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Estimating the age of fire in the Cape flora of South Africa from an orchid phylogeny

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Fire may have been a crucial component in the evolution of the Cape flora of South Africa, a region characterized by outstanding levels of species richness and endemism. However, there is, to date, no critical assessment of the age of the modern fire regime in this biome. Here, we exploit the presence of two obligate post-fire flowering clades in the orchid genus *Disa*, in conjunction with a robust, well-sampled and dated molecular phylogeny, to estimate the age by which fire must have been present. Our results indicate that summer drought (winter rainfall), the fire regime and the fynbos vegetation are several million years older than currently suggested. Summer drought and the fynbos vegetation are estimated to date back to at least the Early Miocene (*ca* 19.5 Ma). The current fire regime may have been established during a period of global cooling that followed the mid-Miocene Climatic Optimum (*ca* 15 Ma), which led to the expansion of open habitats and increased aridification. The first appearance of *Disa* species in the grassland biome, as well as in the subalpine habitat, is in striking agreement with reliable geological and palaeontological evidence of the age of these ecosystems, thus corroborating the efficacy of our methods. These results change our understanding of the historical mechanisms underlying botanical evolution in southern Africa, and confirm the potential of using molecular phylogenies to date events for which other information is lacking or inconclusive.

Keywords: fire; Cape flora; Orchidaceae; *Disa*; palaeoecology

1. INTRODUCTION

Fire influences global ecosystem patterns and processes and must have had a pronounced effect on the evolution of these biotas [1,2]. Without fire, the distribution of ecosystems around the world would be substantially different [3,4]. Vast areas of flammable biomes such as Mediterranean ecosystems, savannas and humid grasslands would turn into forest, particularly in Africa [4]. Although fossil charcoal appears in the geological record soon after the emergence of terrestrial plants [5,6], the age of the current fire regimes is largely unknown [5], but is presumed to be not much older than 6–8 million years (Ma) [4].

Palaeoclimatological and palaeoecological reconstructions are mostly based on plant and animal fossil data (e.g. [7–9]). Although geochemical and geomorphological information is also used (e.g. [10,11]), this comes mainly from marine basins and the link between marine and terrestrial realms is not always clear [12]. For southern Africa the Neogene fossil record is remarkably poor and does not allow for confident reconstruction of the climate, vegetation [13], or the age of the current fire regimes [4]. Other methodologies and independent lines of evidence would thus be welcome to augment our

patchy understanding of past ecosystems. Here, we use the evolutionary history of the orchid genus *Disa* (subfamily Orchidoideae) based on neontological data, to reconstruct past climates and environments.

A reconstruction of the palaeoenvironments of the Cape floristic region (CFR) of southern Africa is particularly interesting since it constitutes, with 9000 species in an area of 90 000 km^2 [14,15], the second most important centre of endemism richness in the world [16]. Most of this richness is found in *fynbos*, a shrubland ecosystem similar to *chaparral* in California and *kwongan* in southwest Australia, characterized by a Mediterranean climate and recurrent fires every 5–50 years [17]. Palaeoclimatic changes have been proposed to have triggered [18–20] and sustained [21,22] plant radiations in the CFR. However, climate alone cannot explain species diversity in the CFR, as the region contains more than twice as many species as expected by global environmental models [23]. A consensus is therefore emerging that the history of diversification in the Cape, possibly the combination of a long history of speciation [24,25] with the existence of long-term stable environment [25,26] might be key to the exceptional modern diversity. Thus, in order to understand the origins of this remarkable diversity, it is essential to correlate speciation history of its elements with the evolution of the relevant modern environments, including fire regime, climate and edaphic conditions [27,28].

Almost half of the species richness in the CFR is accounted for by only 33 'Cape floral clades' [13], of which the large orchid genus *Disa* is one. This is a

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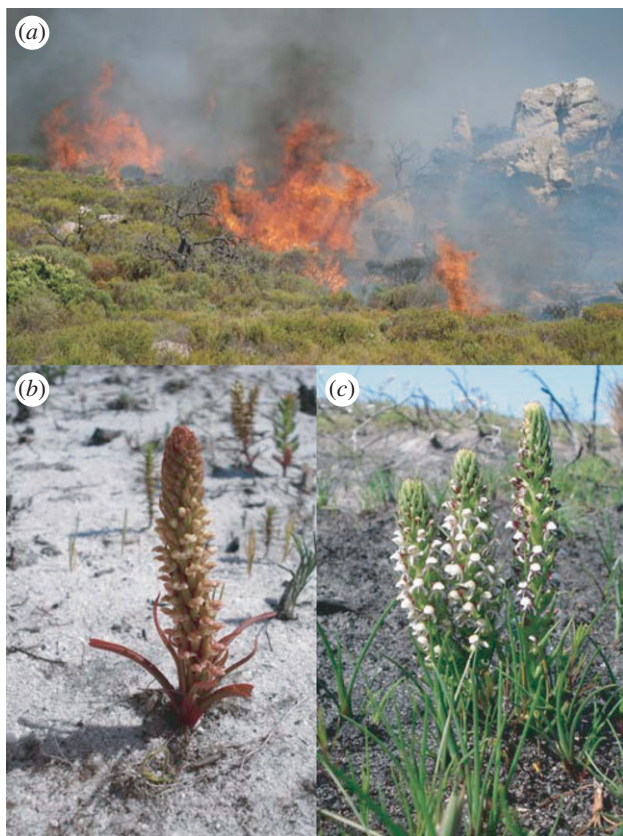
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Figure 1. Fire constitutes an essential element of the Cape floristic region (CFR). Like many other plants in this ecosystem, some *Disa* species flower only the first year after a fire swept through the landscape. (*a*) Fynbos fire at Slangkop, South Africa; (*b*) *D. conferta* (section *Monadenia*) and (*c*) *D. obtusa* (section *Disella*), both obligate fire-dependent species, endemic to the CFR.

particularly charismatic group of orchids, and *Disa uniflora*, the 'Pride of Table Mountain', is well known and often used as an emblem (e.g. of the Mountain Club of South Africa). Of 180 species [29], 100 occur in the CFR and 86 are restricted to it [15]. The various species can be found from the coastal sands to the summits of the highest mountains, yet they are never common [30]. Most are geophytes, well adapted to a fire-prone environment. Indeed, many species are obligate fire-dependent and will only flower in the first year after fire (figure 1), although the mechanism or potential triggers for this are not understood. *Disa* is an ideal clade with which to explore the evolution of Cape environments, as a robust phylogeny of the genus is available, which contains 70 per cent of all species, representing all taxonomic sections, geographical areas and major habitats ([31]; see also electronic supplementary material, figure S1).

Here, we apply the results from a recent study on the ages of major orchid clades [32], based on all reliable orchid fossils described so far, to calibrate the rate-corrected phylogeny of *Disa* and so infer ages for ancestral states of the biomes, rainfall regimes and habitats in which *Disa* occurs. We focus particularly on those attributes for which no independent dates are available, such as the age of the *fynbos* vegetation, winter rainfall and current fire regime, to evaluate how these dates affect our interpretation of the evolutionary history of the Cape flora. We test the assumption that the ecological evolution

in the genus is phylogenetically conservative, and therefore suitable for a reconstruction of Neogene African environments. Finally, we test our methods by determining whether the molecular dates of the first occupation of various habitats in Africa fit the known ages of these habitats (e.g. alpine Drakensberg and grassland).

2. MATERIAL AND METHODS

Phylogenetic relationships were inferred for seven outgroup and 136 ingroup taxa, representing 70 per cent of all recognized *Disa* species and infraspecific taxa. One nuclear and two plastid gene regions were sequenced and combined in a data matrix containing 4094 characters. In a parsimony analysis, 87 nodes of 142 (61%) were supported with a bootstrap support value of 75 per cent or higher, whereas the topology resulting from a Bayesian inference analysis had 101 (71%) nodes with a posterior probability of 0.95 or above (see [31] for details).

For the molecular dating analysis, we applied two widely used algorithms: penalized likelihood implemented in r8s [33] and a Bayesian relaxed clock implemented in BEAST [34]. In both cases, topological and branch length uncertainty was taken into account. A single calibration point was used: the crown age of *Disa*, whose median age was estimated to 19.45 Ma (95% highest posterior density, HPD: 10.2–30 Ma) by Gustafsson *et al.* [32]. In that study, the dating analysis of Ramirez *et al.* [35], based on a fossil from Dominican amber, was extended with the inclusion of two newly described macrofossils assigned to genera *Earina* and *Dendrobium* from New Zealand [36]. In addition, in Gustafsson *et al.* [32] a Bayesian relaxed clock was employed, and the age of fossil *Liliacites* was not used as a maximum age constraint for crown Asparagales, an assumption made by Ramirez *et al.* [35] that received no support in the cladistic analysis of Doyle *et al.* [37]. To take into account the compound error associated with secondary calibrations, in the BEAST analysis the age prior for crown *Disa* was set as a normally distributed subset of the age range obtained for the same node in Gustafsson *et al.* [32].

Disa species were coded for occurrence in biomes, rainfall seasonality and habitat (20 states in total; see electronic supplementary material for details). For the character optimization analysis, we used binary coding (absence/presence) to allow for polymorphic states at internal nodes [38]. Maximum likelihood optimization of ancestral states was performed in MESQUITE v. 2.6 [39]. Initially, all characters were optimized using these assumptions over the maximum *a posteriori* tree. To take into account phylogenetic uncertainty, each character was then optimized over a sample of 1000 penalized likelihood chronograms, and average frequencies across trees calculated for each node of the maximum *a posteriori* tree.

We tested for phylogenetic conservative characters by calculating the number of steps each character required for a parsimony reconstruction, and comparing this to the distribution of minimum lengths for the same character reshuffled 1000 times using Mesquite, while keeping the proportions of the states constant. If the number of steps of the observed distribution was outside 95 per cent of the randomized state distributions, the Null hypothesis that the character states were randomly distributed was rejected. See electronic supplementary material for a detailed description of the methods used.

Table 1. Comparison of age estimates (Ma) for key nodes in the *Disa* phylogeny, depending of the molecular dating method used.

clade in figure 2	taxonomic section	penalized likelihood			BEAST		
		mean	lower	upper	mean	lower	upper
C1	*Disa*	10.5	9.01	12.2	9.68	7.41	12.1
C2	—	8.62	6.95	10.1	8.12	5.95	10.4
C3	*Disella + Monadenia*	14.0	12.9	15.2	10.9	8.51	13.4
C4	*Disella*	12.5	11.0	13.9	9.09	6.69	11.4
C5	*Monadenia*	12.9	11.5	14.3	9.32	6.88	12.0

3. RESULTS

Chronograms inferred by penalized likelihood and BEAST are presented in electronic supplementary material, figures S2 and S3, respectively. In general, median ages estimated by BEAST tended to be younger than those estimated by penalized likelihood, as observed in other studies (e.g. [40]). However, the 95% confidence intervals (CI)/HPD were mostly overlapping for any given node. We report penalized likelihood estimates in the text, but for key nodes we indicate mean ages and CIs from both methods in table 1.

The relative likelihood values for the character state optimization over all nodes of the maximum *a posteriori* tree are reported in the electronic supplementary material, table S1. Attribute shifts supported by an average ($n = 1000$ trees) of greater than or equal to 0.95 for biome, rainfall seasonality and habitat are shown in electronic supplementary material, figures S4–S6, respectively. Shifts supported by an average relative likelihood greater than or equal to 0.70 but less than 0.95 are indicated with an asterisk. The root node of *Disa* was unambiguously optimized to *fynbos* biome and to winter rainfall, both with a mean relative likelihood (λ_m) of 0.99. The obligate fire-dependent clades *Monadenia* (node C_5 in figure 2; 11.5 Ma, lower bound of the 95% CI of the crown group age) and *Disella* (node C_4 in figure 2; 11 Ma, lower bound of the 95% CI of the crown group age) both optimized to fire ($\lambda_m = 0.96$ and 0.98, respectively; figure 2). The most recent common ancestor of these two sister clades (node C_3 in figure 2; 12.9 Ma, lower bound of the 95% CI of the crown group age) was most probably also fire-dependent, given its high likelihood ($\lambda_m = 0.92$) and because this scenario would require only a single habitat transition instead of two. Significance tests showed that biomes, rainfall seasonality (except all year rainfall) and most of the habitats (except subalpine and southeast cloud zone) are phylogenetically conservative. The null hypothesis that these states were randomly distributed across the tree was rejected (electronic supplementary material, table S2). Detailed results are reported in electronic supplementary material.

4. DISCUSSION

Our results suggest that the *fynbos* biome as a whole, as well as the winter rainfall climate of the western and southern part of the CFR, are far older than has generally been assumed [41–44] and may date back to approximately 19.5 Ma (crown age of *Disa* from [32]). Our results further suggest that fires in the CFR were sufficiently predictable to select for fire-stimulated flowering by about 12.9 Ma (C_5 in figure 2; $\lambda_m = 0.96$; 95% CI = 11.5–14.3 Ma) or even earlier (clade C_3, approx. 14 Ma, $\lambda_m = 0.92$, 95% CI = 12.9–15.2 Ma). Interestingly, these ages fall within a period of global climate cooling subsequent to the mid-Miocene Climatic Optimum, approximately 15 Ma [11,45] (see temperature curve in figure 2) and with the start of the radiation of many species-rich clades (e.g. *Moraea* [21], *Satyrium* [25], *Pelargonium* [22], *Protea* [46] and *Muraltia* [25,47]) or with a sharp increase in species numbers such as in Bruniaceae [48]. Thus, fire could have provided the environment during the Late Miocene–Pliocene in which the diversification of the current hyperdiverse clades of the Cape flora was initiated.

The development of a fire regime requires at least two preconditions: flammable vegetation (e.g. *fynbos*, grassland, *chaparral*, *Eucalyptus* forest), and a long dry period such as in a winter rainfall climate where summers are hot and dry. Our data confirm that both these conditions were met. That the *fynbos* biome was already established by approximately 19.5 Ma is not unexpected and is supported by other plant groups. Verboom *et al.* [25] analysed 17 clades typical of the *fynbos* and identified three where the stem node was even older (*Moraea*, Iridaceae, 26.3 Ma; *Ehrharta*, Poaceae 40.9 Ma and Restionaceae, 61.3 Ma). Furthermore, the crown age of the Cape floral clades Crotalarieae p.p., Podalyriae (Fabaceae) and Leucadendriinae (Proteaceae) were estimated to be 46.3, 44.6 and 22–39 Ma, respectively [46,49]. Cape floral elements belonging to Restionaceae, Proteaceae and Ericaceae are already present in the Arnot Pipe deposits of Banke, which are confidently dated back to between 64 and 71 Ma [50]. These three families are also present in the lignite deposits of the Knysna area but the dating of these vary from as old as the Oligocene to as young as the Miocene [51–53]. Thus, many of the characteristic clades of *fynbos* were already present by the Middle/Late Miocene, indicating that the *fynbos* vegetation type might also have been present.

Based on the presumed intensification of the Benguela cold water upwelling, the modern Benguela current system had been dated to *ca* 10 Ma [54,55]. This was thought to have led to increased aridification of the Cape and to the inception of a Mediterranean type climate with most rainfall concentrated in winter by about 5.5–5 Ma [20,42,44]. However, the heightened productivity in the Benguela current, which was used to infer increased upwelling, is mirrored by similar increases in other non-upwelling parts of the oceans [56], and is now suggested to be part of global response to
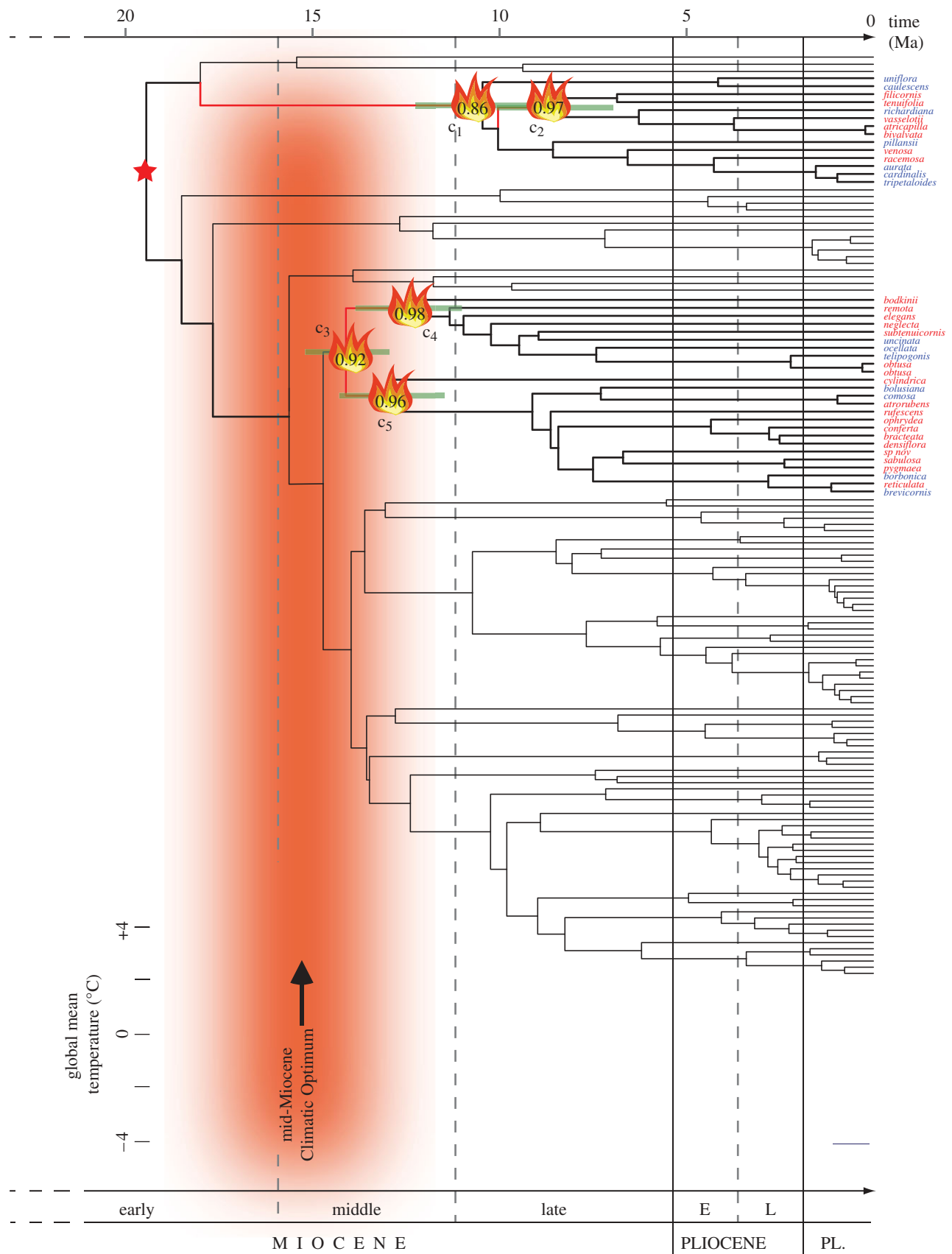
Figure 2. Temporal evolution of *Disa* and earliest optimizations of fire. Maximum *a posteriori* tree from the Bayesian analysis of 136 taxa, with branch lengths proportional to mean ages estimated from a sample of 1000 independently dated chronograms using penalized likelihood. The oldest shifts to fire habitats are outlined by the fire symbol (with relative average likelihood values inside), and the corresponding branches along which shifts occurred highlighted in red. Species marked in red are obligate fire-dependent. Green bars indicate 95% confidence intervals of critical nodes (clades $C_1 - C_5$; see table 1 for precise values). The red star indicates the calibration point. Temperature curve from Zachos *et al.* [45]; timescale from Gradstein & Ogg [77].

palaeoceanographic changes, rather than the result of a localized increase in the upwelling of cold nutrient-rich water. Linder [13] critically reviewed all fossil data and concluded that there is no direct evidence to suggest the establishment of the winter-rainfall regime at the Miocene–Pliocene boundary.

There is ample fossil charcoal (fusain) evidence for the presence of fires, almost since the first land plants were established [5,6]. The many Cretaceous fossil angiosperms preserved as charcoalified remains testify to the early connection between fires and angiosperms. However, fire during the Cenozoic is less well studied and understood [5]. A substantial increase in charcoal in marine sediments indicates that fires became more widespread during the Late Miocene, coinciding with the simultaneous spread of tropical savannas and humid C_4 grasslands in Asia, Africa and America approximately 6–10 Ma [4,57]. Fire ecologists recognize different types of fire regimes and fires typically associated with the above vegetation recur on a 1–5 year cycle [57], whereas, for instance, those associated with Australian eucalypt forests occur on a cycle of 10–100 years [58]. The eucalypt fire system dates back to at least the Middle Miocene [59,60]. Mediterranean vegetation fire, such as those in the heathlands of the Cape *fynbos*, burn on a cycle of 5–50 years [17]. Little information is available on the age of fire in Mediterranean ecosystems. The first positive charcoal evidence of fires in southwestern Australia dates back to the mid-Pliocene. Atahan *et al.* [61] documented that 3.15 Ma deposits from Yallalie, showing a mix of heath and sclerophyll woodland with some remaining rainforest elements, had a fire frequency of between 5 and 13 years. No information on the age of fire regime in the Cape *fynbos* exists [13]. Consequently, the minimum date inferred here for fire in *fynbos* is quite possible, and is consistent with the globally available fossil data.

Eight out of 10 tested habitats are not randomly distributed on the phylogeny, implying phylogenetic niche conservatism for these habitat attributes (electronic supplementary material, table S2). Such niche conservatism has been repeatedly demonstrated [62–66], and in this case implies that the most recent common ancestor of almost all monophyletic sections [31] can be significantly optimized to a characteristic habitat: section *Phlebidia*, *Vaginaria* and *Coryphaea* are typically lithophytic or grow in rocky soil; *Disella* and *Monadenia* flower in the first year after fire; *Reticulibractea*, *Trichochila*, *Stenocarpa*, *Spirales*, *Ovalifoliae* and *Schizodium* occur in mature heath vegetation, while *Aconitoideae* and *Micranthae* are grassland clades. Section *Stenocarpa* can further be neatly divided into two clades, one which is fynbos adapted and the other which occurs in grassland. The only ecologically heterogeneous clade is comprised of sections *Disa* and *Atromaculiferae*, which is sister to the rest of the genus and cannot be assigned to one particular habitat. Thus, the basal split within the genus seems to have resulted in one small clade that has occupied a variety of habitats, whereas the rest of the genus progressively filled particular niches. Nevertheless, the extent of niche conservatism shown by most sections of *Disa* gives us confidence in projecting habitat attributes onto ancestral nodes.

Fossil and geological evidence show that the grassland and subalpine habitat were all established before they were occupied by *Disa* (electronic supplementary material, figure S6) indicating that our optimization methods are reliable. We dated grasslands to be at least 12.4 Ma ($\lambda_m = 0.98$; 95% CI = 11.1–13.8 Ma) suggesting they were present in Africa from at least the Middle Miocene. Although the crown Poaceae are estimated to be approximately 96 Ma on the basis of molecular evidence [67] and the oldest, unequivocal African records of grass pollen found in Niger Delta date back to the Palaeocene [68], African grasslands and savannas only became more extensive from the Middle Miocene [8,69]. In the Niger Delta, grass cover expanded at *ca* 16 Ma and became widespread by the Late Miocene [70]. At Fort Ternan (Kenya), a grassland mosaic that supported Afromontane forest, alpine meadows and marsh was present 14 Ma [71,72]. By the Middle Miocene, Zambezian grassy woodlands were already widespread [42]. Our inferred date of first grassland occupation is thus strikingly consistent with the timing of grassland expansion. In fact, it seems that *Disa* occupied grassland almost as soon as it became available, which is not surprising as ground orchids in general are almost pre-adapted to this type of habitat. Grasslands lack competing shrubs and trees, and orchids, because of their tubers, are able to survive the regular fires that shape and maintain this vegetation. Similar cases of opportunistic immigration linked to species diversification have been recently proposed for other habitats and taxa in the Southern Hemisphere, as a consequence of the expansion of open habitats following the mid-Miocene Climatic Optimum. These include the orchid genus *Hoffmannseggella* in eastern South America [73] and *Livistona* palms in Australia [74]. Since climate cooling appears to have fostered the diversification of these clades in the past, current global warming may constitute an underestimated threat to the long-time conservation of plants in similar environments.

The occupation of the subalpine habitat by *Disa* is Plio-Pleistocene in age. The first occupation in East Africa by *D. stairsii* dates back to 4.3 Ma (95% CI = 2.9–5.8 Ma), while the earliest occupation in the Drakensberg (*D. fragrans*) is 4.1 Ma (95% CI = 2.7–5.6 Ma), both around the Miocene–Pliocene boundary. Most of the subalpine species endemic to the Drakensberg are, however, of Pleistocene origin. The subalpine zone of the Drakensberg was only established between 5 and 3 Ma when the eastern escarpment of southern Africa was uplifted by about 900 m to its current height [75,76]. In eastern Africa, this habitat is also of recent origin and was formed mostly as a result of rifting and volcanic activity during the Pliocene/Pleistocene [75].

5. CONCLUSIONS

The dating on the first occurrence of *Disa* in the alpine and grassland habitat is congruent with independently obtained geological and palaeoclimatological data. Indeed, here the fit of events is quite remarkable. The remaining optimizations are not in conflict with independent data, largely because there is none. Here, it provides new insights, namely that the radiation of many of the clades of the modern Cape flora was initiated in an environment with winter rainfall, and that diversification of these clades correlates with the establishment of a

modern fire regime during a period of global temperature decrease, subsequent to the mid-Miocene Climatic Optimum.

There remains a serious lack of knowledge about the fundamental role of fire in global ecosystem patterns and processes [1]. Fossil data may be frustratingly rare, as it is the case of the Cape flora, and is almost always time-consuming to assemble. We believe that this investigation, as well as Simon *et al.* [2], has shown that mapping ecological characters on to robust, dated phylogenies may be a useful technique to supplement palaeontological information in understanding the development of fire adapted biomes globally.

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