma.

11 The graminoid order, Poales, is the oldest group of monocots that shows fire-  
12 related traits. The fire-prone Anarthriaceae-Restionaceae clade separated from its non-  
13 fire-prone sister 101 Ma and, by the time the two families diverged 91 Ma, soil-stored  
14 seeds with fire-stimulated germination were firmly established in Restionaceae in the  
15 Cape subclade and less certainly (because fewer studies have been conducted) in the  
16 Australasian subclade (Fig. 2c, [29]). The antiquity of fire-proneness among restios is  
17 supported from fossil evidence in the Cape [30] and Australia [10], and from ancestral  
18 trait assignment – the entire clade is fire-prone. *Baloskion tetraphyllum*, arising 27 Ma in  
19 SW Australia, is the only restio whose smoke cue has been examined and KAR<sub>1</sub> shown to  
20 be effective [31].

21

## 22 **Overall trends through time**

23

24 We searched the literature for studies on molecular phylogenies of clades occurring in  
25 fire-prone parts of the world to which fire-related traits had been assigned or were  
26 assignable by us, assuming we could a) recover the phylogeny from GenBank data and b)  
27 determine the applicable trait for each analysed taxon. The result was 134 records  
28 (speciation events: node to node or terminal node) for the origin of fire-stimulated  
29 flowering (28), seed release (31) and germination (75) (Table S1). The 85 records for  
30 Australasia included 48 from SW Australia and three from the Chatham Islands; 37 were  
31 for Africa, especially the Cape; and 12 for the northern hemisphere, especially North  
32 America and Europe. These covered 13 families and 85 genera. The analysis was  
33 conducted at the genus or more-basal taxonomic order in the hierarchy, though

1 sometimes subgenera were included when their traits were distinctive. Where only a few  
2 species were known within a genus but there was no conflict among species in applicable  
3 traits this trait was treated as diagnostic for the genus.

4 All speciation events involving traits considered fire-related occurred under fire-  
5 prone conditions. **Primary directional selection** (D1, trait innovation in the ancestral  
6 stem of the clade coinciding with the onset of fire-proneness, Fig. 1) accounted for 15.7%  
7 of events. **Secondary directional selection** (D2, trait innovation in an already fire-prone  
8 lineage indicating either a fire-regime change or some other constraint becoming limiting)  
9 also accounted for 15.7% of events. **Stabilizing selection** (S, the fire-adapted trait is  
10 conserved through successive speciation events such that speciation must be due to DNA  
11 changes elsewhere) accounted for 68.6% of events. Not a single instance of the trait  
12 arising among non-fire-prone ancestors followed by fire-proneness arising later among  
13 descendants was recorded. The oldest record is for the instigation of serotiny associated  
14 with crown fire in the fire-prone Cupressaceae clade, *Callitris* (Australia)–*Actinostrobus*  
15 (SWA)–*Widdringtonia* (S Africa) at 106 Ma (D1, [32]). The most recent innovation at 1.2  
16 Ma is for the single species, *Protea simplex*, flowering after fire in delayed response to the  
17 advent of summer fires in the E-S African grasslands (D2, [18]).

18 Examining the trends over geological time, reproductive fire-related traits have a  
19 continuous history over the last 110 My (Fig. 3). Speciation events increased  
20 exponentially over that time, peaking at 20–10 Ma then falling markedly (by 60%) in the  
21 most recent 10 My. Trait innovations (transitions from one trait to another across a node)  
22 have occurred in each 10-My interval, and trait consolidation (transfer of a given trait  
23 from one node to the next) has occurred in eight of 11 intervals. There is a tendency for a  
24 greater fraction of speciation events to involve innovations at the older time periods. Trait  
25 stabilization gradually increased from ancient to more recent times, particularly in the  
26 period 50–10 Ma, peaking at 20–10 Ma (Early-Mid-Miocene and encompassing the Mid-  
27 Miocene Optimum, 17–14 Ma) where it accounted for 83% of events. Below trend events  
28 occurred at 40–30 Ma (Mid-Eocene to Early Oligocene), due to a drop in innovations, and  
29 at 10–0 Ma (Mid-Miocene, Pliocene, Quaternary), due to a drop in consolidations.

30 While there has been little use of correlation techniques, the few undertaken show  
31 a close association between fire-proneness and evolution of the fire-adapted trait through  
32 time. Thus, each of three seed storage syndromes evolved closely and synchronously with  
33 fire-prone habitats among the Proteoideae (serotiny, soil-stored seeds, ant-dispersed

1 seeds) [15]. For the genus *Protea*, serotiny evolved with ongoing presence of the  
2 moderately fire-prone shrublands in the Cape, while non-serotiny evolved with ongoing  
3 presence of frequent fire in the savanna grasslands to the east, as serotiny decreases  
4 fitness in such an environment [18]. It is interesting to note that this transition took 3.5  
5 My to complete. Assuming that the summer-rainfall grasslands already existed then [33],  
6 this gives an idea of how long it took winter-rainfall proteas to adapt to a radically  
7 different fire regime (D2 selection). Once this was achieved, rapid speciation of the  
8 adapted nucleus occurred (S selection).

9

## 10 **Smoke-stimulated germination**

11

12 While we were unable to detect any case where the evolution of a fire-adapted trait  
13 preceded the onset of fire-proneness, there are many instances in the literature where the  
14 experimental application of smoke actually induced germination of species not native to  
15 fire-prone habitats. These records need to be resolved as either exceptions to the rule or  
16 explainable by the three alternative mechanisms (under non-fire-prone) noted in Fig. 1.

17       Smoke-water strongly promotes germination of the epiphytic orchid, *Oberonia*  
18 *ensiformis*, that is otherwise zero in its absence [34]. Does this point to a chemical cue in  
19 smoke that reflects an obscure fire-prone past or would it normally be supplied anyway in  
20 its non-fire-prone environment? *Oberonia* is in the semibasal, subtribe Malaxideae, within  
21 the subfamily Epidendroideae [35], whose even more basal subtribes are terrestrial and  
22 increasingly fire-prone, especially Neottieae [36]. Further, its terrestrial sister clade,  
23 Orchidoideae, is not only highly fire-prone but most of its subtribes display FSF, indicating  
24 a long association with fire (Fig. 2b). This means that this species may carry a dormant,  
25 smoke-responsive, gene mechanism inherited from its past that is not currently expressed  
26 (**non-selection** scenario, Fig. 1). But for its known fire-prone background, this unexpected  
27 fire-response might be regarded as a pre-adaptation.

28       Research on the annual weed, *Arabidopsis thaliana* (Brassicaceae), showed that  
29 smoke-stimulated germination could occur under certain circumstances in response to the  
30 presence of butenolide-related karrikins (KAR), a universal component of smoke [37].  
31 Flematti et al. [4] noted that: "The discovery that karrikins are active in *Arabidopsis*, even  
32 though it is not known to be a smoke-responsive or fire-adapted species, led to the  
33 speculation that karrikins could be mimicking an endogenous signaling molecule...". But this

1 statement fails the test of logic: if KAR is in smoke, and *Arabidopsis* responds to KAR, then  
2 *Arabidopsis* must be smoke-responsive (also see [38]). Further, there are over 750  
3 accessions from indigenous or naturalized locations in the fire-prone Mediterranean Basin  
4 [39], including Spain, Italy, Canary Islands, Israel and Turkey, and California, N rim of Africa,  
5 Cape of S Africa and N American prairies and pine forests, and hundreds of collections that  
6 are utilized but whose origin is unknown  
7 (<https://www.arabidopsis.org/portals/education/aboutarabidopsis.jsp#world>). In addition, although the  
8 phenology of germination and flowering varies greatly between ecotypes, they all produce  
9 seeds by summer [41], consistent with a summer-dry (fire-prone) climate. Finally, as  
10 inhabitants of disturbed sites generally, smoke/KAR-responsive seeds are characteristic of  
11 the entire Brassicaceae [41,42]. The closest sister families are also fire-prone and smoke-  
12 stimulated germination is well-demonstrated in the ancestral Gyrostemonaceae [43,44].  
13

## 14 **Conclusions**

15  
16 None of the 134 speciation events we examined could be interpreted as the outcome of  
17 selection in a non-fire-prone environment (exaptation) resulting from fire-mimicking  
18 selection (non-fire-derived constraint in common with some component of fire, Fig. 1), as  
19 there were no reversals of the fire-prone → adaptation trait sequence (Figs. 2,3).  
20 Even so, directional selection implies that the two phenomena—imposition of a fire-prone  
21 environment and trait innovation—act contemporaneously though not necessarily  
22 simultaneously even if they appear to coincide at the same node in the phylogeny: because  
23 adaptation involves selection (extinction) followed by genetic change, a time lag is  
24 inevitable [16]. Such subtleties of timing are not an issue when fire-proneness straddles  
25 more than one node in the phylogeny, as is usually the case (Fig. 2, S1). Once adapted to  
26 the new fire regime, the new trait is simply carried from one node to the next (stabilized  
27 selection), and our collation indicates that over two-thirds of speciation events were of  
28 this type. There has been no period over the last 100 My when both types of fire-adapted  
29 trait have not evolved, although trait proliferation peaked in the increasingly drought  
30 (and thus fire)-prone Miocene (Fig. 3).

31 Even when smoke was imposed on species occurring naturally in non-fire-prone  
32 habitats and they revealed positive responses, further probing showed that their sibling  
33 ecotypes, sisters and/or ancestors had evolved in fire-prone environments. Research on

1 the biochemistry of germination in *Arabidopsis* is pointing to the presence of a karrikin-  
2 receptive protein (KAI2) among flowering plants generally [45]) that also implicates fire  
3 in directing the evolution of KAR-sensitivity generally. Far from being exceptions to the  
4 rule, fire remains central to understanding smoke-sensitivity even in (apparently) non-  
5 fire-prone environments. This means that the plants inherited the relevant gene  
6 mechanism for implementing the fire-adapted trait even though it may not be expressed  
7 currently or under selection, as this is context-driven.

8

## 9 **Future research**

10

11 The data on which this Opinion article is based favour Southern Hemisphere floras so it is  
12 clear that more dated phylogenies and trait assignments for fire-prone regions in the  
13 Northern Hemisphere are required to redress the balance and generalize the outcomes.  
14 The accuracy of the evolutionary predictions would be enhanced by incorporating fossil  
15 lineages into the phylogenies; the techniques for this are now available and fossils are  
16 beginning to be incorporated to improve the ability to identify the origin of taxa and their  
17 ancestral traits [5].

18 Reasons for reluctance to accept the role of fire in the evolution of plant traits  
19 include a) lack of evidence from the fossil record—this is being tackled by a change in the  
20 priorities of palaeontologists who are starting to look for fossil charcoal and fire-related  
21 traits [10,19]; b) lack of comparative experiments that show superior fitness in the  
22 presence of fire compared with other possible limiting factors such as drought (see  
23 Causley et al. [46] for a rare exception), except there are increasing numbers of studies  
24 that seek to identify what component of fire is the specific cause of the response [41],  
25 though possible interaction effects are rarely considered; c) lack of trust in, or familiarity  
26 with, these new ‘indirect’ ancestral trait reconstruction methods, that is already proving  
27 not to be justified [10,19]; and d) a general mind set that views fire as a destructive rather  
28 than a rejuvenating force in nature [47] that means fire is ignored or rejected as a possible  
29 explanation for newly documented evolutionary events .

30 The final frontier is linking fire cues to genetic mechanisms that effectively bypass  
31 the fire-proneness-prerequisite issue as this must be true by definition. Great progress  
32 has been made with the karrikin-component of smoke and the germination of *Arabidopsis*  
33 [45] without fully appreciating its ancestral link with fire. However, the topic is

1 completely open for exploring the other fire cues and fire-related traits as discussed here.  
2 It starts by acknowledging that fire preceded (but later co-occurred with) drought as the  
3 dominant selective force in extant fire-prone systems.

#### 4 5 **Acknowledgments**

6  
7 We acknowledge support by the Australian Research Council (DP12013389 and  
8 DP130103029). We thank Mike Crisp for comments on the draft manuscript, the editor  
9 and the three reviewers for challenging our ideas and the way we expressed them.

10

11

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23  
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26

## 27 **Figures**

28

29 Fig. 1. Flow diagram showing the mechanisms involved in the evolution of fire-adapted  
30 traits from fire-related traits in fire-prone habitats, and the contextual relationships  
31 between primary, secondary and stabilizing selection (highlighted in pink), and the  
32 evolution of apparently fire-adapted traits in non-fire-prone habitats, via non-selection and  
33 fire-mimicking selection leading to exaptations, as alternative explanations for their

1 evolution (highlighted in blue). Arrows indicate the direction of selection and consequent  
2 change in traits over time. While different processes occur in the fire-prone and non-fire-  
3 prone environments, there is no particular meaning in their relative placement in this  
4 scheme.

5 Fig. 2. Four schematic phylogenies that demonstrate the clade was fire-prone prior to the  
6 evolution of the targeted fire-adapted trait. a. Evolution of Pinaceae (Northern  
7 Hemisphere). Note that serotiny appeared 46 My after *Pinus* had first been exposed to fire.  
8 Adapted from [16] and updated from [19]. b. Evolution of orchids (Orchidaceae, South  
9 Africa) with fire-stimulated flowering (FSF) showing they have arisen from non-fire-prone  
10 ancestors with loss of FSF in some more recent lineages. Note that *Disa* was fire-prone  
11 before FSF appeared later in two lineages. Adapted from [19,20] with dating support from  
12 [20,23]. c. Evolution of Restionaceae (South Africa, Australasia), showing that fire-  
13 stimulated seed release and germination do not arise before the family is fire-prone.  
14 Adapted from [28] with dating support from [10,17,20,31]. d. Evolution of Proteaceae  
15 (Australia, South Africa) showing it was fire-prone > 15 My before seed-storage traits  
16 appeared in the family. Adapted from [17] with dating support from [10,26,31].  
17 \*Leucadendrinae other than *Leucadendron*. Flame symbol = early evidence of fire-  
18 proneness (date given); blue lines = lineages with initial fire-adapted trait; green lines =  
19 lineages with a later evolving fire-adapted trait.

20 Fig. 3. Frequency of three fire-adapted reproductive traits (fire-stimulated flowering, seed  
21 release and germination) at 10-My intervals over the last 100 My, based on 134 records  
22 from 40 papers (Table S1). The outcomes of three types of selection in response to fire are  
23 distinguished: primary and secondary directional selection and stabilizing selection, to  
24 show all have played significant roles in understanding the evolution of fire-adapted traits.

Supplementary material

Table S1

Figure S1

Figure 1

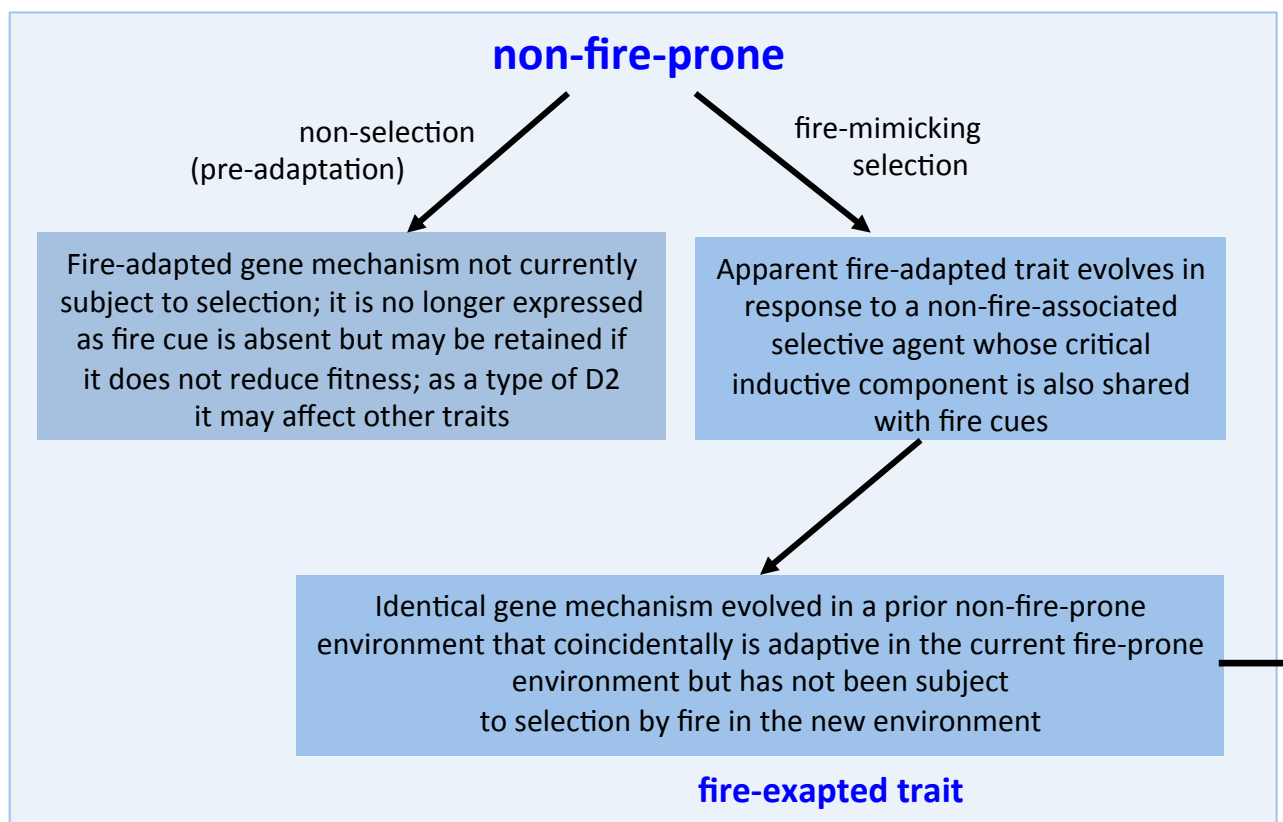
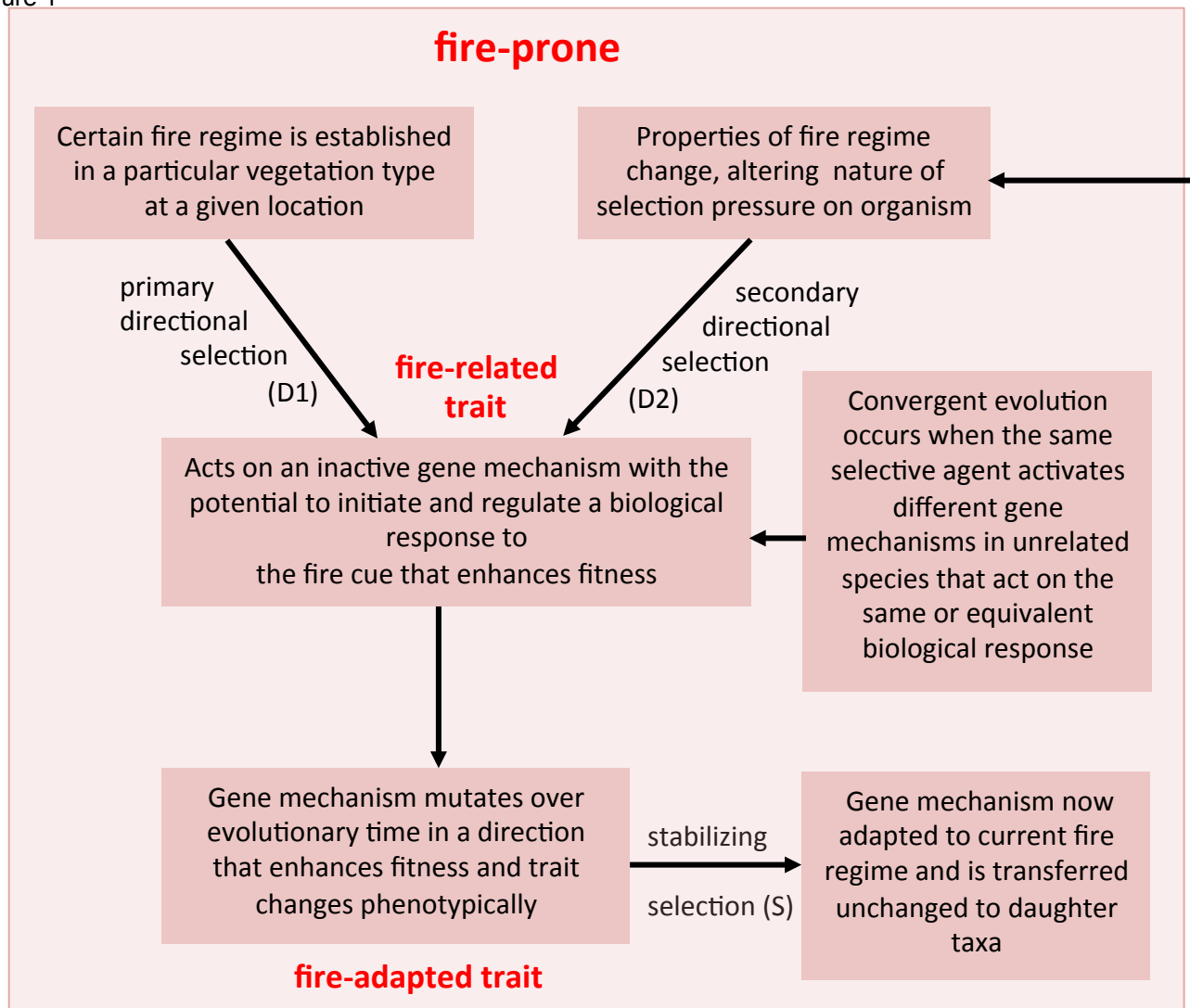
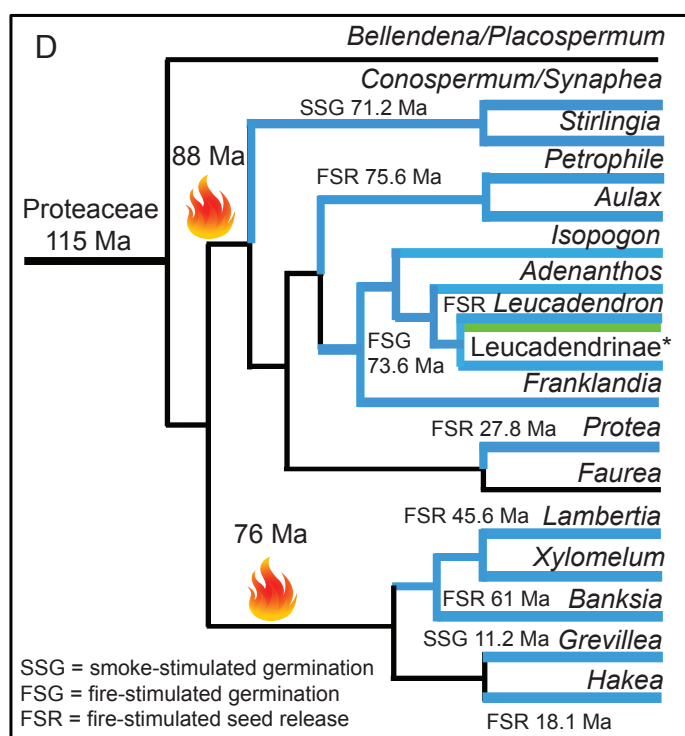
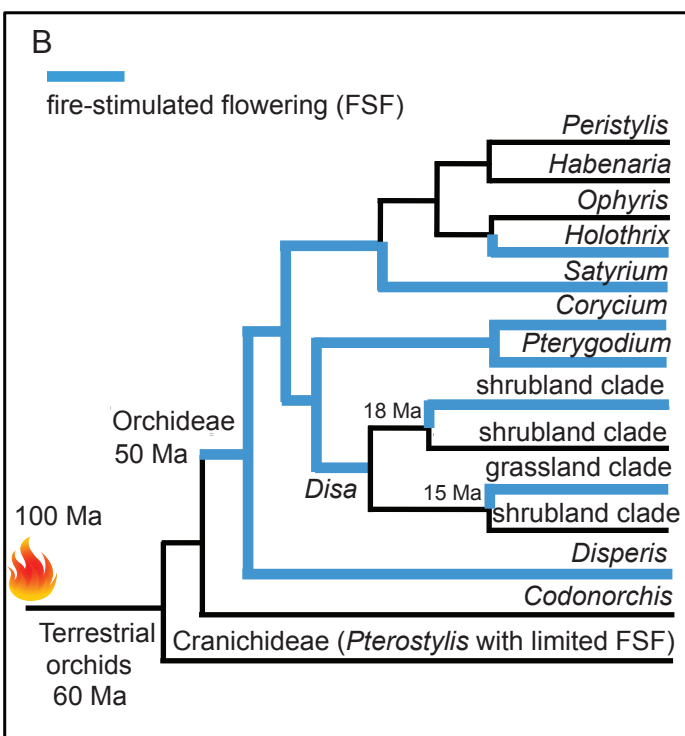
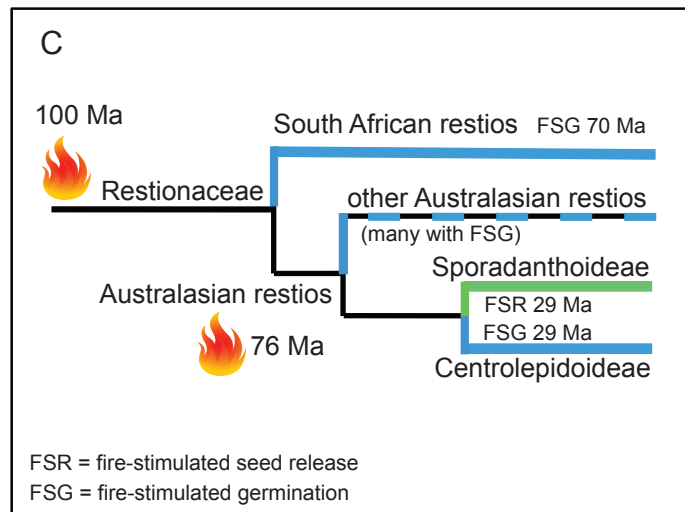
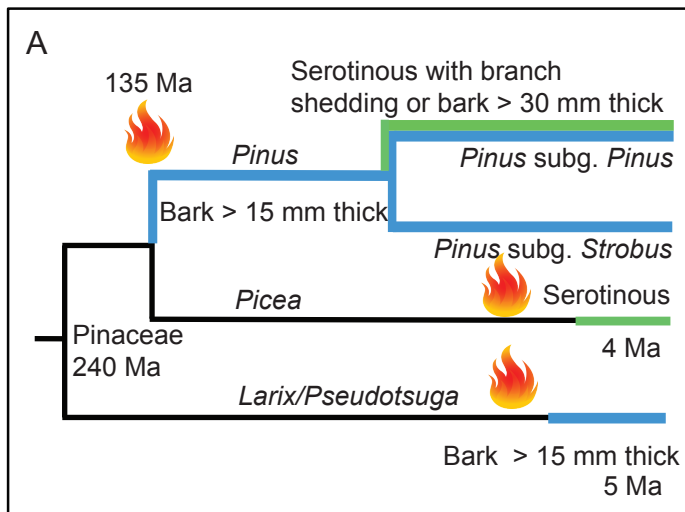


Figure 2



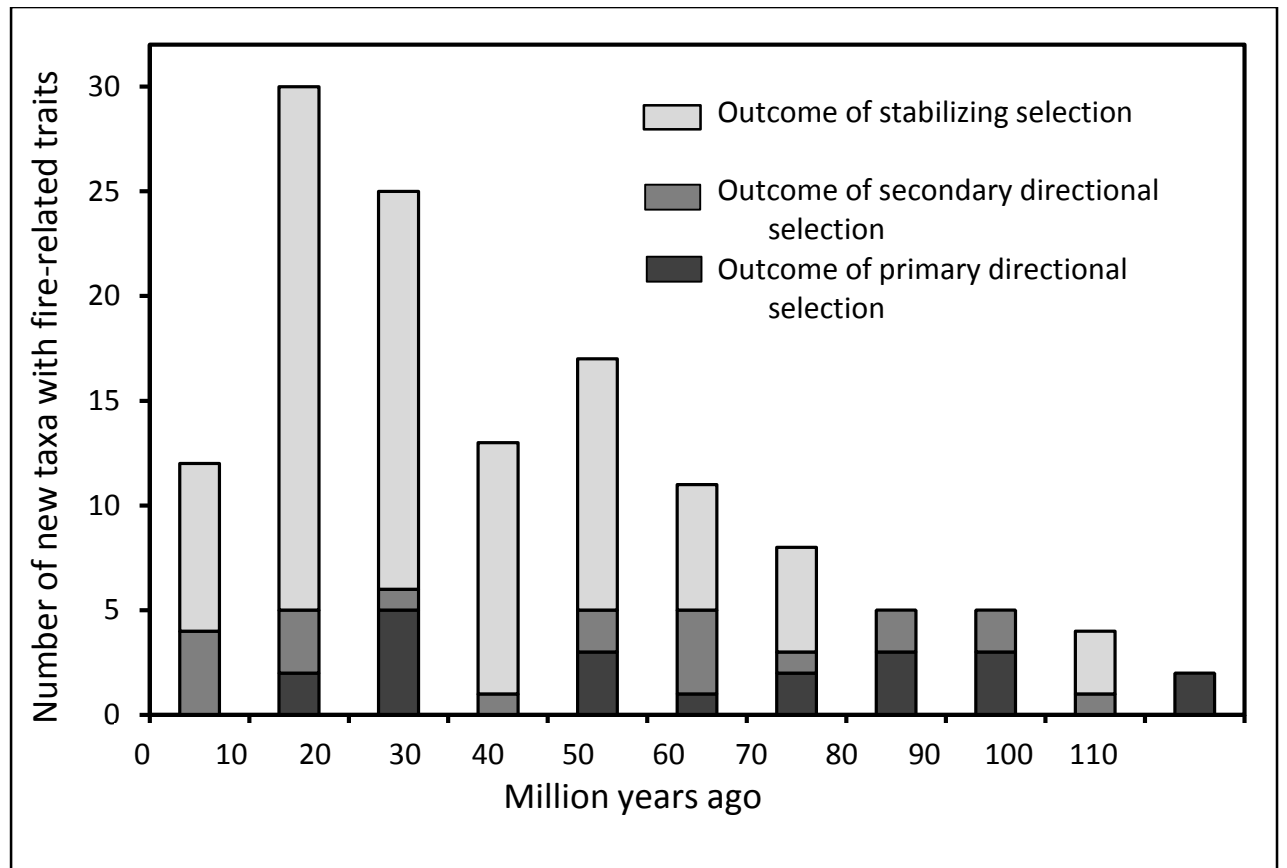


Fig. 4



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**Outstanding questions:**

- Is fire-proneness also a necessary prerequisite for the evolution of fire-killed species (with seed storage) and resprouters (not considered here)?
- Does fire-proneness precede evolution of fire-adapted traits in all fire-prone biomes other than those examined here?
- Can techniques be developed that enable separation of the onset of fire-proneness and origin of fire-related traits along a branch (i.e., node to node) within a phylogeny?
- How quickly are non-expressed, gene mechanisms for producing fire-adapted traits lost from clades?
- Will the karrikin-coupling protein, KAI2, prove to be universally present among seed plants? Will this mean that all land plants ultimately have a fire-prone past?
- Can particular fire-adapted traits be traced to particular loci in the genome?
- Are skeptics able to articulate what they would accept as evidence that fire has played a key role in directing evolution of at least some of the Earth's terrestrial flora?





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