

Opinion

Fire-Proneness as a Prerequisite for the Evolution of Fire-Adapted Traits

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

Fire and Plant Trait Evolution

Plant properties that are characteristically associated with fire-prone environments are called fire-related traits [1], whereas fire-adapted traits are not only associated with fire or one of its byproducts (heat, smoke, combustion products, nutrient release, increased diurnal temperature fluctuations) but have also been shown to increase plant fitness as a consequence [2]. A species is regarded as fire-prone if the habitat in which it occurs ignites at least once during its lifespan: it is an environmental trait of the species of interest. The concept can be extended to lineages and clades if it can be shown that their members also have a history of exposure to fire. If a trait has evolved in response to selection by fire then it follows that the environment of a plant must have been fire-prone before the appearance of that trait thereby providing the context in which selection occurs. An alternative is that the trait is an adaptive response to a limiting factor that is unrelated to fire but has elements in common with it, giving the erroneous impression that it is a fire-adapted trait. This can be viewed as the outcome of multi-agent selection, or, in the present context, fire-mimicking selection (Figure 1). Examples include ethylene in smoke that may induce post-fire flowering [3], but ethylene is also released by decomposing plant matter that could be an alternative source; nitrate salts that promote germination are not only released from litter and vegetation in response to ignition but also following soil disturbance and exposure of soil organic matter to sunlight after gap creation [4].

This is to be distinguished from convergent evolution wherein the same selective agent acts on different gene processes that control evolution of the same or equivalent trait in unrelated clades. Examples of convergent evolution include fire-stimulated germination in disparate clades that is promoted by different chemical components of smoke (e.g., karrikins, ethylene, glyceronitrile, nitrogen dioxide) that act independently on different parts of the genetic pathway that leads to post-fire germination [5]. As a result of fire-mimicking selection, a trait may evolve in a non-fire-prone environment, in response to a non-fire-related limiting factor, but be expressed, and have

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Ancestral trait reconstruction using accurately 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 fossil charcoal and reconstruction techniques.

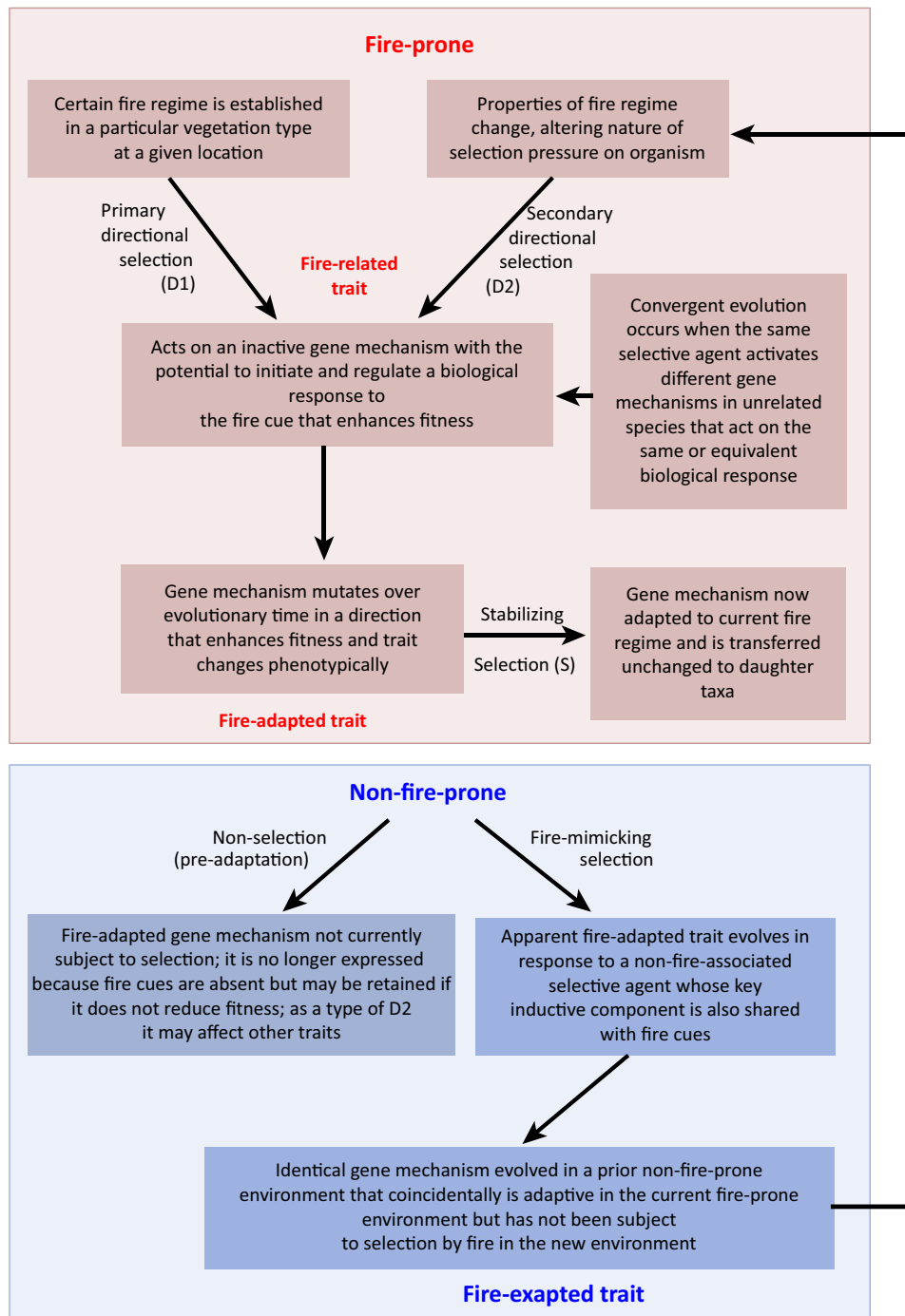
Ascertaining whether or not the onset of exposure to fire preceded the advent of putatively fire-adapted traits enables the identification of unique adaptations to fire.

Fire-mimicking (multi-agent) selection and associated exaptations are alternative explanations of apparent fire-adapted traits that require selection via drought or non-unique components of fire to precede selection by fire.

Smoke-stimulated germination among plants in non-fire-prone habitats may not be an anomaly if it can be shown that they possess a dormant gene mechanism inherited from a fire-prone past.

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Figure 1. Flow Diagram Showing the Mechanisms Involved in the Evolution of Fire-Adapted Traits from Fire-Related Traits in Fire-Prone Habitats, and the Contextual Relationships between Primary, Secondary, and Stabilizing Selection (Highlighted in Pink), and the Evolution of Apparently Fire-Adapted Traits in Non-Fire-Prone Habitats, Via Non-Selection and Fire-Mimicking Selection Leading to Exaptations, as Alternative Explanations for Their Evolution (Highlighted in Blue). Arrows indicate the direction of selection and consequent change in traits over time. While different processes occur in the fire-prone and non-fire-prone environments, there is no particular meaning in their relative placement in this scheme.

equal fitness benefit in, the new fire-prone environment without undergoing additional selection. This conserved adapted response to a prior agent of selection is called an exaptation; it is a fire-exapted trait (Figure 1). If fire-mimicking selection and associated exaptations apply, then fire-proneness will postdate the appearance of the trait in geological time. Our objective in this Opinion article is to document which sequence of events actually occurred in earlier times – are fire-adapted traits true adaptations or merely exaptations?

Fire-related traits have sometimes been viewed as adaptations to drought, but exaptations to fire, in the belief that the former preceded the latter [2]. There is a parallel here with fire-mimicking selection because the dryness and heat associated with summer drought can be viewed as a mild form of the drying and heating effect of fire. However, the biological and fitness effects of fire and drought on biota can never be considered synonymous, as would be required for the identification of exaptations. Drought-prone environments are inextricably associated with fire in current ecosystems but not necessarily in earlier times when high atmospheric oxygen levels often dictated flammability of plant material [2]. While this makes determining which constraint is ancestral a difficult task, such a distinction is essential in the present context. Organisms may appear to tolerate intensities of constraints that they have not experienced in their past (pre-adaptation) but this might merely reflect ignorance about their evolutionary history. Besides, as soon as the trait appears it will be subject to selection by the more constraining agent, leading to further adaptation and making such labels as exaptation and pre-adaptation redundant.

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

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

Methodology and Corroboration

An understanding of the evolution of fire-adapted traits requires knowledge of fire-proneness of the supporting vegetation and the presence of the fire-adapted trait of interest over geological time. The existence of charcoal (fusain) is a proxy for fire but has often been overlooked in the fossil record where pollen identification is the usual focus of the study. In claiming to identify a fire scar for the first time among fossil trees, Byers *et al.* [13] was surprised that this had not been observed before, and suggested that disciplinary ‘tunnel-vision’ was the reason. Where possible, we have used fossil charcoal to indicate the antiquity of fire-proneness, but fossil charcoal has four drawbacks even when researchers are looking for it: (i) charcoal is rarely preserved in

strata because fires occur remotely from wetlands where preservation is favored, (ii) fossil records are only a 'snapshot' in time, (iii) there is no guarantee that the lineages of interest actually co-occurred with sources of charcoal recorded in the general area or even at the same site, and (iv) the mere presence of charcoal gives no indication of the associated fire regime nor whether it was sufficient to have an evolutionary impact. Identifying fire-related traits is even more problematic: these are rarely preserved in the fossil record, especially the phenological traits as examined here. Thus, cones are invariably preserved in the 'open' condition [2] with no clue as to the circumstances causing the death of the supporting stem leading to seed release (drought, senescence, physical damage, fire?). The interannual timing of flowering with respect to fire-stimulated flowering cannot be gauged from the pollen record because it is too coarse. Regarding soil-stored seeds, Baskin *et al.* [14] note: 'we know of no study that shows seed coat anatomy of fossil seeds of families whose extant members have physical dormancy'.

In view of the limited fossil evidence for the presence of fire or fire-related traits, a major breakthrough in determining the origin of fire-adapted traits has occurred over the past 5 years. This involves the use of molecular phylogenies that have a time dimension added to all internal nodes of the phylogeny (chronogram) and for which the state of the fire-related traits (present/absent) of their component species or genera is known. The probability of a given trait existing at a given node in the phylogeny is determined by comparing with pre-existing probabilities elsewhere in its lineage and adjacent lineages (by Bayesian Markov–Chain Monte Carlo ancestral-state reconstruction methods) [2,15–18]. While it is accepted that the trait may vary along a given branch of the chronogram, probabilities can only be applied to the nodes. Another limitation of the approach is that it assumes that the extant species are genetically (and thus trait) representative of all species that have ever existed in the clade; in other words there have been no extinctions of major lineages that might lead to bias in the assignments. Thus, traits are traced back in time until the probability of their existence is no longer significant and that date is treated as its origin.

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

Recent attempts at identifying ancestral traits and fire conditions through the phylogeny using the above approaches have prompted paleontologists to seek fossil evidence to test the predictions. For example, from their trait-assignment analysis, Lamont and He [17] concluded

that the Proteoideae were exposed to fire by 88 million years ago (Ma) and possessed soil or on-plant stored seeds by 76 Ma. Thus, Carpenter *et al.* [10], reporting some 4 years later, examined fossils assignable to Proteoideae in central Australia for the period 75–65 Ma and showed that charcoal was indeed abundant then. However, they were not able to identify macrofossil seeds or cones that might support the purported existence of seed storage. Similarly, He *et al.* [16] concluded that *Pinus* became fire-prone at an estimated 126 Ma (Figure S1), and, from trait assignment, thick bark arose at about the same time. Falcon-Lang *et al.* [18], also in a report 4 years later, located the oldest pine fossil known (Nova Scotia, Canada), which they dated to 140–133 Ma, and showed that it was often preserved as charcoal and that the abundant resin ducts would have made the species highly flammable. Because there is some debate about interpreting what probability to assign internodes with Monte Carlo procedures, we note that only by treating the stem as having the same fire regime as assigned to the crown node did we correctly identify the actual fire condition of ancestral *Pinus*. Thus, in the two cases where it has been possible to assess the indirect methodology as outlined here, it has been fully supported.

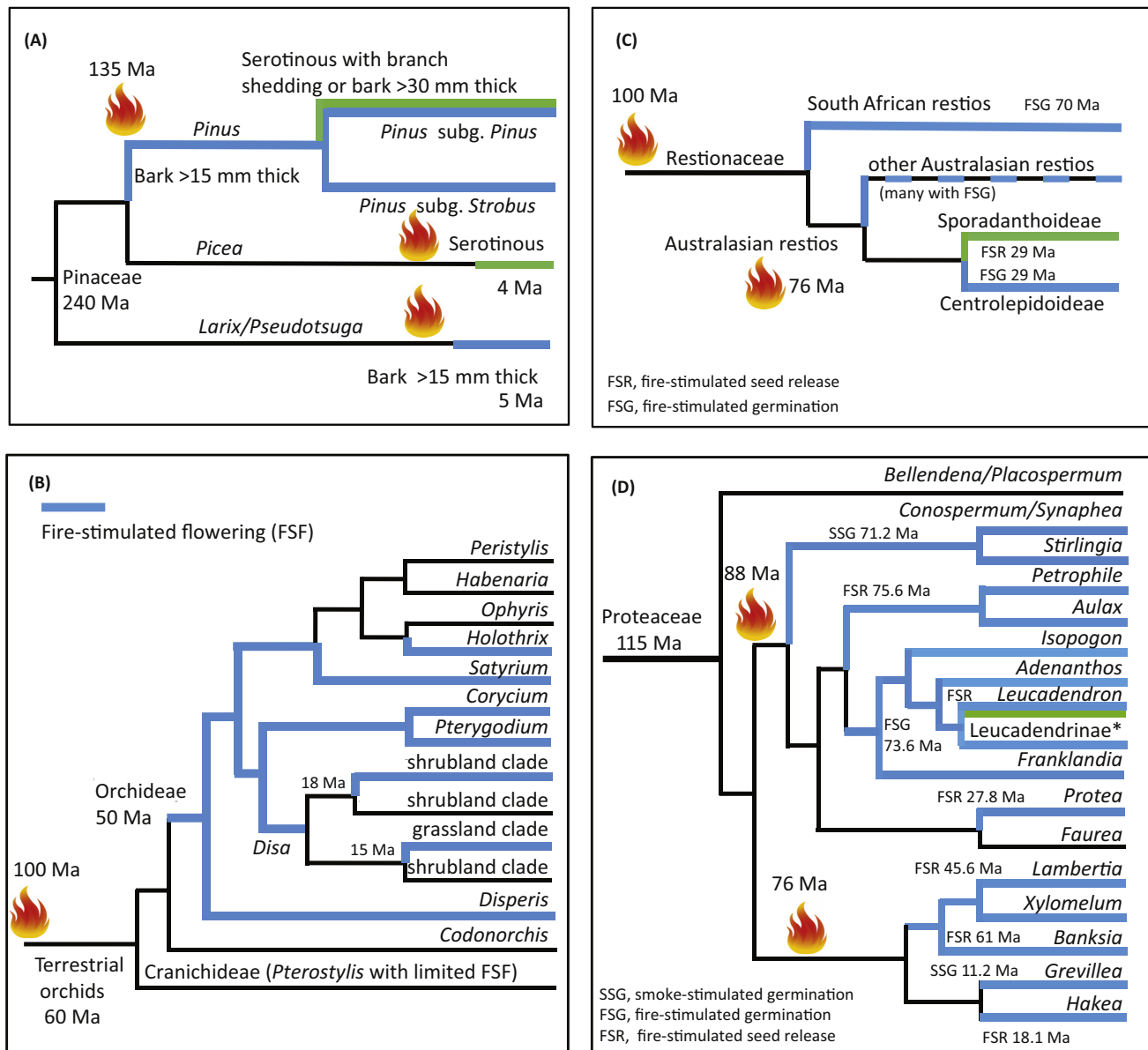
Fire-Stimulated Flowering (FSF)

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

Fire-Stimulated Seed Release

He *et al.* [16] estimated by trait assignment, and Falcon-Lang *et al.* [23] later confirmed directly from charcoal, that pines were probably fire-prone (at least to surface fires) from their very beginning in the Lower Cretaceous, 135 Ma (Figure 2A), with $P = 0.63$ that the lineage already possessed thick bark. By contrast, at 159 Ma, $P = 0.22$ that the clade was fire-prone and $P = 0.18$ that it possessed thick bark. *Pinus* developed even thicker bark over the next 46 Myr, with 100% certainty that the crown was also fire-prone by 89 Ma. By the Upper Cretaceous, subgenus *Pinus* was characterized by either retention of intact cones (serotiny) and their supporting side branches (with consequent seed release in response to crown fire), or even thicker bark with shedding of side branches (with consequent deterrence of the fire ‘ladder’ effect) and no serotiny. By contrast, subgenus *Strobus* remained fitfully fire-prone and did not develop such fire-adapted traits. Not only is speciating into non-fire-prone vegetation a later development among pines, some lineages have also oscillated from one fire type to the other during their evolutionary history (Figure S1).

Although the Proteaceae arose ~115 Ma it did not become fire-prone until 88 Ma via the subfamily Proteoideae (Figure 2D) [17]. Seed storage could be detected by 81 Ma but whether in the soil or on the plant was not clear. By 73.5 Ma, the *Petrophile* (Australia)–*Aulax* (Cape) lineage had developed closed cones with fire-stimulated seed release. Serotiny appeared independently as the ancestral condition in *Leucadendron* [24], with smoke-stimulated germination being a later development among some lineages (R. Newton, unpublished). Woody cones or fruits, with fire-stimulated seed release, also appeared as the ancestral state among *Protea* (28 Ma), *Lambertia*–*Xylomelum* (46 Ma), *Banksia* (62 Ma), and *Hakea* (18 Ma) (Figure 2D), with the last



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Figure 2. Four Schematic Phylogenies Demonstrating that the Clade was Fire-Prone Before the Evolution of the Targeted Fire-Adapted Trait. (A) The evolution of the Pinaceae (Northern Hemisphere). Note that serotinity appeared 46 Myr after *Pinus* had first been exposed to fire. Adapted from [16] and updated from [23]. (B) The evolution of orchids (Orchidaceae, South Africa) with fire-stimulated flowering (FSF) showing that they arose from fire-prone ancestors with loss of FSF in some more-recent lineages. Note that *Disa* was fire-prone before FSF appeared later in two lineages. Adapted from [19,23] with dating support from [19,46]. (C) The evolution of the Restionaceae (South Africa, Australasia), showing that fire-stimulated seed release and germination did not arise before the family was fire-prone. Adapted from [27] with dating support from [10,17,19,30]. (D) The evolution of the Proteaceae (Australia, South Africa) showing that they were fire-prone >15 Myr before seed-storage traits appeared in the family. Adapted from [17] with dating support from [10,25,30]. **Leucadendrinae* other than *Leucadendron*. Key: flame symbol, early evidence of fire-proneness (date given); blue lines, lineages with initial fire-adapted trait; green lines, lineages with a later evolving fire-adapted trait.

three lineages in subfamily Grevilleoideae, each independently becoming fire-prone directly from rainforest ancestors, followed by evolution of serotinity along the ancestral stem. *Protea* is interesting because it occupied shrubland with a moderate fire frequency at 13.6 Ma and $P = 0.91$ that it was serotinous, but by 8.3 Ma one lineage had invaded subtropical grassland with frequent fire and $P = 0.84$ that it was no longer serotinous (seed storage is redundant if not

maladaptive when annual fires are likely) [17]. Thus, over 5.2 Myr, the lineage passed from summer to late-winter fires and from serotiny to non-serotiny.

Fire-Stimulated Germination

At the same time as serotiny unequivocally appeared for the first time in Proteaceae, so too did fire-stimulated germination, 15 Myr after becoming fire-prone (Figure 2D). The fire-prone *Grevillea–Hakea* complex separated from its rainforest sister *Buckinghamia* at ~35 Ma [24] but not until 18 Ma did *Hakea*, with canopy-stored seeds, separate from its sister lineage within *Grevillea*, that retained the ancestral trait of soil-stored seeds. Similarly, the entire family Haemodoraceae has been fire-prone since its inception 89.5 Ma, but soil-stored seeds appeared 8.5 Myr later in the subfamily, Conostyloideae [25]. Germination in this group is essentially smoke-stimulated [26,27], with the chemical inducer, glyceronitrile, becoming effective at least at 42 Ma and, nested within that, the karrikin, KAR₁, at 18 Ma.

The graminoid order, Poales, is the oldest group of monocots that shows fire-related traits. The fire-prone Anarthriaceae–Restionaceae clade separated from its non-fire-prone sister 101 Ma and, by the time the two families diverged 91 Ma, soil-stored seeds with fire-stimulated germination were firmly established in Restionaceae in the Cape subclade, and less certainly (because fewer studies have been conducted) in the Australasian subclade (Figure 2C) [28]. The antiquity of fire-proneness among restios is supported from fossil evidence in the Cape [29] and Australia [10], and from ancestral trait assignment – the entire clade is fire-prone. *Baloskion tetraphyllum*, arising 27 Ma in SW Australia, is the only restio whose smoke cue has been examined and where KAR₁ has been shown to be effective [30].

Overall Trends through Time

We searched the literature for studies on molecular phylogenies of clades occurring in fire-prone parts of the world to which fire-related traits had been assigned or were assignable by us, assuming we could (i) recover the phylogeny from GenBank data and (ii) determine the applicable trait for each taxon analyzed. The result was 134 records (speciation events: node to node or terminal node) for the origin of fire-stimulated flowering (28 records), seed release (31) and germination (75) (Table S1). The 85 records for Australasia included 48 from SW Australia and three from the Chatham Islands; 37 were for Africa, especially the Cape; and 12 for the northern hemisphere, especially North America and Europe. These covered 13 families and 85 genera. The analysis was conducted at the genus or more-basal taxonomic order in the hierarchy, although sometimes subgenera were included when their traits were distinctive. Where only a few species were known within a genus, but there was no conflict among species in applicable traits, this trait was treated as diagnostic for the genus.

All speciation events involving traits that are considered to be fire-related occurred under fire-prone conditions. Primary directional selection (D1, trait innovation in the ancestral stem of the clade coinciding with the onset of fire-proneness, Figure 1) accounted for 15.7% of events. Secondary directional selection (D2, trait innovation in an already fire-prone lineage, indicating either a fire-regime change or some other constraint becoming limiting) also accounted for 15.7% of events. Stabilizing selection (S, the fire-adapted trait is conserved through successive speciation events such that speciation must be due to DNA changes elsewhere) accounted for 68.6% of events. Not a single instance was recorded of the trait arising among non-fire-prone ancestors followed by fire-proneness arising later among descendants. The oldest record is for the instigation of serotiny associated with crown fire in the fire-prone Cupressaceae clade, *Callitris* (Australia)–*Actinostrobus* (SWA)–*Widdringtonia* (S Africa) at 106 Ma (D1, [31]). The most recent innovation at 1.2 Ma is for the single species, *Protea simplex*, flowering after fire in delayed response to the advent of summer fires in the E–S African grasslands (D2, [18]).

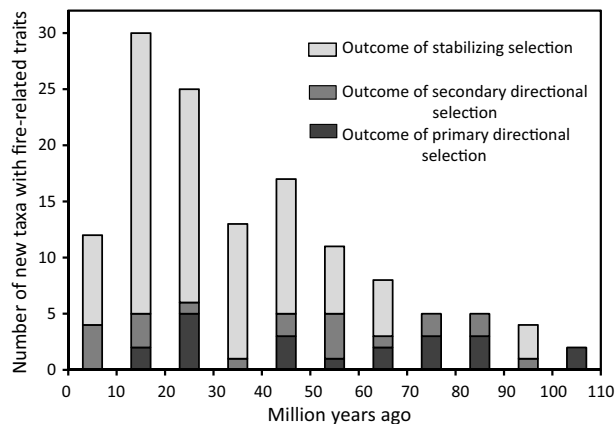


Figure 3. Frequency of Three Fire-Adapted Reproductive Traits (Fire-Stimulated Flowering, Seed Release, and Germination) at 10 Myr Intervals over the Past 100 Myr Based on 134 Records from 40 Reports (Table S1). The outcomes of three types of selection in response to fire are distinguished: primary and secondary directional selection and stabilizing selection, to show all have played significant roles in understanding the evolution of fire-adapted traits.

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

While there has been little use of correlation techniques, the few undertaken show a close association between fire-proneness and the evolution of the fire-adapted trait through time. Thus, each of three seed-storage syndromes evolved closely and synchronously with fire-prone habitats among the Proteoideae (serotiny, soil-stored seeds, ant-dispersed seeds) [15]. For the genus *Protea*, serotiny evolved with ongoing presence of the moderately fire-prone shrublands in the Cape, while non-serotiny evolved with ongoing presence of frequent fire in the savanna grasslands to the east because serotiny decreases fitness in such an environment [18]. It is interesting to note that this transition took 3.5 Myr to complete. Assuming that the summer-rainfall grasslands already existed at that time [32], this gives an idea of how long it took winter-rainfall protea species to adapt to a radically different fire regime (D2 selection). Once this was achieved, rapid speciation of the adapted nucleus occurred (S selection).

Smoke-Stimulated Germination

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

Smoke-water strongly promotes germination of the epiphytic orchid, *Oberonia ensiformis*, that is otherwise zero in its absence [33]. Does this point to a chemical cue in smoke that reflects an obscure fire-prone past or would it normally be supplied in its non-fire-prone environment?

Oberonia is in the semibasal, subtribe Malaxideae, within the subfamily Epidendroideae [34], whose even more basal subtribes are terrestrial and increasingly fire-prone, especially Neottieae [35]. Further, its terrestrial sister clade, Orchidoideae, is not only highly fire-prone but most of its subtribes display FSF, indicating a long association with fire (Figure 2B). This means that this species may carry a dormant, smoke-responsive, gene mechanism inherited from its past that is not currently expressed (non-selection scenario, Figure 1). However, but for its known fire-prone background, this unexpected fire-response might be regarded as a pre-adaptation.

Research on the annual weed, *Arabidopsis thaliana* (Brassicaceae), showed that smoke-stimulated germination could occur under particular circumstances in response to the presence of butenolide-related karrikins (KAR), a universal component of smoke [36]. Flematti *et al.* [4] noted that: ‘The discovery that karrikins are active in *Arabidopsis*, even though it is not known to be a smoke-responsive or fire-adapted species, led to the speculation that karrikins could be mimicking an endogenous signaling molecule...’. However, this statement fails the test of logic: if KAR is in smoke, and *Arabidopsis* responds to KAR, then *Arabidopsis* must be smoke-responsive (also see [37]). Further, there are over 750 accessions from indigenous or naturalized locations in the fire-prone Mediterranean Basin [38], including Spain, Italy, Canary Islands, Israel, and Turkey, as well as in California, the northern rim of Africa, the Cape of South Africa, and North American prairies and pine forests, and in hundreds of collections that are utilized but whose origins are unknown (www.arabidopsis.org/portals/education/aboutarabidopsis.jsp#world). In addition, although the phenology of germination and flowering varies greatly between ecotypes, they all produce seeds by summer [39], consistent with a summer-dry (fire-prone) climate. Finally, as inhabitants of disturbed sites generally, smoke/KAR-responsive seeds are characteristic of the entire Brassicaceae [39,40]. The closest sister families are also fire-prone, and smoke-stimulated germination is well-demonstrated in the ancestral Gyrostemonaceae [41,42].

Concluding Remarks and Future Directions

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

Even when smoke was imposed on species occurring naturally in non-fire-prone habitats, and they revealed positive responses, further probing showed that their sibling ecotypes, sisters, and/or ancestors had evolved in fire-prone environments. Research on the biochemistry of germination in *Arabidopsis* is pointing to the presence of a KAR-receptive protein (KAI2) among flowering plants generally [43] that also implicates fire in directing the evolution of KAR-sensitivity. Far from being exceptions to the rule, fire remains central to understanding smoke-sensitivity even in (apparently) non-fire-prone environments. This means that the plants inherited the relevant gene mechanism for implementing the fire-adapted trait even though it may not be expressed currently or under selection, because this is context-driven.

Outstanding Questions

Is fire-proneness also a necessary pre-requisite for the evolution of fire-killed species (with seed storage) and resprouters (not considered here)?

Does fire-proneness precede the 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 the 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 KAR-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 specific loci in the genome?

Are skeptics able to articulate what they would accept as evidence that fire has played a key role in directing the evolution of at least some of the Earth's terrestrial flora?

Future Research

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

Reasons for reluctance to accept the role of fire in the evolution of plant traits include (i) lack of evidence from the fossil record – this is being tackled by a change in the priorities of paleontologists who are starting to look for fossil charcoal and fire-related traits [10,23]; (ii) lack of comparative experiments that show superior fitness in the presence of fire compared with other possible limiting factors such as drought (see Causley *et al.* [44] for a rare exception); although increasing numbers of studies are seeking to identify what component of fire is the specific cause of the response [39], possible interaction effects are rarely considered; (iii) lack of trust in, or familiarity with, these new ‘indirect’ ancestral trait reconstruction methods, that is already proving not to be justified [10,23]; and (iv) a general mind-set that views fire as a destructive rather than a rejuvenating force in nature [45], and consequently fire is ignored or rejected as a possible explanation for newly documented evolutionary events.

The final frontier is linking fire cues to genetic mechanisms that effectively bypass the fire-proneness-prerequisite issue because this must be true by definition. Great progress has been made with the KAR component of smoke and the germination of *Arabidopsis* [43,47], but without fully appreciating its ancestral link with fire. However, the topic is completely open for exploring the other fire cues and fire-related traits as discussed here (see Outstanding Questions). It starts by acknowledging that fire preceded (but later co-occurred with) drought as the dominant selective force in extant fire-prone systems.

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Supplemental Information

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References

- Paula, S. *et al.* (2009) Fire-related traits for plant species of the Mediterranean Basin. *Ecology* 90, 1420
- He, T. *et al.* (2016) A 350-million-year legacy of fire adaptation among conifers. *J. Ecol.* 104, 352–363
- Araújo, G.M. *et al.* (2013) Fire drives the reproductive responses of herbaceous plants in a Neotropical swamp. *Plant Ecol.* 214, 1479–1484
- Mojzes, A. *et al.* (2015) Is the positive response of seed germination to plant-derived smoke associated with plant traits? *Acta Oecol.* 65–66, 24–31
- Flematti, G.R. *et al.* (2013) Karrikin and cyanohydrin smoke signals provide clues to new endogenous plant signaling compounds. *Mol. Plant.* 6, 29–37
- Hopper, S.D. *et al.* (2016) Biodiversity hotspots and Ocbil theory. *Plant Soil* 403, 167–216
- Keeley, J.E. *et al.* (2011) Fire as an evolutionary pressure shaping plant traits. *Trends Plant Sci.* 16, 406–411
- Mucina, L. and Wardell-Johnson, G.W. (2011) Landscape age and soil fertility, climatic stability, and fire regime predictability: beyond the OCBIL framework. *Plant Soil* 341, 1–23
- Groom, P.K. and Lamont, B.B. (2015) *Plant life of Southwestern Australia: Adaptations for Survival*, De Gruyter Open
- Carpenter, R.J. *et al.* (2015) Fossil evidence for open, Proteaceae-dominated heathlands and fire in the Late Cretaceous of Australia. *Am. J. Bot.* 102, 2092–2107
- Dayrell, R.L. *et al.* (2016) Phylogeny strongly drives seed dormancy and quality in a climatically buffered hotspot for plant endemism. *Ann. Bot.* Published online August 27, 2016. <http://dx.doi.org/10.1093/aob/mcw163>
- Litsios, G. *et al.* (2014) Effects of a fire response trait on diversification in replicated radiations. *Evolution* 68, 453–465
- Byers, B.A. *et al.* (2014) First known fire scar on a fossil tree trunk provides evidence of Late Triassic wildfire. *Palaeogeog. Palaeoclim. Palaeoecol.* 411, 180–187
- Baskin, J.M. *et al.* (2000) Taxonomy, anatomy and evolution of physical dormancy in seeds. *Plant Sp. Biol.* 15, 139–152
- He, T. *et al.* (2011) Banksia born to burn. *New Phytol.* 191, 184–196
- He, T. *et al.* (2012) Fire-adapted traits of *Pinus* arose in the fiery Cretaceous. *New Phytol.* 194, 751–759

17. Lamont, B.B. and He, T. (2012) Fire-adapted Gondwanan angiosperm floras arose in the Cretaceous. *BMC Evol. Biol.* 12, 223
18. Lamont, B.B. *et al.* (2013) Adaptive responses to directional trait selection in the Miocene enabled Cape proteas to colonize the savanna grasslands. *Evol. Ecol.* 27, 1099–1115
19. He, T. *et al.* (2016) A Cretaceous origin for fire adaptations in the Cape flora. *Sci. Rep.* 6, 34880
20. Lamont, B.B. and Downes, K.S. (2011) Fire-stimulated flowering among resprouters and geophytes in Australia and South Africa. *Plant Ecol.* 212, 2111–2125
21. Bytebier, B. *et al.* (2011) Estimating the age of fire in the Cape flora of South Africa from an orchid phylogeny. *Proc. Roy. Soc. B* 278, 188–195
22. Crisp, M.D. *et al.* (2014) Clock model makes a large difference to age estimates of long-stemmed clades with no internal calibration: a test using Australian grasses. *BMC Evol. Biol.* 14, 263
23. Falcon-Lang, H.J. *et al.* (2016) The oldest *Pinus* and its preservation by fire. *Geology* 44, 303–306
24. Tonnabel, J. *et al.* (2014) Convergent and correlated evolution of major life-history traits in the angiosperm genus *Leucadendron* (Proteaceae). *Evolution* 68, 2775–2792
25. Sauquet, H. *et al.* (2009) Contrasted patterns of hyperdiversification in Mediterranean hotspots. *Proc. Nat. Acad. Sci. U.S.A.* 106, 221–225
26. Hopper, S.D. *et al.* (2009) Molecular phylogenetics of Haemodoraceae in the Greater Cape and Southwest Australian floristic regions. *Mol. Phylogenet. Evol.* 51, 19–30
27. Downes, K.S. *et al.* (2015) Fire-related cues and the germination of eight *Conostylis* (Haemodoraceae) taxa, when freshly collected, after burial and after laboratory storage. *Seed Sci. Res.* 25, 286–298
28. Downes, K.S. (2014) Do fire-related cues, including smoke-water, karrikinolide, glyceronitrile and nitrate, stimulate the germination of 17 *Anigozanthos* taxa and *Blancoa canescens* (Haemodoraceae)? *Aust. J. Bot.* 62, 347–358
29. Muir, R.A. *et al.* (2015) Lower Cretaceous deposit reveals first evidence of a post-wildfire debris flow in the Kirkwood Formation, Algoa Basin, Eastern Cape, South Africa. *Cretaceous Res.* 56, 161–179
30. Ma, G-H. *et al.* (2006) Comparative enhancement of germination and vigor in seed and somatic embryos by the smoke chemical 3-methyl-2H-furo[2,3-C]pyran-2-one in *Baloskion tetraphyllum* (Restionaceae). *In Vitro Cell. Develop. Biol. Plant* 42, 305–308
31. Mao, K. *et al.* (2013) Distribution of living Cupressaceae reflects the breakup of Pangea. *Proc. Nat. Acad. Sci. U.S.A.* 109, 7793–7798
32. Osborne, C.P. (2008) Atmosphere, ecology and evolution: what drove the Miocene expansion of C4 grasslands? *J. Ecol.* 96, 35–45
33. Malabadi, R.B. *et al.* (2012) Smoke saturated water promoted in vitro seed germination of an epiphytic orchid *Oberonia ensiformis* (Rees) Lindl. *Res. Plant Biol.* 2, 32–40
34. Givnish, T.J. *et al.* (2015) Orchid phylogenomics and multiple drivers of their extraordinary diversification. *Proc. Roy. Soc. B* 282, 20151553
35. Bernardos, S. *et al.* (2007) Composition, geographical affinities and endemism of the Iberian Peninsula orchid flora. *Nordic J. Bot.* 25, 227–237
36. Nelson, D.C. *et al.* (2009) Karrikins discovered in smoke trigger *Arabidopsis* seed germination by a mechanism requiring gibberellic acid synthesis and light. *Plant Physiol.* 149, 863–873
37. Pennacchio, M. *et al.* (2007) Allelopathic effects of plant-derived aerosol smoke on seed germination of *Arabidopsis thaliana* (L.) Heynh. *Res. Lett. Ecol.* 2007 6508
38. Hoffmann, M.H. *et al.* (2003) Analysis of molecular data of *Arabidopsis thaliana* (L.) Heynh. (Brassicaceae) with geographical information systems (GIS). *Mol. Ecol.* 12, 1007–1019
39. Daws, M.I. *et al.* (2007) Butenolide from plant-derived smoke enhances germination and seedling growth of arable weed species. *Plant Growth Regul.* 51, 73–82
40. Long, R.L. *et al.* (2011) Seeds of Brassicaceae weeds have an inherent or inducible response to the germination stimulant karrikinolide. *Ann. Bot.* 108, 933–944
41. Baker, K.S. *et al.* (2005) Dormancy release in Australian fire ephemeral seeds during burial increases germination response to smoke water or heat. *Seed Sci. Res.* 15, 339–348
42. Beilstein, M.A. *et al.* (2010) Dated molecular phylogenies indicate a Miocene origin for *Arabidopsis thaliana*. *Proc. Nat. Acad. Sci. U.S.A.* 107, 18724–18728
43. Waters, T. (2014) The karrikin response system of *Arabidopsis*. *Plant J.* 79, 623–631
44. Causley, C.L. *et al.* (2016) Fitness benefits of serotiny in fire- and drought-prone environments. *Plant Ecol.* 217, 773–779
45. Pyne, S.J. (2016) Fire in the mind: changing understandings of fire in Western civilization. *Phil. Trans. R. Soc. B* 371, 20150166
46. Gustafsson, A.L.S. *et al.* (2010) Reassessing the temporal evolution of orchids with new fossils and a Bayesian relaxed clock, with implications for the diversification of the rare South American genus *Hoffmannseggella* (Orchidaceae: Epidendroideae). *BMC Evol. Biol.* 10, 177
47. Donohue, K. *et al.* (2005) Environmental and genetic influences on the germination of *Arabidopsis thaliana* in the field. *Evolution* 59, 740–757