Extinction pulse at Eocene–Oligocene boundary drives diversification dynamics of two Australian temperate floras

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The diversification dynamics of the Australian temperate flora remains poorly understood. Here, we investigate whether differences in plant richness in the southwest Australian (SWA) biodiversity hotspot and southeast Australian (SEA) regions of the Australian continent can be attributed to higher net diversification, more time for species accumulation, or both. We assembled dated molecular phylogenies for the 21 most species-rich flowering plant families found across mesic temperate Australia, encompassing both SWA and SEA regions, and applied a series of diversification models to investigate responses across different groups and timescales. We show that the high richness in SWA can be attributed to a higher net rate of lineage diversification and more time for species accumulation. Different pulses of diversification were retrieved in each region. A decrease in diversification rate across major flowering plant lineages at the Eocene–Oligocene boundary (ca 34 Ma) was witnessed in SEA but not in SWA. Our study demonstrates the importance of historical diversification pulses and differential responses to global events as drivers of present-day diversity. More broadly, we show that diversity within the SWA biodiversity hotspot is not only the result of recent radiations, but also reflects older events over the history of this planet.

1. Introduction

Biodiversity is unevenly distributed across the globe. In an analysis of both richness and threats, Myers et al. [1] and Williams et al. [2] identified 36 global biodiversity hotspots. Comprising less than 5% of the Earth’s land surface and more than 44% of total plant species [1], some of these hotspots have been studied extensively to investigate the origins and maintenance of their unusually high species diversity. High species richness in some of these regions (e.g. the Andes [3–5], the succulent karoo [6], Qinghai-Tibetan plateau [7]) has been attributed to rapid radiation of multiple lineages coinciding with recent major geological and climatic events. In contrast, high species richness in other hotspots, such as the mediterranean climate regions of South Africa and southwestern Australia, have been attributed to a combination of recent radiations [8,9], lower extinction rates [10–12] and sustained accumulation of lineages over time [13–15]. The southwest Australian (SWA) hotspot has the lowest average net diversification rate and a disproportionate number of old lineages compared with other regions of high plant diversity [3,13]. However, few species-rich lineages from SWA have been considered in these studies. In addition, most species-rich genera and families in SWA are widely distributed across temperate southern Australia, occurring also in mesic southeastern Australia (SEA) (figure 1m). Differences in richness and diversification...
The two southern Australian mesic regions are currently separated by the arid interior and Nullarbor Plain (figure 1m). A clear floristic distinction between these regions has been noted in multiple phytogeographic studies [16–20]. The SWA flora has been described by many authors [16,19,21–25] as ‘extraordinary’ and ‘remarkable’, on a continental and global scale. The SWA region is a centre for species richness [25,26], endemism [27–29] and phylogenetic diversity [20,30,31]. Approximately 48% of the region’s 8000 plant species are endemic [25], whereas SEA contains fewer plant species (4810) and a lower level of endemism (14%) [32].

Here, we seek to investigate whether the greater plant richness in SWA is best attributed to (1) a higher net diversification rate (diversification rate hypothesis), (2) more time for species accumulation (clade age hypothesis) or (3) a combination of both. We assembled a dataset of dated phylogenies for species and genera from 21 plant families across the 21 families used in this study, including in cases where explicit information about a clade’s diversity through time is lacking (i.e. lacking well-sampled phylogenies or a detailed fossil record). Since absolute extinction rates through time cannot be accurately estimated without an extensive fossil record, we calculated both extinction and lambda-mu (i.e. speciation–extinction) for each clade using present-day species diversity and estimated clade age using the method-of-moments approach [39]. This approach was selected because it could be applied to all 21 families used in this study, including in cases where explicit information about a clade’s diversity through time is lacking (i.e. lacking well-sampled phylogenies or a detailed fossil record). Since absolute extinction rates through time cannot be accurately estimated without an extensive fossil record, we calculated both extremes of no extinction (i.e. pure birth model; \( \kappa = 0 \)) and high relative extinction rates (\( \kappa = 0.9 \)) (see electronic supplementary material S1 for details). Further statistical analyses from this

Figure 1. Representative diversity of the Australian temperate flora (a) Eucalyptus macrorrapha Hook.; (b) Gompholeobium cyaninum Chappill; (c) Hyalosperma cotula (Benth.) Paul G. Wilson; (d) Thysanotus R.Br.; (e) Banksia buwero R.Br.; (f) Stenanthemum nanum Rye; (g) Anigozanthos mangles D. Don; (h) Drosera barbigera Plach.; (i) Gompholeobium confertum (DC.) Crisp; (j) Drosera erythrorhiza subsp. squamosa (Benth.) N. G. Marchant & Lowrie; (k) Synephe sp. Darkin (F. Hort et al. 586); (l) Thelymitra epipactoides F. Muell.; (m) map of Australia shaded by topographic contours, with southwest and southeast defined by their respective IBRA regions (see methods in the electronic supplementary materials). Photographs: F. J. Nge. (Online version in colour.)

2. Methods

(a) Assembly of data and criteria for selection

The SEA and SWA regions were defined following Cook et al. [33] using the Interim Biogeographic Regionalisation for Australia (IBRA7) bioregional classification scheme: http://www.environment.gov.au/topics/land/national-reserve-system/science-maps-and-data/australias-bioregions-ibra) (electronic supplementary material, table S1a). The 21 most species-rich flowering plant families with substantial endemic lineages in both regions were selected (table S1b). These families include 71% (5645/8000 spp.) and 75% (3607/4810 spp.) of extant plant species in SWA and SEA, respectively, allowing us to detect region-wide signals of these two areas more broadly and accurately (see electronic supplementary materials S1 and S2 for details).

(b) Dating and molecular analyses

We analysed diversification of genera as a proxy for lineage diversification across older timescales, in contrast to other studies that only focused on more recent diversification dynamics using densely sampled species phylogenies. A similar approach, using higher taxa to address incomplete sampling, has been applied in other studies and has yielded sufficient signal to detect changes in diversification rates through time and across different lineages [34–36]. A comprehensive study incorporating species-level diversification dynamics is warranted but is not currently possible owing to the paucity of data available across the 21 families. The stem age for each genus was estimated from time-calibrated phylogenies compiled from the literature, or in TimeTree [37] where available (see electronic supplementary material S1 for details). We were able to obtain stem ages for all Australian temperate genera (i.e. 100% sampling at the genus level) across the 21 families except for Myrtaceae (62%) (see electronic supplementary material, S2). Stem ages are preferred over crown ages as they include extinction events prior to a crown radiation and hence provide a better estimate of overall net diversification during the entire history of the clade [38].

(c) Diversification rate analyses

We calculated the rate of diversification \( \lambda - \mu \) (i.e. speciation–extinction) for each clade using present-day species diversity and estimated clade age using the method-of-moments approach [39]. This approach was selected because it could be applied to all 21 families used in this study, including in cases where explicit information about a clade’s diversity through time is lacking (i.e. lacking well-sampled phylogenies or a detailed fossil record). Since absolute extinction rates through time cannot be accurately estimated without an extensive fossil record, we calculated both extremes of no extinction (i.e. pure birth model; \( \kappa = 0 \)) and high relative extinction rates (\( \kappa = 0.9 \)) (see electronic supplementary material S1 for details). Further statistical analyses from this
dataset were conducted in R [40] (see electronic supplementary material S1 for details).

(d) BAMM diversification analyses
We identified three major groups (Acacia, Eucalyptus and Ericaceae: Epacridioideae) with relatively well-sampled species phylogenies (more than 50% of described species) across both regions as suitable for diversification rate analyses using more sophisticated methods. We used BAMM v. 2.5.0 [41] to estimate speciation and extinction rates through time and to identify significant rate shifts in diversification (for details, see electronic supplementary material S1). Sequence data from published studies were sourced from online repositories (electronic supplementary material, table S2).

(e) Lineage-through-time and diversification-through-time
Lineage-through-time plots were generated manually by summing the number of lineages through time, according to the stem ages of genera at each cumulative time interval for both regions. Plots were generated for (a) the set of all 21 families, (b) endemic genera specific to each region and genera not found in the other region, (c) monotypic genera and (d) for each of the families or infra-families where there were data available for more than one genus.

Lineage-diversification-through-time plots at the genus level were generated manually by summing the number of lineages that diverged (based on stem age) in each time-bin. Time-bin intervals were 5 Myr from 0 to 40 Ma, and 10 Myr from 40 to 70 Ma (to account for fewer data points during that period). Plots were generated for (a) the summation of all 21 families, (b) monotypic genera and (c) each of eight families chosen owing to high disparity between the southwest biodiversity hotspot and temperate plant families show that the present-day species-rich, but nevertheless contribute to the region’s diversity. Similarly, SWA contains greater numbers of not only species-rich and endemic genera, but also species-poor lineages (including monotypic genera; electronic supplementary material, tables S9 and S10).

SWA lineages that are shown here to have been relatively unaffected by the E–O boundary event have had more time for species accumulation than in SEA (figure 2 and electronic supplementary material, figure S9). Indeed, the region contains a larger proportion of old genera and has an average lower diversification rate than SEA, as diversification is correlated not only with species diversity but also the timing of divergence (i.e. stem age; figure 3 and electronic supplementary material, tables S5 and S8).

(b) Group-specific responses
Our study shows that while generalized, region-wide differences between diversification dynamics in SWA and SEA can be detected across multiple plant groups, there are also significant group-specific dynamics. The SWA contains a combination of old and young radiations: e.g. a mature radiation sensu Linder [43] of Chamelaucieae (Myrtaceae) occurred during the Oligocene in SWA (figure 2f), while radiations of other lineages (e.g. SWA Leucopogon clade, Eucalyptus subg. Symphyomyrtus and E. subg. Eucalyptus) occurred much more recently after the Miocene (see electronic supplementary material, figure S0). Similarly, while there is no observable decline in average net speciation in SEA at the E–O boundary (figure 2i), a clear decline is evident in some lineages (e.g. Proteaceae, Mirbelieae, Restionaceae and Cyperaceae; figure 2c,d,g,h).

Major radiations of temperate Australian lineages are not limited to SWA, as several groups have also radiated recently in SEA. A significant increase in diversification rate in the Pliocene (ca 4 Ma) was detected from our BAMM analyses for the Botrycephalae clade in Acacia (Fabaceae) (see electronic supplementary material, figure S0a). A significant increase in diversification rate was detected in the Miocene for Eucalyptus subg. Symphyomyrtus in both SEA and SWA regions (see electronic supplementary material, figure S0c). Other genera such as Pomaderris (Rhamnaceae), Epacris (Ericaceae) and Leiocarpa (Asteraceae) were also identified as unusually species rich in SEA under the high extinction rate model from the methods-of-moments approach (electronic supplementary material, figure S5).

The Gnaphalieae tribe (Asteraceae) showed an increase in diversification in the Pliocene in both regions, with further diversification of monotypic genera across the southwest boundary and arid interior during the Pleistocene (figure 2j). A recent diversification was also found in Tasmanian endemic genera within Ericaceae, most of which diverged during the Late Miocene and Pliocene (figure 2c).
Figure 2. Lineage-diversification-through-time plots, with number of generic divergences across 10 Myr (from 70 to 40 Myr) and 5 Myr (from 40 to 0 Myr) time-bins for the SWA and SEA temperate regions. Points are actual values across each time-bin (green diamonds, southwest; orange squares, southeast). The trend lines show the moving average across each time-bin (green dotted, southwest; orange smooth, southeast). (a) Total genera; (b) monotypic genera; (c) Proteaceae; (d) Mirbelieae, Fabaceae; (e) Ericaceae; (f) Chamelaeceae, Myrtaceae; (g) Restionaceae; (h) Cyperaceae; (i) Rutaceae; (j) Asteraceae. Grey horizontal trend lines track the change in mean sea surface temperature, adapted from [42]. (a–i) Vertical grey lines mark the Eocene–Oligocene boundary; (j) the vertical grey bar marks the Mid-Miocene Climatic Optimum. Illustrations were sourced from www.plantillustrations.org and modified under the Creative Commons Attribution 2.0 license: (b) Cephalotus follicularis, (c) Banksia coccinea, (d) Gompholobium, (e) Styphelia triflora, (f) Darwinia macrostegia, (g) Restionaceae, (h) Schoenus nigricans, (i) Boronia heterophylla and (j) Rhodanthe manglesii. (Online version in colour.)
4. Discussion

Diversification dynamics across the two southern Australian mesic regions are primarily driven by different diversification pulses that correlate with major shifts in regional and global climate. Our meta-analysis indicated a net decrease in diversification rate for the SEA flora compared with SWA at the E–O boundary (ca 33.9 Ma). Available palaeobotanical evidence also points to higher extinction levels in SEA compared with SWA around the E–O boundary than in the preceding time period, with the flora becoming distinctly impoverished across southern mainland Australia and Tasmania at that time ([44] and references therein). However, the interpretation from the fossil record is derived from available pollen records during that time, which are incomplete and only allow for a general interpretation of changes in floral composition. Thus, they cannot be used to assess for changes in lineage diversification of specific families as was done here with extant taxa in this study. Regional extinctions elsewhere in the world have been inferred during this time period, triggered by a sharp drop in global surface temperature marking the commencement of extensive glaciation in Antarctica ([45,46], e.g. [47]), caused by the formation of the Antarctic Circumpolar Current following the opening of the Tasmanian Gateway and Drake Passage [48–52]. A sharp drop in regional temperature at the E–O boundary is well documented for southeastern Australia, with transient glaciation in Tasmania and a 7°C drop in sea surface temperature adjacent to southwest Victoria [44,53].

By contrast, temperatures in SWA remained relatively mild at this time, regulated by warm water gyres in the Eucla Basin and the warm proto-Leeuwin current that fringed the western edge of the continent [44,54]. For this reason, lineages in SWA appear to have been less severely affected by cooling at the E–O boundary than in SEA, reflected in the relatively higher levels of net diversification among plant groups in that region. This is supported by available palaeorecords indicating a diverse Mid-E–O macroflora in SWA that was distinctively different in floristic composition from that in SEA at that time [55]. However, further quantitative studies are required that explicitly compare floristic diversity and composition of SWA and SEA palaeorecords. Such studies will allow us to draw links between molecular phylogenetic datasets derived from extant lineages and direct evidence from extinct lineages.

Higher levels of extinction in SEA than SWA during the Pleistocene have also been postulated as an important factor driving the lower current diversity in SEA compared with SWA [10,11]. We were unable to address this adequately in this study given the scale of our analysis. An apparent slowing of diversification from the Pliocene to Pleistocene across all groups in both regions from our meta-analysis is likely to be artefactual and not reflective of actual diversification dynamics (electronic supplementary material, figure S7). Differences across more recent timescales would have occurred between clades within genera. More detailed studies with denser taxon sampling are required to test for different responses across genera and families in the two regions across more recent timescales. Particular attention should be paid to diversification around the Mid-Miocene (18–16 Ma), with our study indicating a decrease in diversification of genera in SWA compared with SEA (figure 2). However, missing taxa (i.e. radiations of clades unaccounted for at our genus-level analyses) increased significantly during that time period (electronic supplementary material, figure S7), limiting our confidence in this result based on our current dataset. More studies with densely sampled phylogenies will address this gap in knowledge.

While evolutionary responses in different plant families may differ, meta-analyses can elucidate general, region-wide responses by summing across lineages. Our study provides a framework for understanding the evolutionary history of different regions using an approach that can be applied to other biodiversity hotspots. It is clear that the drivers for unusually high species diversity in different biodiversity hotspots are not the same. Our study demonstrates that drivers of species disparity across different regions can be uncovered by incorporating multiple comparable lineages across two or more regions, and highlights the importance of including responses at different evolutionary scales. Current literature concentrates on recent radiations in a few key lineages as an explanation for high species richness in young biodiversity hotspots [3,56]. Studies of species-level diversification patterns have failed to uncover significant differences in diversification rates between the SWA hotspot and other regions [33,57]. Indeed, results from this study and others [58] have shown that many contemporary temperate Australian plant lineages have higher diversification rates within genera in SEA than SWA. Studies incorporating responses beyond species-level radiations, such as the present study, are needed to uncover the drivers of present-day diversity in older biodiversity hotspot regions such as SWA, other Mediterranean regions of the southern hemisphere and the tropics, as their biotas have much older origins. In the case of temperate Australia, the pulse event around the E–O boundary has resulted in a lower starting point and lag-time for diversification in SEA. As a result, for lineages shared between regions, SEA has fewer extant genera and species despite having similar rates of diversification to SWA from the Oligocene to the Late Miocene. This signal can still be detected even with subsequent radiations from the surviving lineages across the two regions, indicating the power of using molecular phylogenies in detecting diversification pulses across different scales [59]. Also, despite the more recent radiations of several major plant lineages (e.g. Acacia and Eucalyptus) in SEA as

![Figure 3. Diversification rate distributions of genera for the SWA and SEA temperate regions under the high extinction model (κ = 0.9). Net diversification rates are net speciation events per Myr per lineage (sp sp$^{-1}$ Myr$^{-1}$).](image-url)
shown from our BANN analyses and in other studies [58], the region is still more depauperate than SWA owing to the compounding effect of lineage diversification. Buried from the E–O boundary extinction pulse, SWA has a higher number of older genera and a lower average diversification rate. Thus, our study shows that higher levels of plant diversity in the SWA biodiversity hotspot can be explained by a higher diversity of generically level taxa (i.e. older long-survived lineages), and more time for species accumulation, in agreement with other studies that focused on Mediterranean hotspot regions [12,13].

This study considered plants only; indeed, a long literature on the unusually high species richness in SWA, beginning with Hooker (1859) [19], has focused almost entirely on plants; the region is usually considered a ‘botanical hotspot’. The general explanation favoured here—relative buffering of SWA from global climatic change at the E–O boundary that caused an extinction pulse in SEA—should also have affected many animal groups, and the pattern we have found could be detectable in low-vagility, temperate, mesic animal lineages, particularly invertebrates. Our hypothesis is thus testable using meta-analyses of animal lineages at different evolutionary scales, as we have done here for plants. Similar studies will also achieve a better understanding of different drivers across hotspot regions elsewhere. Focusing on plant-level diversification patterns may be sufficient in uncovering drivers across many regions in the Northern Hemisphere, owing to the relatively young age of the northern biota, but a different approach as shown here is warranted for other regions, tailored to incorporate diversification events throughout the regions’ histories.

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**Data accessibility.** Data available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.tht76hdvg [60].

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