Evol Ecol (2017) 31:603–617
DOI 10.1007/s10682-017-9905-4

IDEAS & PERSPECTIVES

African geoxyles evolved in response to fire; frost came later

Byron B. Lamont[1] · **Tianhua He**[1] · **Juli G. Pausas**[2]

Received: 5 May 2017 / Accepted: 23 May 2017 / Published online: 27 May 2017

Abstract It has been proposed in separate studies that fire or frost were the critical selective agents in the evolution of subshrub geoxyles (SGs) in African subtropical grasslands. We attempt to resolve this controversy by examining the evolution of SGs among the entire genus *Protea* that is widespread throughout southern/central Africa. We show that SGs are not confined to grasslands but occur in a wide range of non-forest vegetation types, including mediterranean shrublands. SG proteas arose 1–11 million years ago but their multiple origins among other geoxyles, confounded by strong intraspecific variability among grassland species, makes it impossible to identify the ancestral growth form. We conclude that the evolutionary history of SG proteas has occurred under lightning-prone conditions that promoted fire and were essentially frost-free; exposure to frost has been limited to certain elevated locations in more recent times. This is supported by many SGs having pyrogenic flowering and lack of seed storage among grassland species.

Keywords Fire · Frost · Geoxyle · Grassland · *Protea* · Shrubland

Subshrub geoxyles in Africa: the current controversy

Identifying the agents of selection responsible for the evolution of critical adaptive traits is a key task in evolutionary ecology. There has been recent controversy about the origins of the resprouting subshrubs in central/southern (summer-rainfall) Africa. Maurin et al. (2014) examined the origin of these suffrutescent (hereafter, subshrub) geoxyles [i.e.,

---

Electronic supplementary material The online version of this article (doi:10.1007/s10682-017-9905-4) contains supplementary material, which is available to authorized users.

✉ Tianhua He
Tianhua.He@curtin.edu.au

[1] Department of Environment and Agriculture, Curtin University, PO Box U1987, Perth, WA 6845, Australia

[2] CIDE-CSIC, Ctra. Naquera km 4.5, 46113 Montcada, Valencia, Spain

plants with underground woody structures, sometimes supplemented by rhizomes, that enable resprouting after dieback, Lindman (1914) in Du Rietz (1931)] and contended that they arose in the late Cenozoic in response to frequent grassland fires. Finckh et al. (2016) responded that their evidence indicated frost instead was the key selective force as frost damage was common and recurrent fire was too recent. Davies et al. (2016) reiterated their previous interpretation but noted that much remained to be known about the evolutionary history of this growth form. Earlier, White (1977) believed that the distribution of geoxylic subshrubs was edaphically controlled, associated with nutrient-poor, seasonally waterlogged sands. Here, we address the stated need for more intensive sampling of lineages and their distribution (Frost 2012; Davies et al. 2016) by examining resprouting in the entire genus *Protea* that has a 28-million-year history (Valente et al. 2010). This genus is widespread in Africa, stretching from the SW tip of the continent to central Africa with outliers reaching Ethiopia in the NE and Guinea in the NW (Valente et al. 2010). We used the distribution and phylogenetic history of *Protea* to examine the claims that a) subshrub geoxyles (SGs) in Africa are restricted to the savanna grasslands with a summer rainfall (White 1977; Maurin et al. 2014) since *Protea* is also widespread elsewhere, and b) that SGs are more likely an adaptive response to the ravages of frost rather than of fire (Finckh et al. 2016). The answers involve knowing: (1) the distribution of proteas in relation to vegetation type, and incidence of fire and frost; (2) the morphological limits and genetic *vs* environmental controls of relevant growth forms; (3) the relative damage caused by fire *vs* frost; (4) whether fire or frost arose first as the key selective agent; and (5) whether SGs are associated with other biological attributes that might give a clue to the critical selective agent.

Maurin et al. (2014) listed 23 proteas, which occur in the subtropical savanna grasslands of central Africa with a predominantly summer rainfall, that they considered to be subshrub geoxyles (SGs). We examined their morphological traits and compared them with the majority of proteas that occurs in the Cape shrublands, with a winter-(to uniform)-rainfall, to see if any SGs also occurred there. Finckh et al. (2016) pointed out that fires at their Angolan Plateau site were human- rather than lightning-caused, and therefore were too recent to have an evolutionary impact. The distributions of all SG proteas were therefore compared against selected temperature, lightning-strike and fire records in an attempt to identify limiting factors in common. This was supplemented by a comparison of species/lineage ages in each region that might provide a clue to the climates under which they arose.

Morphology and habitat-type of subshrub geoxylic proteas

Six of the 23 endemic grassland proteas listed by Maurin et al. (2014) as SGs were included in the Valente et al. (2010) phylogeny and we added two more from the Valente analysis (Table S1). The heights of these two species were not significantly different from the other 23 ($P = 0.650$, t test). We then identified 17 apparent SG species that are confined to the Cape shrublands (Table S1) among those used by Valente. Heights of the shrubland proteas were not significantly different from the grassland species though with a tendency to be lower and less variable (Table 1). All 42 proteas resprout from rootstocks most of which are best described as lignotubers (Frost 2012; Lamont et al. 2013), i.e., swollen woody structures that can store buds anywhere on their upper surface, supported by a woody primary root of much narrower dimensions (Clarke et al. 2013). Maurin et al.

Table 1 Traits for subshrub geoxylic proteas (see Supplementary Table S1 for details) and all subshrub geoxyle species analysed by Maurin et al. (2014)

Trait	Grassland/savanna proteas ($n = 17$)	Shrubland proteas ($n = 25$)	All species in Maurin ($n = 35$)
Climate–rainfall	Summer	Winter (uniform)	Summer
Mean height (m)	0.66	0.41*	– (mostly rhizomatous so must be short)
Minimum height (m)	0.10	0.15	–
Maximum height (m)	1.00	1.20	–
Rhizomatous (%)	12	70.5	66.5
Creeping/decumbent (%)	28	6	10
Erect/suberect (%)	60	23.5	13.5
Branches undivided (%)	80	82	–
Sparsely branched (%)	20	17.5	–
Deciduous/stems ephemeral (%)	12	23.5	7? (poorly known)
Fire-stimulated flowering (%)	25? (poorly known)	35	44
Seed storage (plant or soil) (%)	0	100	0

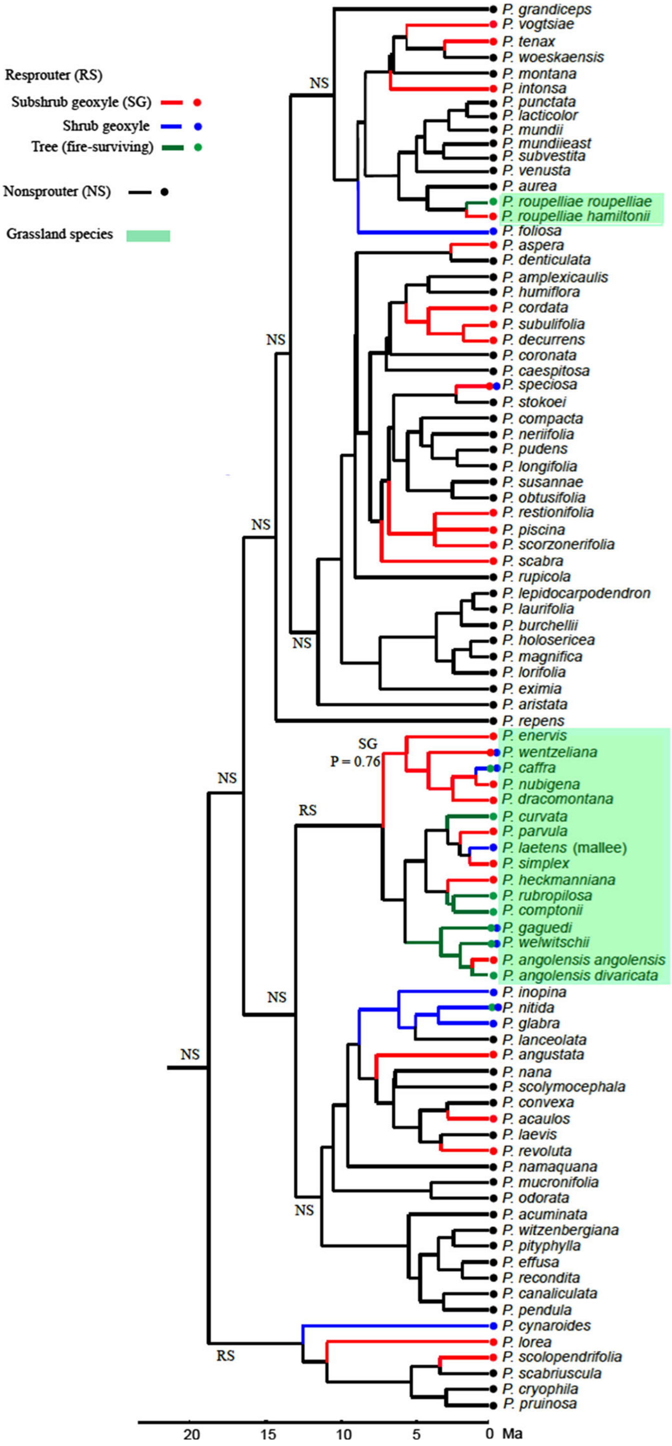
* $P = 0.082$ (unequal variances)

– means that data not supplied nor available in the literature

All arose from rootstocks (usually lignotubers) sometimes with creeping stems or rhizomes

(2014) treated all the SG species they recorded among 22 families as arising from xylopodia, i.e., swollen woody structures with a few apical buds supported by swollen roots, but only recorded previously from Brazil where unrelated SGs abound (Rizzini and Heringer 1961; Simon et al. 2009). However, the morphological descriptions/images for 30 SGs analysed by Maurin et al. (2014) that we examined were more likely to possess lignotubers and woody rhizomes with two having taproot tubers without any hard wood (data not provided here). Unlike the grassland geoxyles, most shrubland proteas have rhizomes arising from burls with fewer having a procumbent or erect habit, more similar to the Maurin et al. (2014) master list (Table 1). However, short, simple or sparsely divided branches, as in Maurin's list, characterize all shrubland and grassland species. Though SGs are often considered to be deciduous or with ephemeral branches (White 1977), this was only occasionally recorded in any of the three lists. Thus, a wide range of woody, bud-storing structures that support low, spreading shoot systems can be identified among SGs (xylopodia, lignotubers, woody rhizomes, taproot tubers, root suckers) so that no one resprouting mechanism accounts for their ability to survive disturbance.

The phylogeny of Valente et al. (2010) included 17 shrubland and 9 grassland proteas that we were able to assign to SGs (Fig. 1). It was of interest to know if subshrub geoxyles (SGs) are more likely to occur in one vegetation type rather than another. We constructed a dated phylogeny for *Protea* based on Valente et al. (2010) and Lamont et al. (2013). We assigned the growth forms, subshrub and shrub geoxyle and fire-surviving tree, and the vegetation type (grassland or shrubland) to all species from Table S1, Chisumpa and Brummitt (1987) and Rebelo (2001). Taking their phylogenetic position into account, we


Resprouter (RS)
Subshrub geoxyle (SG)
Shrub geoxyle
Tree (fire-surviving)
Nonsprouter (NS)
Grassland species
NS
NS
NS
NS
NS
SG
P = 0.76
RS
NS
NS
NS
RS
P. grandiceps
P. vogtsiae
P. tenax
P. woeskaensis
P. montana
P. intonsa
P. punctata
P. lacticolor
P. mundii
P. mundiieast
P. subvestita
P. venusta
P. aurea
P. roupelliae roupelliae
P. roupelliae hamiltonii
P. foliosa
P. aspera
P. denticulata
P. amplexicaulis
P. humiflora
P. cordata
P. subulifolia
P. decurrens
P. coronata
P. caespitosa
P. speciosa
P. stokoei
P. compacta
P. neriifolia
P. pudens
P. longifolia
P. susannae
P. obtusifolia
P. restionifolia
P. piscina
P. scorzonerifolia
P. scabra
P. rupicola
P. lepidocarpodendron
P. laurifolia
P. burchellii
P. holosericea
P. magnifica
P. lorifolia
P. eximia
P. aristata
P. repens
P. enervis
P. wentzeliana
P. caffra
P. nubigena
P. dracomontana
P. curvata
P. parvula
P. laetens (mallee)
P. simplex
P. heckmanniana
P. rubropilosa
P. comptonii
P. gaguedi
P. welwitschii
P. angolensis angolensis
P. angolensis divaricata
P. inopina
P. nitida
P. glabra
P. lanceolata
P. angustata
P. nana
P. scolymocephala
P. convexa
P. acaulos
P. laevis
P. revoluta
P. namaquana
P. mucronifolia
P. odorata
P. acuminata
P. witzenbergiana
P. pityphylla
P. effusa
P. recondita
P. canaliculata
P. pendula
P. cynaroides
P. lorea
P. scolopendrifolia
P. scabriuscula
P. cryophila
P. pruinosa
20
15
10
5
0
Ma

◀**Fig. 1** Chronogram for *Protea* showing grassland (highlighted in *green*) and shrubland (not highlighted) species, with lineages for nonsprouting shrubs (*black*), and resprouting subshrubs (*red*), shrubs (*blue*) and trees (*green*) indicated

tested for any correlated shift of SGs between the habitat of grassland and shrubland (see Notes S1, Supplementary Material for details). There was no contingent association between presence of SG proteas and their location (shrubland *vs* grassland) ($\log_{e}$-BF $= -10.1$, i.e., $P \gg 0.05$, see Notes S1, Supplementary Material). Thus, we conclude that SGs among proteas are not confined to subtropical grasslands (with a summer rainfall) but are also prominent in sclerophyllous shrublands predominantly under a mediterranean climate (with a summer drought and winter rainfall). This is also true for *Leucospermum* (Proteaceae) with one species listed by Maurin et al. (2014) but 11 SGs also in the Cape shrublands (Rebelo 2001). Similarly, a unique lineage of seven prostrate, serotinous banksias (Proteaceae), six resprouting via woody rhizomes (Witkowski and Lamont 1997), occurs in shrublands and woodlands of mediterranean southwestern Australia, while the single species in savanna is a fire-tolerant tree without seed storage (He et al. 2011). It is also worth noting that the original examples of 'dwarf-shrubs' and 'herbaceous' geoxyles listed by Lindman (1914) [in Du Rietz (1931)] were from Europe, e.g. *Helianthemum chamaecistus* (with a root-crown). Other early researchers also noted the presence of SGs in non-grasslands: e.g. *Myrica elliptica* in the low shrublands of the Outeniqua Range, 350 km east of Cape Town (Fig. 2, Burtt Davy 1922), and the xylopodial-bearing *Pterocaulon interruptum* on the east coast of Brazil "far away from the savanna" (Lindman 1900). Thus, SGs generally are not confined to grasslands but occur in a wide range of open vegetation types.

Distribution of subshrub geoxylic proteas and environment

For the nine subshrub geoxylic proteas in grasslands analysed by Valente et al. (2010), individual species ranged 15–78% of their populations on loam to 5–85% on sand while one occurred on clay and another on peat (collated from Rebelo 2009). Thus, edaphic constancy within and between SGs is negligible and soil type is unlikely to explain their distribution (contrast White 1977), nor their evolution (supported by Lamont et al. 2013). SGs of the Cape occur within 120 km of the coast but the SE African SGs occur up to 320 km inland near Polokwane (Pietersburg) (Fig. 2). Most shrubland and grassland *Protea* SGs occur in the area outside the mean winter isotherm of 10 °C, i.e., the area with 0–20 annual frost days (Finckh et al. 2016), including some species in the frost-free zone (<50 km from the coast). The 7 °C isotherm surrounds the area with 35–70 annual frost days and includes about 20% of the grassland populations. It is clear that most SGs do not occur in particularly frost-prone areas but some do. Since the distribution of SG proteas strongly reflects the distribution of proteas generally (see map in Valente et al. 2010) we conclude that there is nothing especially frost-tolerant about them that can account for their belowground, bud-storage efficacy.

The symptoms of both fire and frost are to cause dieback of adult plants and death of young plants in particular. However, dieback of adults due to frost is never as severe as that due to fire because the bases of aerial stems survive and a few axillary buds remain intact (Holdo 2005, Fig. 2c in Finckh et al. 2016). This is attributable to the insulating effect of the highly flammable litter and living grass layers, and the fact that winter-dormant buds, as occur with grassland proteas (Smith and Granger 2017), are highly resistant to freezing

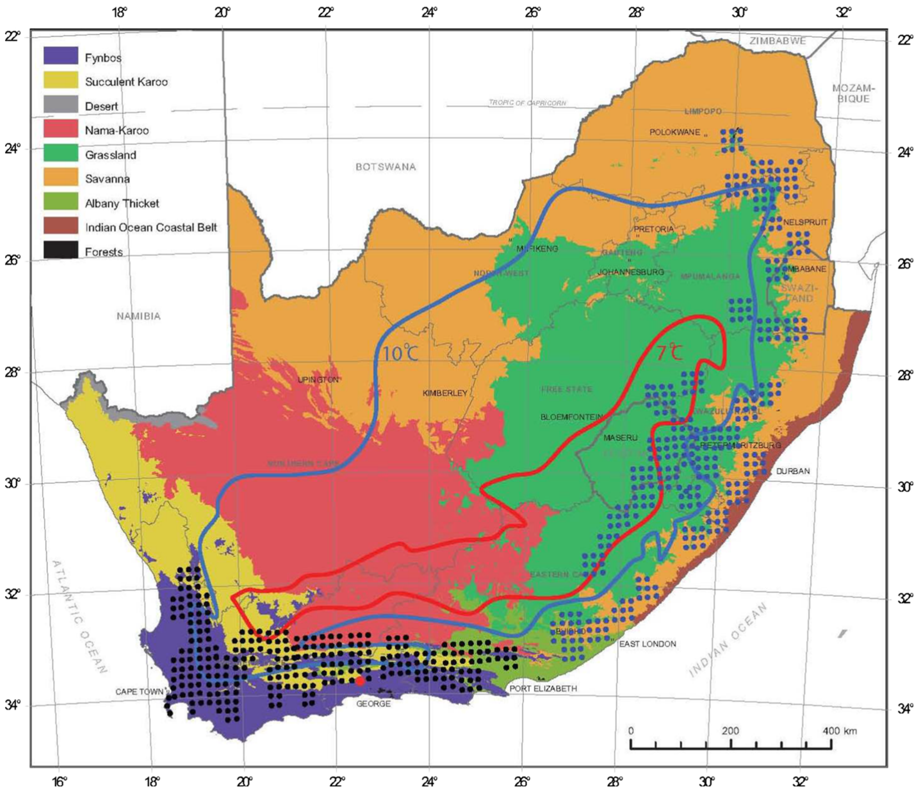


Fig. 2 Biome map of South Africa (from Mucina and Rutherford 2006) to which has been added the distribution of subshrub geoxylic proteas in shrubland (*black dots*) and grassland (*blue dots*) (from Rebelo 2001). The orange dot is the location of a subshrub geoxyle, *Myrica elliptica* (Myricaceae), outside the grasslands as reported by Burtt Davy (1922). Also added are two selected isotherms for mean winter temperatures (http://www.south-africa-tours-and-travel.com/south-africa-climate.html). Note that the coastal strip to 50 km inland is usually frost-free (Finckh et al. 2016)

(Ristic and Ashworth 1997). Receiving 60 frost-days annually in the Drakensberg Mountains, the non-SG species, *Protea roupelliae* subsp. *roupelliae*, had a 40% survival rate over eight years since establishment when unburnt, but only 4% survival when burnt annually (Smith and Granger 2017). Adult proteas are moderately resistant to frost (Rebelo 2009). Thus, *P. cynaroides*, experiences a 50% reduction in leaf chlorophyll fluorescence at 5.2 °C (Bannister and Lord 2006) but complete recovery would occur from the unharmed axillary buds. The same species would lose all its aboveground mass if burnt but there is full recovery from fire via buds in the lignotuber (Rebelo 2001). Wakeling et al. (2012) showed that some dieback of acacia seedlings in the South African Highveld occurred from 1350 m elevation (23 frost days during the trial) but mortality only exceeded the savanna sites (located at <1000 m) from 1650 m (37 frost days). So frost can kill young plants but there are vast areas of grassland (from 1000 to 1650 m) where frost is not severe enough to kill seedlings. Even young, fire-killed proteas show some frost-resistant traits (Prunier et al. 2012). Wakeling et al. (2012) concluded that the absence of trees in the grasslands was not due to frost *per se* but to slow growth rates that rendered the young plants more vulnerable to the frequent risk of incineration. That SG proteas do occur in grasslands must therefore depend on their rapid development of belowground bud-storing structures that are primarily resistant to fire.

In the absence of human intervention, savanna/grassland fires are initiated by lightning (Kennedy and Potgieter 2003). Finckh et al. (2016) noted that the incidence of lightning was low in Angolan grasslands during winter (dry season) when they were most likely to burn (that we also confirm, Fig. S1), reducing support for the SG as a fire-related trait. However, most current fires are deliberately lit, whereas prior to human occupation of the area, fires were historically most likely to occur at the start of the wet season (September–November) when lightning is abundant (Fig. S1; Kennedy and Potgieter 2003). The South African Highveld is fire-prone from March to November (Smith and Granger 2017) with abundant lightning in March–April and especially October–November with some in August–September that even now probably contributes to early dry and wet season fires (Fig. 3). Despite human intervention, there is still a close association between the incidence of lightning strikes and fire frequency (Manry and Knight 1986). Plotting lightning isolines on a vegetation map of South Africa shows that, in fact, the southeast grasslands are the most lightning-prone part of South Africa (Fig. S2; also see Keeley et al. 2012). The western Cape is in the range 100–250 lightning strikes/50 × 50 km^2/annum increasing to 3500 strikes at the eastern extreme. Most of the grasslands are in the range 1750–4400 strikes. Coupled with their high flammability, this renders the grasslands strongly fire-prone. While it is confounded with management fires, grasslands typically burn at 2–3-year intervals (Roques et al. 2001) and many SGs may even burn annually (Medwecka Kornas 1980). Indeed, with the long human occupation of the area (300,000 years; Archibald et al. 2012) and the switch to winter fires further inhibiting recovery (Kennedy and Potgieter 2003), resprouting shrubs would not only be continually pruned back to the subshrub category (Hoffmann and Solbrig 2003) but it is sufficient time for some ecological (e.g. spatial redistribution) and evolutionary changes to have taken place. This variable incidence of fire might well explain why it has been possible to recognize so many subspecific ranks among woodland/grassland proteas.

Fires are less frequent in the Cape shrublands (Fig. 3a, b), typically at 10–20-year intervals (Rundel et al. 2016) and here peak incidence of lightning and fire activity coincide (Fig. 3). Not only has the Cape had a shorter history of human occupation (165,000 years; Brown et al. 2009) but the timing conducive to human-lit fires (again the dry season) coincides with the occurrence of lightning (Fig. 3), thus reducing the impact of humans on the presence of SGs there. The abundance of fixed-form, lignotuberous-rhizomatous proteas in the Cape might be more attributed to the presence of a mediterranean climate, with its severe summer droughts and intense, moderate-interval fires (Lamont et al. 2013; Causley et al. 2016) with frosts rare except in the mountain ranges. In conclusion, while the incidence of lightning and frost varies greatly in the Cape, it is clear that any site where SG proteas occur was far more likely to be burnt by lightning-initiated fires than to experience frost by the late Quaternary.

Age and evolution of subshrub geoxylic proteas

Protea originated in the Cape 27.8 million years ago (Ma) under fire-prone conditions (Lamont et al. 2013; Fig. 1). Using continuous-time Markov model of trait evolution for discrete traits (Pagel and Meade 2006), we reconstructed the evolutionary trajectory of key traits in *Protea* (see Notes S1, Supplementary Material). The ancestral condition was

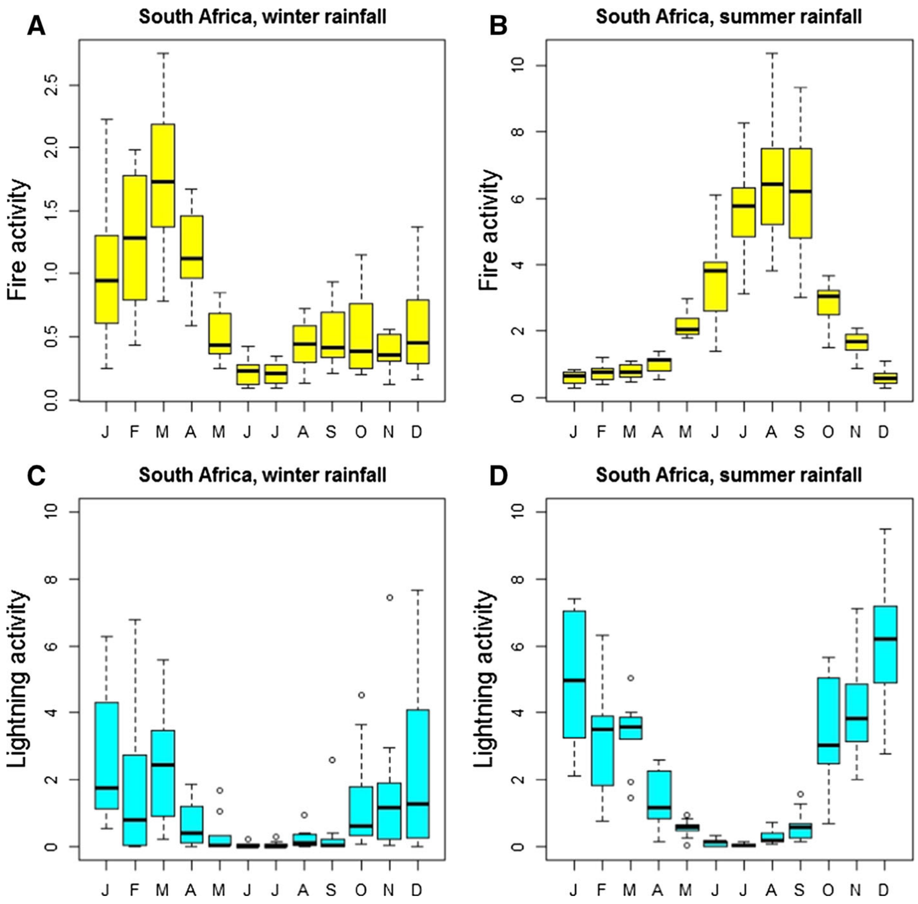


Fig. 3 Monthly fire activity (**a**, **b**) and lightning activity (**c**, **d**) for the winter (**a**, **c**) and summer (**b**, **d**) rainfall regions of South Africa, estimated from remote sensing data. The Y-axis indicates relative activity and therefore has no units; variability about the mean refers to 15 and 9-year variability for fire and lightning, respectively (details in Notes S1, Supplementary Material). Equivalent information for the Angolan Plateau (as studied by Finckh et al. 2016) is shown in Fig. S1

nonsprouting (fire-killed) though a resprouting lineage appeared early, at 18.7 Ma. The rest of the clade remained nonsprouting until the resprouting grassland subclade emerged 12.7 Ma. This did not diversify until a SG lineage arose 7.0 Ma with a sister lineage whose ancestral state is unclear as it is currently a mixture of resprouting SGs, shrubs and trees. Thus, SGs have arisen several times throughout the evolutionary history of the genus. Overall, shrubland SGs are twice the age of grassland SGs (Table 2) with *P. lorea* in the shrublands the oldest at 10.8 Ma. Similarly, shrubby resprouters (that we treat as geoxyles with a larger growth form, Table 3) are older in the Cape (by 3.7 My) with *P. cynaroides* oldest at 12.4 Ma. Thus, resprouting shrubs have a longer history than SGs in the Cape (by 1.6 My), though there is no indication that shrub geoxyles were the ancestors of the subshrub geoxyles. This contrasts with the grasslands where resprouting subshrubs, shrubs and trees have similar mean ages, in the range 3–2 Ma, again with no indication of any evolutionary sequence (Table 2). Such a mixture of phylogenetic relationships,

Table 2 Mean ages (plus max(imum) and min(imum) ages) in million years of subshrub and shrub geoxylic *Protea* species/lineages (defined in Fig. S4) in shrubland (s, winter-rainfall) and grassland (g, summer-rainfall) habitats derived from the chronogram in Fig. 1

Protea growth form	Habitat	*N*	Mean	Max	Min	*P* (*t* test)
Subshrub geoxyles	Shrubland	14	4.9	10.8	1.5	0.0344 (1 − t, unequal)
	Grassland	6	2.5	6.9	1.0	
Shrub geoxyles	Shrubland	5	7.8	12.4	2.5	0.0098 (1 − t, equal)
	Grassland	5	2.1	3.6	0.8	
a. Subshrub geoxyles	s + g	20	4.2	10.8	1.0	0.2637 (1 − t, equal)
b. Shrub geoxyles	s + g	10	5.0	12.4	0.8	
c. Subshrub geoxyles (Maurin)	a vs c (g)	32	3.6	15.2	0.3	0.5386 (2 − t, unequal)
1. Subshrub geoxyles	Grassland	6	2.5	6.9	1.0	
2. Shrub geoxyles	1 vs 2 (g)	5	2.1	3.6	0.8	0.7272 (2 − t, equal)
3. Trees	1 vs 3 (g)	4	3.0	5.6	0.9	0.7572 (2 − t, equal)

t test refers to a comparison between habitats; 1 − t (ailed) tests applied when the directional hypothesis was supported numerically and 2 − t (ailed) when they were not or not applicable. (un)equal refers to variances. Maurin = ages from Maurin et al. (2014) excluding proteas. Growth forms and habitats from Table S1 and Rebelo (2001)

Table 3 Key to resprouting types showing the four subdivisions of geoxyles and contrasting them with geophytes, aeroxyles and caudiciform plants

1. Resprouter with non-woody underground parts that bear a few concealed buds (bulb, corm, primary rhizomes, swollen stems/roots), above-ground parts ephemeral or, if present, incinerated by fire—geophyte
2. Resprouter with woody underground parts (lignotuber, xylopodium, secondary rhizomes/roots) that bear a few to many concealed buds, sometimes also above ground and many equisized stems—geoxyle
   1. *Subshrub* (≤1 m tall)—all above-ground parts sparsely branched and ephemeral, or, if present or woody, incinerated by fire
   2. *Shrub* (>1–2.5 m tall)—strongly branched stems woody but mostly incinerated by fire
   3. *Mallee* (>2.5 m tall)—strongly branched stems woody and survive fire
   4. *Tree (clonal)* (>2.5 m tall)—trunk and branches woody and survive fire
3. Resprouter with woody trunk and strongly branched stems that bear many aerial concealed buds—*Tree (non-clonal)* (>2.5 m tall)—trunk and main branches survive fire—aeroxyle

Caudiciform plants whose non-woody trunks and apical buds survive fire and may exist for many years below ground before emerging (cycads, palms, grasstrees, treeferns, some aloes) have been omitted from this scheme

Note that some individual species may range from subshrubs to trees depending on growing conditions (Table S2)

confounded by strong intraspecific variability (Table S2), makes it impossible to identify the ancestral growth form among grassland proteas. Thus, we are not able to support the contention that SGs are necessarily derived from forest-dwelling relatives (Maurin et al. 2014). Mean ages of the 32 grassland SGs in 21 families (from Maurin et al. 2014, excluding *Protea*) were not significantly different (3.6 Ma) than for *Protea* with 20 SGs (4.2 Ma, Table 2).

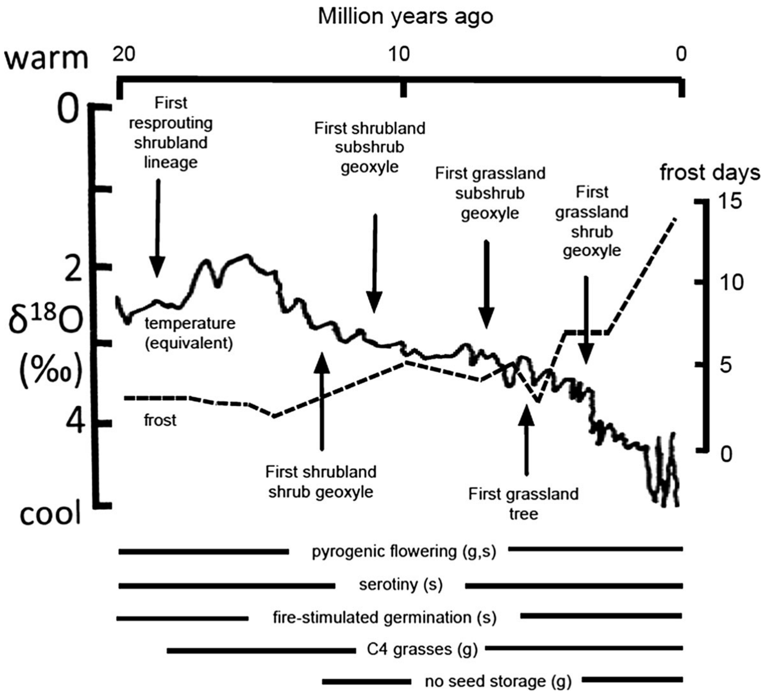


Fig. 4 Key times in the early evolution of resprouting proteas (from Fig. 1) relative to isotopic oxygen levels as a surrogate for mean world temperatures (adapted from Zachos et al. 2008) and mean of the estimated frost days pa in northern Germany at the same time (Utescher et al. 2009). The presence of surrogates for fire over the same period is indicated by the continual presence of lineages in the African grasslands (g) or shrublands (s) with pyrogenic flowering (Bytebier et al. 2011; He et al. 2016), serotiny (Lamont and He 2012), fire-stimulated germination (Lamont and He 2012, He et al. 2016), C4 grasses (Edwards et al. 2010) and lack of seed storage (Lamont et al. 2013)

Prevailing environment during early evolution of subshrub geoxylic proteas

Fire—South Africa has a long history of fire that has recently been traced to the Upper Cretaceous (Muir et al. 2015; He et al. 2016) when fire also directed evolution of the reproductive biology of the proteoid Proteaceae (Lamont and He 2012). By 20–15 Ma many plant traits tied to the presence of fire were present (Fig. 4). Terrestrial orchids in both the shrublands and grasslands (Bytebier et al. 2011) and bloodroots (Haemodoraceae) in the shrublands (He et al. 2016) were already displaying fire-stimulated flowering. Speciation of Restionaceae at the generic level, whose soil-stored diaspores are stimulated to germinate by fire (essentially smoke), peaked in the period 35–5 Ma (He et al. 2016). Confirmation of fire-proneness at these times comes from charcoal records in the highly mixed vegetation (with 6 Proteaceae pollen types) of Saldanha Bay, 100 km N of Cape Town, 25–20 Ma (Roberts et al. 2017) and the Namibian grasslands, 1600 km north of Cape Town, 9–3 Ma (Hoetzel et al. 2013). The association of fire with C4 grasslands is well-established (Scheiter et al. 2012) and these can be traced from 18 Ma in Africa, especially from 10 Ma (Edwards et al. 2010). The ancestral protea possessed on-plant seed storage (serotiny) where the key to its fitness advantage is fire-stimulated seed release and seedling recruitment in the post-fire environment (Lamont and Enright 2000; Causley et al. 2016). However, Lamont et al. (2013) showed that proteas were only able to invade the grasslands from the Cape once resprouting was combined with the loss of serotiny (and seed storage in general that is not only redundant but possibly maladaptive in an environment where fires are likely every year), which was achieved by 12.7 Ma. Thus, the first SGs arose in shrublands and grasslands that were both highly fire-prone but with quite different fire-properties (Fig. 3).

Climate—Diversification in *Protea* began just prior to the Mid-Miocene Climatic Optimum 15 Ma (Fig. 4), escalating from 6 Ma but declining markedly with the onset of glaciation 2.5 Ma in the Pleistocene (Fig. 1). Most extant resprouting species/lineages arose under conditions much warmer than currently, including the first SGs in shrubland and grassland (Fig. 4). The warmer the annual average temperatures, the less likely frosts will occur (Alexander et al. 2006). Utescher et al. (2009) estimated that annual ground frost days in northern Germany, with a temperature regime not unlike the mountain ranges where some SG proteas occur (Rebelo 2001), were close to zero from 15 Ma (mean temperature of coldest month >10 °C) and only began to rise substantially from 4 Ma when >50% of SG lineages had already arisen (Table 2). Thus, Sciscio et al. (2016) determined a mean annual temperature of 21 °C at 11.6 Ma in the Cape Peninsula (possessing several SGs) compared with a current temperature of 17 °C that even now is frost-free. The mean age of shrubland SGs coincided with the Miocene–Pliocene boundary and the grassland SGs with the Pliocene–Pleistocene boundary, so climates must have been less warm and only frost-prone during evolution of some upland grassland proteas (Fig. 4). Nevertheless, pollen records indicate that the vegetation was *Protea*-dominated savanna rather than grassland at this time, more akin to the current savanna to the north and west that is frost-free and has a history from the Pliocene (Vrba 1985; Hoetzel et al. 2013; Finckh et al. 2016). In fact, several SG proteas listed by Maurin et al. (2014) (*P. welwitschii* subsp. *hirta*, *P. wentzeliana*, *P. enervis*, *P. angolensis* var. *angolensis*, *P. inyanganiensis*) occur in this savanna-type (Hyde et al. 2016, so cannot be mapped in our Fig. 2). We conclude that the evolutionary history of SG proteas has occurred under strongly fire-prone conditions that were essentially frost-free and that exposure to frost has been limited to certain elevated (Highveld) locations in more recent times.

Covariation of the subshrub geoxylic habit with other reproductive traits

The incidence of fire-stimulated flowering in grassland savannas is exceptionally high among the world floras (Lamont and Downes 2011; Platt et al. 1988). This may be related to the abundance of herbaceous and geophytic species among which this trait is best represented. There can be no better proof of the effectiveness of fire as an agent of selection than pyrogenic flowering (He et al. 2016). While it is historically poorly recorded, and it is difficult to locate plants unburnt for any length of time in grasslands, our lists of SGs, including that of Maurin et al. (2014) (Table 1), show levels (25–44%) much higher than for fire-prone floras generally, e.g. 10% in Australian heathlands (Lamont and Keith 2017). Though it is far from universal, this confirms that many geoxyles have had a long association with fire that has promoted evolutionary changes in their sexual reproductive phenology as well as in their vegetative recovery.

There is no fitness advantage in storing seeds in an ecosystem where germination is likely every year as fires that create suitable conditions for germination and establishment are likely every year (Gignoux et al. 2009). Indeed, if there is a ‘cost’ associated with storage, it might even be maladaptive. Inspection of the species used by Maurin et al. (2014) shows that almost all have succulent fruits (with non-dormant seeds) or require no pretreatment for germination (Table 1; Weiersbye and Witkowski 2002). Similarly, Lamont et al. (2013) showed that the only way proteas could invade the savanna grasslands from the Cape was to reverse the near-universal trait of canopy seed storage in the shrublands to universal non-storage. Dayrell et al. (2017) also demonstrated that there is little soil seed storage in the Brazilian savannas but attributed it to the reliable wet seasons of so-called OCBIL (old, climatically-buffered, infertile landscape) systems. This interpretation cannot be accepted, for such major OCBILS as the Cape and southwestern Australia are characterized instead by their extremely high levels of seed storage (Enright et al. 2007)—the difference in levels of seed storage between these regions can in fact be attributed to their contrasting fire regimes. If frost was the dominant constraint in grasslands then soil storage would have been favoured historically, for the seed store remaining allows a second chance at seedling recruitment following initial failure (the so-called bet-hedging advantage). Even so, our detection of SG proteas in both shrublands (with seed storage) and grasslands (without seed storage) means that the likelihood of seed storage is not relevant to understanding the general biology of SGs.

Conclusions

We followed up the pairwise comparisons of subshrub (suffrutescent) resprouters with their taller sisters in many families by Maurin et al. (2014) with a full analysis of an entire genus, *Protea*, to test ideas on the relative importance of fire and frost in their evolution. Treating the subshrub geoxyle at a strictly morphological level, we find that they are just as likely to occur in the mediterranean shrublands as in the savanna grasslands of southern/central Africa. Since the distribution of the SG growth form reflects the distribution of proteas generally, it is not an adaptation to a particular fire regime, as this may vary greatly in terms of seasonality, frequency and intensity throughout its range. This greatly reduces the likelihood of frost as the key selective agent but not of lightning-caused fires where they occur, both now and historically. We show that shrubland subshrub geoxyles appeared much earlier than grassland subshrub geoxyles, consistent with the delay in migration of proteas from the Cape north and east to the subtropical grasslands, but that their evolutionary longevity still matches with species examined by Maurin et al. (2014). Thus, the background of subshrub geoxylic proteas in African grasslands is somewhat different from those in South America, morphologically (no xylopodia) and historically, with a fire-prone rather than a rainforest past (Simon et al. 2009). This may not be true for some other clades in the shrublands that have non-fire-prone affinities (such as *Searsia*, *Euclea*, *Olea*, *Rapanea*, Richard Cowling, pers. comm.)

Subshrub geoxylic proteas arose ultimately from nonsprouting (fire-killed) serotinous shrub lineages, usually in parallel with the origins of resprouting shrubs and trees without any later reversals to the parent type. The SG growth form is part of a continuum of size under the general umbrella of geoxyles that is not always fixed at species rank but subject to the vagaries of fire that continually reduces stature and promotes lignotuber evolution and enlargement (Notes S2: Why are SG proteas so short?). Any occasional observed resistance by SGs to frost can be attributed to their prior adaptation to ancestral fire. Above-average occurrence of pyrogenic flowering and universal absence of seed storage are correlated traits with resprouting that confirm the over-riding impact of fire. Frost can be considered a mild form of disturbance (compared with fire) in terms of its effects on plants so that there is no need for any morpho/physiological change in the underground bud-storing structures even in the presence of severe frost—SGs are already exapted to frost. However, while seedlings cannot adapt to fire, they may develop some frost-resistance in frost-prone populations (Prunier et al. 2012) so that mechanisms unrelated to resprouting may have enabled some frost-resistance to evolve more recently.

Acknowledgements We thank Richard Cowling, Tony Rebelo, David Ackerly and three other reviewers for comments on the manuscript. This work was supported by the Australian Research Council (DP120103389 and DP130013029).

References

Alexander LV, Zhang X, Peterson TC, Caesar J, Gleason B, Klein Tank AMG, Tagipour A (2006) Global observed changes in daily climate extremes of temperature and precipitation. J Geophys Res 111:D05109. doi:10.1029/2005JD006290

Archibald S, Staver AC, Levin SA (2012) Evolution of human-driven fire regimes in Africa. Proc Natl Acad Sci 109:847–852

Bannister P, Lord JM (2006) Comparative winter frost resistance of plant species from southern Africa, Australia, New Zealand, and South America grown in a common environment (Dunedin, New Zealand). NZ J Bot 44:109–119

Brown KS, Marean CW, Herries AIR, Jacobs Z, Tribolo C, Braun D, Roberts DL, Meyer MC, Bernatchez J (2009) Fire as an engineering tool of early modern humans. Science 325:859–862

Burtt Davy J (1922) The suffrutescent habit as an adaptation to environment. J Ecol 10:211–219

Bytebier B, Antonelli A, Bellstedt DU, Linder HP (2011) Estimating the age of fire in the Cape flora of South Africa from an orchid phylogeny. Proc R Soc B 278:188–195

Causley CL, Fowler WM, Lamont BB, He T (2016) Fitness benefits of serotiny in fire- and drought-prone environments. Plant Ecol 217:773–779

Chisumpa SM, Brummitt RK (1987) Taxonomic notes on tropical African species of *Protea*. Kew Bull 42:815–853

Clarke PJ, Lawes MJ, Midgley JJ, Lamont BB, Ojeda F, Burrows GE, Enright NJ, Knox KJE (2013) Resprouting as a key functional trait: how buds, protection and reserves drive persistence after fire. New Phytol 197:19–35

Davies TJ, Daru BH, Bank M, Maurin O, Bond WJ (2016) Multiple routes underground? Frost alone cannot explain the evolution of underground trees. New Phytol 209:910–912

Dayrell RL, Garcia QS, Negreiros D, Baskin CC, Baskin JM, Silveira FA (2017) Phylogeny strongly drives seed dormancy and quality in a climatically buffered hotspot for plant endemism. Ann Bot 119:267–277

Du Rietz GE (1931) Life-forms of terrestrial flowering plants. Acta Phytogeogr Suec 3:1–95

Edwards EJ, Osborne CP, Strömberg C, Smith SA, C4 Grasses Consortium (2010) The origins of C4 grasslands: integrating evolutionary and ecosystem science. Science 328:587–591

Enright NJ, Mosner E, Miller BP, Johnson N, Lamont BB (2007) Soil versus canopy seed storage and plant species coexistence in species-rich shrublands of southwestern Australia. Ecology 88:2292–2304

Finckh M, Revermann R, Aidar MP (2016) Climate refugees going underground–a response to Maurin et al. (2014). New Phytol 209:904–909

Frost PGH (2012) The responses and survival of organisms in fire-prone environments. In: Booysen PDV, Tainton NM (eds) Ecological effects of fire in South African ecosystems, vol 48. Springer, Berlin

Gignoux J, Lahoreau G, Julliard R, Barot S (2009) Establishment and early persistence of tree seedlings in an annually burned savanna. J Ecol 97:484–495

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

He T, Lamont BB, Manning J (2016) A Cretaceous origin for fire adaptations in the Cape flora. Sci Rep 6:34880

Hoetzel S, Dupont L, Schefuss E, Rommerskirchen F, Wefer G (2013) The role of fire in Miocene to Pliocene C4 grassland and ecosystem evolution. Nat Geosci 6:1027–1030

Hoffmann WA, Solbrig OT (2003) The role of topkill in the differential response of savanna woody species to fire. For Ecol Manag 180:273–286

Holdo RM (2005) Stem mortality following fire in Kalahari sand vegetation: effects of frost, prior damage, and tree neighbourhoods. Plant Ecol 180:77–86

Hyde MA, Wursten BT, Ballings P, Coates Palgrave M (2016) Flora of Zimbabwe: records of *Protea welwitschii*. http://www.zimbabweflora.co.zw/speciesdata/species-display.php?speciesid=120800. Retrieved 6 Oct 2016

Keeley JE, Bond WJ, Bradstock RA, Pausas JG, Rundel PW (2012) Fire in Mediterranean ecosystems: ecology, evolution and management. Cambridge University Press, Cambridge

Kennedy AD, Potgieter ALF (2003) Fire season affects size and architecture of *Colophospermum mopane* in southern African savannas. Plant Ecol 167:179–192

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

Lamont BB, Enright NJ (2000) Adaptive advantages of aerial seed banks. Plant Species Biol 15:157–166

Lamont BB, He T (2012) Fire-adapted Gondwanan Angiosperm floras arose in the Cretaceous. BMC Evol Biol 12:223

Lamont BB, Keith D (2017) Heathlands and associated shrublands. In: Keith D (ed) Vegetation of Australia, 3rd edn. Cambridge University Press, Cambridge, pp 339–368

Lamont BB, He T, Downes KS (2013) Adaptive responses to directional trait selection in the Miocene enabled Cape proteas to colonize the savanna grasslands. Evol Ecol 27:1099–1115

Lindman CAM (1900) Vegetationen i Rio Grande do Sul (Sydobrasilien). Nordin and Josephson, Stockholm

Manry DE, Knight RS (1986) Lightning density and burning frequency in South African vegetation. Vegetatio 66:67–76

Maurin O, Davies TJ, Burrows JE, Daru BH, Yessoufou K, Muasya AM, van der Bank M, Bond WJ (2014) Savanna fire and the origins of the 'underground forests' of Africa. New Phytol 204:201–214

Medwecka Kornas A (1980) *Gardenia subacaulis* a pyrophytic suffrutex of the African savanna. Acta Botanica Acad Sci Hung 26:131–138

Mucina L, Rutherford MC (2006) The vegetation of South Africa, Lesotho and Swaziland. South African National Biodiversity Institute, Pretoria

Muir RA, Bordy EM, Prevec R (2015) Lower Cretaceous deposit reveals first evidence of a post-wildfire debris flow in the Kirkwood Formation, Algoa Basin, Eastern Cape, South Africa. Cretac Res 56:161–179

Pagel M, Meade A (2006) Bayesian analysis of correlated evolution of discrete characters by reversible-jump markov chain monte carlo. Am Nat 167:808–825

Platt WJ, Evans GW, Davis MM (1988) Effects of fire season on flowering of forbs and shrubs in longleaf pine forests. Oecologia 76:353–363

Prunier R, Holsinger KE, Carlson JE (2012) The effect of historical legacy on adaptation: do closely related species respond to the environment in the same way? J Evol Biol 25:1636–1649

Rebelo AG (2001) A field guide to the Proteas of Southern Africa. Fernwood Press, Nova Scotia

Rebelo AG. 2009. Protea Atlas Project. South African National Biodiversity Institute, Kirstenbosch, South Africa. http://www.proteaatlas.org.za. Retrieved 26 Aug 2009

Ristic Z, Ashworth EN. 1997. Mechanisms of freezing resistance of wood tissues: recent advancements. In Basra AS, Basra RK. (Eds) Mechanisms of environmental stress resistance in plants. Amsterdam, the Netherlands. pp. 123–136

Rizzini C, Heringer E (1961) Underground organs of plants from southern Brazilian savannas, with special reference to the xylopodium. Phyton 17:105–124

Roberts DL, Neumann FH, Cawthra HC, Carr AS, Scott L, Durugbo EU, Humphries MS, Cowling RM, Bamford MK, Musekiwa C, MacHutchon M (2017) Palaeoenvironments during a terminal Oligocene or early Miocene transgression in a fluvial system at the southwestern tip of Africa. Glob Planet Change 150:1–23

Roques KG, O'Connor TG, Watkinson AR (2001) Dynamics of shrub encroachment in an African savanna: relative influences of fire, herbivory, rainfall and density dependence. J Appl Ecol 38:268–280

Rundel PW, Arroyo MTK, Cowling RM, Keeley JE, Lamont BB, Vargas P (2016) Mediterranean biomes: evolution of their vegetation, floras and climate. Ann Rev Ecol Evol Syst 47:383–407

Scheiter S, Higgins SI, Osborne CP, Bradshaw C, Lunt D, Ripley BS et al (2012) Fire and fire-adapted vegetation promoted C4 expansion in the Late Miocene. New Phytol 195:653–666

Sciscio L, Tsikos H, Roberts DL, Scott L, van Breugel Y, Damste JS, Schouten DR, Grocke DR (2016) Miocene climate and vegetation changes in the Cape Peninsula, South Africa: evidence from biogeochemistry and palynology. Palaeogeogr Palaeoclimatol Palaeoecol 445:124–137

Simon MF, Grether R, de Queiroz LP, Skema C, Pennington RT, Hughes CE (2009) Recent assembly of the Cerrado, a neotropical plant diversity hotspot, by in situ evolution of adaptations to fire. Proc Natl Acad Sci 106:20359–20364

Smith FR, Granger JE (2017) Survival and life expectancy of the tree *Protea roupelliae* subsp. *roupelliae* in a montane grassland savanna: effects of fire regime and plant structure. Aust Ecol. doi:10.1111/aec.12459

Utescher T, Mosbrugger V, Ivanov D, Dilcher DL (2009) Present-day climatic equivalents of European Cenozoic climates. Earth Planet Sci Lett 284:544–552

Valente LM, Reeves G, Schnitzler J, Mason IP, Fay MF, Rebelo TG, Chase MW, Barraclough TG (2010) Diversification of the African genus *Protea* (Proteaceae) in the Cape biodiversity hotspot and beyond: equal rates in different biomes. Evolution 64:745–760

Vrba ES (1985) Early hominids in southern Africa: updated observations on chronological and ecological background. In: Tobias PV (ed) Hominid Evolution. Alan R Liss, New York, pp 195–200

Wakeling JL, Cramer MD, Bond WJ (2012) The savannah-grassland ‘treeline’: why don’t savanna trees occur in upland grasslands? J Ecol 100:381–391

Weiersbye IM, Witkowski ETF (2002). Seed fate and practical germination methods for 46 perennial species that colonize gold mine tailings and acid mine drainage-polluted soils in the grassland biome. In: Seydack AHW Vorster T, Vermeulen WJ, van der Merwe IJ (Eds). Multiple use management of natural forests and woodlands: policy refinements and scientific progress. Proceedings of the Natural Forests and Savanna Woodlands Symposium III, KNP, Department of Water Affairs and Forestry Indigenous Forest Management, Pretoria. pp. 221–255

White F (1977) The underground forests of Africa: a preliminary review. Gard Bull 29:57–71 (Singapore)

Witkowski ETF, Lamont BB (1997) Does the rare *Banksia goodii* have different vegetative, reproductive or ecological attributes from its widespread co-occurring relative *B. gardneri*? J Biogeogr 24:469–482

Zachos JC, Dickens GR, Zeebe RE (2008) An early Cenozoic perspective on greenhouse warming and carbon-cycle dynamics. Nature 451:279–283