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A congruent molecular signature of vicariance across multiple plant lineages

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Abstract

Explaining disjunct distributions, or why closely related organisms are often separated by apparently severe barriers such as oceans or deserts, is a great challenge for historical biogeography. Competing explanations are long-distance dispersal across a barrier, and vicariance, in which disjunct taxa are descended from an ancestral population that was split by formation of the barrier. Vicariance explanations are testable by their prediction that near-simultaneous speciation should have occurred across multiple lineages of organisms between the disjunct areas because the origin of a barrier would potentially disrupt gene flow within multiple species. To date, there have been few studies providing evidence for multiple synchronous ancient divergences across a barrier whose origin coincides with the timing of the speciation events. Here, we use relaxed molecular-clock dating to investigate the timing of south-western (SW) versus south-eastern (SE) divergences in 23 pairs of plant lineages in southern Australia. Sixteen of the divergences correlate with the origin, 13–14 million years (Myr) ago, of the arid treeless Nullarbor Plain. The Nullarbor Plain currently forms a substantial barrier to SW–SE migration but during the last 45 Myr this region has experienced multiple episodes of marine inundation and subaerial exposure. Thus, there have been multiple events that could have caused either isolation and speciation, or secondary contact, among the taxa of southern Australia. The strong molecular signal of coincident speciation in many diverse lineages during a short period provides the best evidence to date linking synchronous speciation to an ancient vicariance event.

Keywords: Vicariance; Dispersal; Cenozoic; Nullarbor Plain; Australia; Angiosperms; Aridification; Biome assembly; Relaxed molecular clock

1. Introduction

For most of the last 30 years, vicariance has been viewed as a primary contributor to distribution patterns of biota that occur disjunctly in widely separated areas (Humphries and Parenti, 1999; Nelson and Platnick, 1981). However, recently many vicariance hypotheses have been challenged by evidence supporting long-distance dispersal between areas (de Queiroz, 2005; McGlone, 2005; Poux et al., 2005; Renner, 2004; Sanmartín and Ronquist, 2004; Winkworth et al., 2005). Vicariance and dispersal should leave different patterns in phylogenies of taxa that occur in disjunct areas (Donoghue and Moore, 2003; Rosen, 1978). Jump dispersal is generally expected to be stochastic, occurring at different times in different lineages and, under most models, predicts no congruence of pattern or timing among phylogenies (but see Cook and Crisp, 2005a). In contrast, vicariance should result in near-simultaneous speciation across multiple groups of organisms because the barrier would potentially disrupt gene flow among multiple populations in the divided communities. Therefore, a vicariance model makes the testable prediction of concerted speciation among multiple taxa in the disrupted areas. For example, phylogenies of co-occurring taxa whose ancestors were widespread in the supercontinent Gondwana before it rifted apart should show the same pattern and timing of speciation events (Humphries and Parenti, 1999; Poux et al., 2005; Sanmartín and Ronquist, 2004). Because New Zealand rifted from the supercontinent first (about

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80 Myr ago), followed later by South America, Antarctica and Australia (about 30 Myr ago), taxa occurring in South America and Australia should have diverged more recently from each other than from their relatives in New Zealand (Humphries and Parenti, 1999; Rosen, 1978).

Traditionally, the hierarchical pattern (but not the timing) of speciation in phylogenies has been compared among taxa to test the prediction of congruence. A recent large comparative study found such evidence of Gondwanan vicariance in animal taxa, with the order of branching reflecting the pattern of break-up of Gondwana (Sanmartín and Ronquist, 2004). In contrast, there appeared to be a preponderance of long-distance dispersal in plant taxa, because the branching patterns did not fit the expectations of continental drift. However, the fossil record is neither sufficiently complete nor detailed in taxonomic resolution to allow testing of the timing of divergences for most taxa. The recent advent of evolutionary rate modelling of DNA sequences (Rutschmann, 2006; Sanderson et al., 2004) allows such testing (Penny and Phillips, 2004; Poux et al., 2005). Most molecular tests to date have concerned single speciation events, comparing their timing with that of geological events that could have caused vicariance. The Australian and New Zealand sister taxa of *Nothofagus* have been determined to have diverged too recently to be the result of Gondwanan vicariance whereas vicariance between Australasian and South American taxa has not been ruled out (Cook and Crisp, 2005b; Knapp et al., 2005). Most comparisons of multiple taxa have found uncoordinated timing with no support for vicariance events affecting multiple taxa (Galley and Linder, 2006; Poux et al., 2005; Waters and Craw, 2006; Winkworth et al., 2005; Yoder and Nowak, 2006). In contrast, a comparison of 33 dated divergences between north American plant taxa and their European or Asian sisters found that most were less than 30-Myr-old, favouring the Beringia land-bridge hypothesis (Donoghue and Smith, 2004). Some studies using coalescent methods have found evidence of vicariance across multiple taxa during the last 5 Myr, in California (Calsbeek et al., 2003) and the Australian wet tropics (Joseph et al., 1995). However, evidence for congruent divergences linked to more ancient events (>10 Myr ago) is lacking.

The south-western (SW) Australian temperate sclerophyll biome (Crisp et al., 2004) is an internationally recognised biodiversity hotspot (Myers et al., 2006) including about 7000 plant species of which 49% are endemic (Hopper and Gioa, 2004). It is isolated by an arid zone from other Australian biomes (Fig. 1). Currently there is a 750-km gap, where the central Australian arid zone extends to the south coast via the Nullarbor Plain, between the SW and the south-eastern (SE) temperate sclerophyll biomes. This plain is an arid limestone plateau and, as its name suggests, is mostly treeless. Instead, it is dominated by low shrubs of the family Chenopodiaceae. In contrast, the sclerophyllous heaths, woodlands and forests of the SW and SE temperate biomes are diverse and characterised by typically Australian families such as Myrtaceae (e.g. eucalypts), Proteaceae, Fabaceae, Ericaceae and Casuarinaceae.

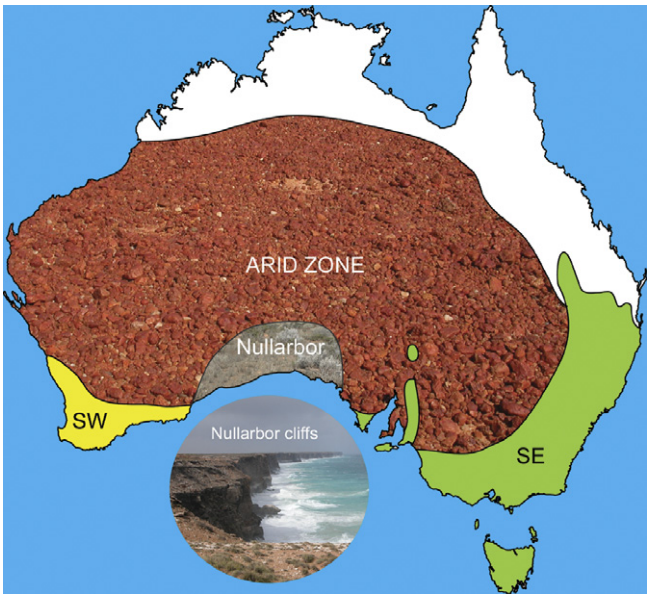

Fig. 1. Location of temperate biomes in southern Australia and the barriers separating them. The south-western (SW) and south-eastern (SE) temperate biomes are separated by the central Australian arid zone and the Nullarbor Plain.

Although the SW and SE biomes have high endemism at species level, past contact is clearly indicated by the many shared genera and species groups (Crisp et al., 2004; Hopper and Gioa, 2004; Mast and Givnish, 2002; Nelson, 1974). Hypothesized barriers to contact between SW and SE biotas, past and present, include marine incursion (Heatwole, 1987; Mast and Givnish, 2002), aridity (Hopper and Gioa, 2004; Mast and Givnish, 2002), and/or calcareous soil (Hopper and Gioa, 2004; Mast and Givnish, 2002; Nelson, 1974). The Cenozoic geological record in southern Australia (Feary and James, 1998; Li et al., 2004; McGowran et al., 2004) shows three rapid drying events, each linked to an expansion of the Antarctic ice sheet, at about 34, 14 and 3 Myr ago ('Chills II–IV', Fig. 2a), and each probably opened an arid barrier between the SW and SE. Between the chilling events, higher sea levels, usually accompanied by a warmer and wetter climate, led to marine incursions into the Nullarbor region (Eucla Basin), with deposition of limestone, in the periods about 42–34, 27–21 and 16–14 Myr ago (Fig. 2b).

This study aimed to determine whether current disjunctions in plant taxa that occur both sides of the Nullarbor Plain correlate with any of these climatic and geological events. To do this, we used relaxed molecular-clock dating to compare divergence times in plant taxa that are shared between the SW and SE biomes. It would be expected that, if the SW–SE divergences were the result of vicariance, there should be clustering of the estimated dates (cf. Yoder and Nowak, 2006). If one or more of the geological/climatic events known for the region were responsible for the disjunct distributions, any clustering should occur

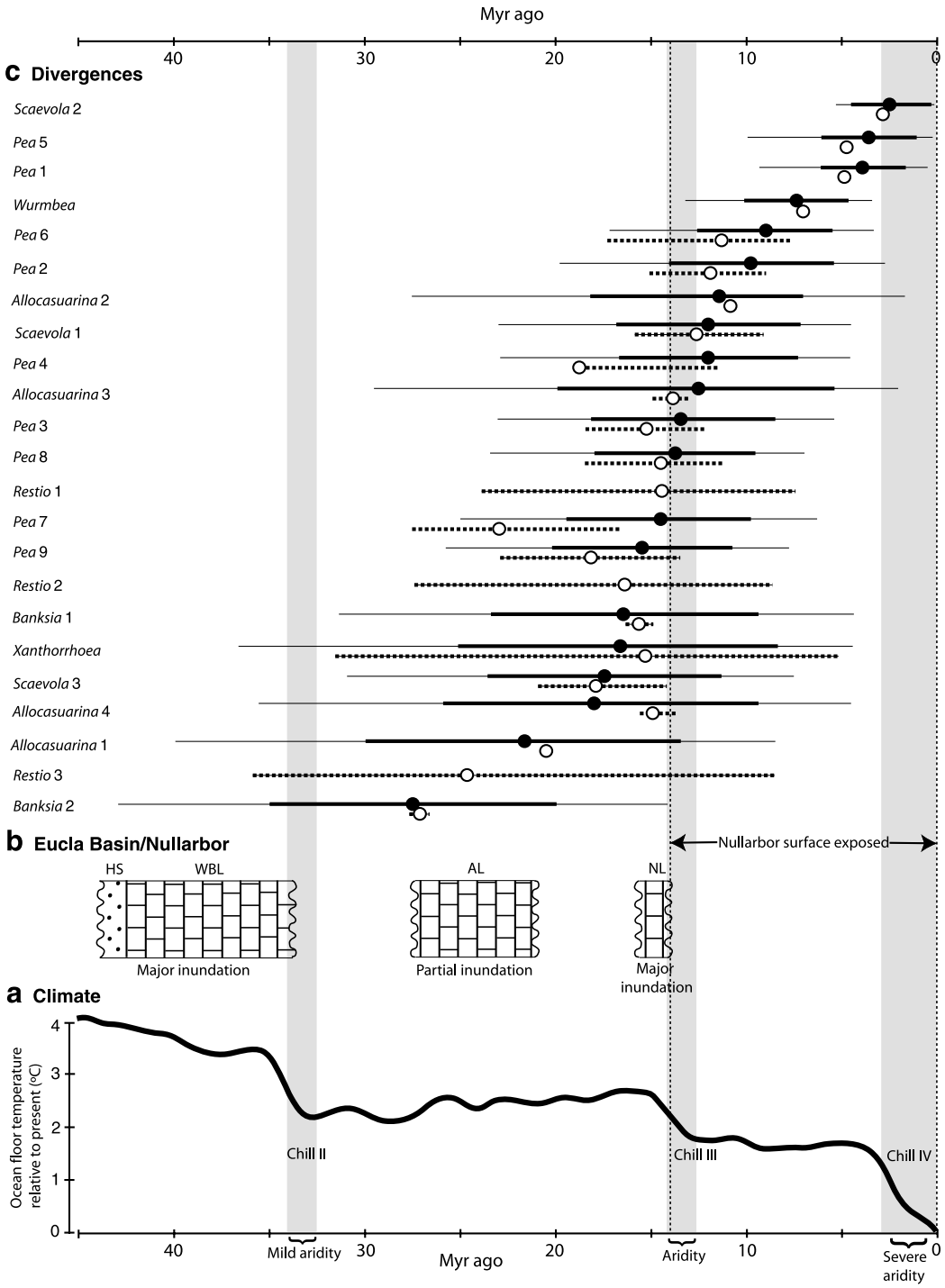

Fig. 2. Divergence times of sister plant lineages in SW and SE Australia in relation to Cenozoic geological and climatic events. (a) Global temperature trend as indicated by ocean-floor temperature relative to present, showing three major cooling events (Chills II–IV) (McGowran et al., 2004) linked to aridification in Australia. (b) Geological history of the Eucla Basin and Nullarbor Plain (Benbow et al., 1995; Feary and James, 1998; Li et al., 2004; McGowran et al., 2004; Sheard and Smith, 1995). Periods of marine inundation with sedimentation are indicated by brick (cool-water carbonates) and sandstone (stipple) patterns (HS, Hampton sandstone; WBL, Wilson Bluff limestone; AL, Abrakurrie limestone; NL, Nullarbor limestone). (c) Divergence times, ranked chronologically, show strong clustering about the period when the Nullarbor region was inundated (16–14 Myr ago), then elevated and aridified (14–13 Myr ago). Estimated dates and errors are given, and taxon labels explained, in Table 3. Vertical grey bars indicate three climatic chilling and drying events and the dotted vertical line indicates the uplift of the Nullarbor Plain about 14 Myr ago. Black dots and open circles show estimates of divergence times using Bayesian and NPRS methods, respectively. Thick bars show standard deviations, and thin and dotted bars show 95% confidence intervals.

during those periods. In contrast, if the current disjunctions between taxa in the SW and SE were the result of independent long-distance dispersal, the distribution of dates should fit a stochastic constant-rate model (Nee et al., 1994).

2. Materials and methods

2.1. Data

We used DNA sequence data from published sources to represent the diversity of angiosperm taxa and life forms that occur in both the SW and SE Australian temperate biomes: *Allocasuarina* (Steane et al., 2003) (Casuarinaceae), *Banksia* (Mast and Givnish, 2002) (Proteaceae), Restionaceae (Briggs et al., 2000), *Scaevola* (Howarth et al., 2003) (Goodeniaceae) and *Wurmbea* (Vinnersten and Reeves, 2003) (Colchicaceae), and from our own work in the pea-flowered Mirbelieae (Orthia et al., 2005) and Bossiaeeae (Fabaceae), and *Xanthorrhoea* (Xanthorrhoeaceae) (Table 1). The last two data sets were supplemented by some unpublished sequences from our laboratory. Sequences were aligned by hand (after concatenation of multiple cpDNA loci where relevant) and ambiguous alignments and ragged ends were removed. For Restionaceae, sequence data were unavailable and instead we extracted a phylogram from Briggs et al. (2000) using TreeThief (Rambaut, 1999). We sampled all unambiguous SW–SE divergences in the study groups. Species-level sampling was sufficiently dense in each of the data sets to reconstruct the basal node of the most recent SW–SE divergences. In this broad study, it was impractical to sample all study groups exhaustively—and unnecessary. For a large clade of species known to be endemic in one biome (e.g. the SW), it is only necessary to sample one or few representative species to 'capture' the node at which it diverged from its sister taxon in the other biome (e.g. SE). Therefore, where SW–SE sister-group relationships were already known, we represented them sufficiently to capture the relevant node without necessarily sampling all members of the sister taxa, nor all of their close relatives. In clades where the exact SW–SE divergences were unclear, we included a larger sample of species from both areas. Where possible, a large sample of outgroup taxa was included to avoid small-sample bias (Cook and Crisp, 2005b; Linder et al., 2005) and to allow use of multiple calibration points.

2.2. Phylogenetic analyses

Base composition did not vary significantly among taxa in any data set and therefore standard models of molecular evolution could be applied. Phylogenies were estimated using Bayesian inference with MrBayes 3.1 (Ronquist and Huelsenbeck, 2003) with a separate GTR + I + G model for each partition, including separate partitions for each codon position in protein-encoding genes, and a standard (discrete state) model with a gamma parameter for indels. Specifying the most parameter-rich model (GTR + I + G) allows the Bayesian search to thoroughly explore parameter space and implicitly includes simpler models (Ronquist and Huelsenbeck, 2003). For each Bayesian analysis, two parallel runs were compared to check for convergence. Consensus topologies from MrBayes were used for the molecular dating analyses. Substitutional saturation was assessed by deviation from linearity of plots of uncorrected ('p') pairwise distances against ML distances derived in PAUP (Swofford, 2002) using the most appropriate model determined by Modeltest (Posada and Crandall, 1998).

2.3. Relaxed molecular clock dating

Violation of an assumption of a molecular clock was assessed using a likelihood-ratio test in PAUP and was detected in all data sets. Subsequently, we used Bayesian rate smoothing as implemented in Multidivtime (Thorne and Kishino, 2002) and non-parametric rate smoothing (NPRS) as implemented in r8s (Sanderson, 2003) to

Table 1
Higher-level taxa, outgroups, sequences and sources of data used in this study

Taxa	Outgroups	No of sequences	Markers sequenced[a]	Aligned sequence length (bases)	Sources
Allocasuarina (Casuarinaceae)	Fagales and Cucurbitales	291	*matK*	771	Steane et al. (2003)
Banksia (Proteaceae)	subfam. Grevilleoideae	45	*rpl16*, *trnL* intron, *trnL-F* spacer	1756	Mast and Givnish (2002)
Fabaceae (tribes Mirbelieae and Bossiaeeae)	Fabaceae *s.l.*	239	*ndhF*	854	Orthia et al. (2005); Crisp and Cook, unpublished.
Australian Restionaceae	Poales	42	*rbcL*, *trnL* intron, *trnL-F* spacer	na	Briggs et al. (2000)
Scaevola (Goodeniaceae)	Asterales	64	ITS (nr DNA)	729	Howarth et al. (2003)
Wurmbea (Colchicaceae)	Colchicaceae	73	*rps16* intron, *atpB-rbcL* spacer, *trnL-F* intron and spacer	3830	Vinnersten and Reeves (2003)
Xanthorrhoea (Xanthorrhoeaceae)	Monocots	161	*ndhF*	861	Givnish et al. (2005); Crisp and Cook, unpublished

[a] Sequences are from chloroplast DNA except where otherwise indicated.

estimate ages and confidence intervals for nodes of interest. These methods differ in their assumptions and optimality criteria, and were used comparatively as a check on whether the estimation of divergence times from our data is sensitive to the biases of particular methods. The Bayesian method requires a fixed topology but uses the underlying sequence data to estimate branch lengths using the F84 model and to incorporate uncertainty in branch-lengths (Thorne and Kishino, 2002). Prior estimates of branch-length related parameter values were set in Multidivtime by using the formulae provided by the authors (Thorne and Kishino, 2002). Standard deviations and 95% credibility intervals were calculated by the program. Multidivtime analyses were each run twice to verify convergence of the Markov chain Monte Carlo chains on a solution. NPRS also requires a fixed topology but does not use the underlying data in any further way. The MrBayes consensus topologies and branch lengths were used for input to NPRS, except for Restionaceae (below). Confidence intervals were calculated using the r8s program (Sanderson, 2003) where possible (the program sometimes failed to find a cross-over point). Because the Australian Restionaceae analysis was based on only a phylogram (with no underlying sequence data), Bayesian rate smoothing was not possible and only NPRS analysis was undertaken.

2.4. Calibration of molecular dates

Where possible, we calibrated nodes using dated fossils; otherwise, we used secondary estimates from previous studies (Table 2). Where a fossil with synapomorphies for a lineage was available, it was treated as a minimum age and placed at the base of the stem (Doyle and Donoghue, 1993). All calibration points at internal nodes were treated as minimum ages. The age of fossils was converted to absolute time using the geologic time scale of Gradstein and Ogg (2004). The calibration point at the root of each phylogeny (Table 2) was treated differently by the two rate-smoothing methods. In the NPRS analyses, the root calibration was treated as a fixed age. In the Bayesian analysis it was used as a prior estimate of the root age (*rttm* parameter) from which the posterior (final) estimate was derived. An estimate of the standard deviation of this age was also given as a prior estimate but the posterior estimate of the age was not limited to within this bound and could have been older or younger. For data sets that included multiple calibration points, the compatibility of the latter was tested by deleting each in turn and estimating their age using the remaining calibration points. Our interpretation of calibration points followed that of previous authors, cited in Table 2, with the following exceptions.

For *Allocasuarina*, *Normapolles* (96 Myr ago) was placed at the divergence between Cucurbitales and Fagales (Magallon and Sanderson, 2001) (calibration point A I in Table 2 and Supplementary Fig. 1) and, alternatively, at the divergence between Fagaceae and its sister group (= the rest of Fagales, including Casuarinaceae, Betulaceae, Jugulandaceae etc., and excluding *Nothofagus*). The latter placement was based on a narrower interpretation of *Normapolles* (Friis et al., 2006) (see Discussion in Cook and Crisp, 2005b).

For *Banksia*, the root (B I) was fixed at the backbone of subfamily Grevilleoideae, which is a strongly supported polytomy or near-polytomy including the well-supported tribe Banksieae (Jordan et al., 2005; Weston and Barker, 2006; Weston, personal communication). Pollens attributed to several lineages in this polytomy first appeared in the late Cretaceous: *Macadamia*/*Helicia* and *Gevuina*/*Hicksbeachia* (85–90 Myr ago); *Carnavonia* (about 85 Myr ago); and *Telopea* (about 75 Myr ago) (Dettmann, 1994). Thus it appears that, sometime around 85–70 Myr ago, multiple sister taxa to the tribe Banksieae came into existence. However, some of these pollens may be convergent with the extant pollens with which they have been matched (Hill et al., 1995; Jordan et al., 1998). Moreover, the 'Macadamia'-type pollen, which appeared first (85–90 Myr) could be plesiomorphic for grevilleoids or even for the family. We therefore used 85 and 70 Myr ago as alternative calibration ages for the grevilleoid backbone.

For *Xanthorrhoea*, the root (X I) was fixed at the divergence between the Alismatales and the rest of the monocots, using a secondary date of 131.5 Myr ago (Janssen and Bremer, 2004). Alternatively, we used the younger estimate of 120 Myr for this node by Bell et al. (2005) who used fossil pollen of Araceae (*Mayoa* and an unnamed coprolite; Friis et al., 2004). However, we consider this placement unlikely because *Mayoa* was matched unequivocally with the Spathiphylleae (*Spathiphyllum* and *Holochlamys*) and the coprolite with Monstereae (e.g. *Monstera*) and Zamioculcadeae (e.g. *Gonatopus*) (Friis et al., 2004, 2006). In our tree, the earliest divergence between the taxa having these two pollen types is at the node separating *Spathiphyllum* and *Arisaema* (Tam et al., 2004). Therefore, we also used this node as a calibration point (X II), set at a minimum age of 120 Myr ago ('Late Barremian–Early Aptian'). It is four nodes higher in our tree than the divergence between Alismatales and the rest of monocots. Note that Bell et al. (2005) incorrectly referred to the divergence between Alismatales and the rest of monocots as the 'monocot crown node'. *Acorus* (not sampled by Bell et al.) is sister to the rest of monocots (Chase, 2004), which include Alismatales, and therefore this divergence is the monocot crown node.

2.5. Tests on distribution of divergence times

After it was found that the SW–SE divergences appeared to cluster around 13–14 Myr ago, we tested whether this pattern differed from the distribution of divergence times among other lineages in the data sets. The Bayesian estimates of divergence times for all nodes in all seven study groups were pooled and, from these, two data sets were extracted for statistical comparison. Data set A ($n = 20$) comprised all the SW–SE divergences. Data set

Table 2
Calibration points for molecular dating

Taxon and calibration point code	Node[a]	Fossils unless secondary (S)[b]	Type of constraint[c]	Age (Myr)	References
Allocasuarina (Casuarinaceae)					
A I	Cucurbitales vs Fagales	*Normapolles*	Root	96	Magallon and Sanderson (2001)
A II	*Nothofagus* vs rest of Fagales	*Nothofagus* pollen and co-existing macrofossils of sister group	Min	83.5	Cook and Crisp (2005b) and references therein
A III	Betulaceae vs Casuarinaceae	*Endressianthus*	Min	71	Cook and Crisp (2005b) and Friis et al. (2003)
A IV	*Alnus* vs *Betula*	Leaves and reproductive structures of both genera	Min	49	Forest et al. (2005)
A V	*Corylus* vs *Carpinus*	Fruits	Min	49	Forest et al. (2005)
A VI	Casuarinaceae crown	*Gymnostoma antiquum*	Min	55	Scriven and Hill (1995)
Banksia (Proteaceae)					
B I	Backbone of subfamily Grevilleoideae	Several pollens	Root	85 or 70	Dettmann (1994) and Jordan et al. (1998)
B II	Stem of tribe Banksieae	Unique pollen	Min	62	Macphail et al. (1994); Martin, 1994
B III	Stem of *Banksia*	*Banksiaephyllum taylori*	Min	58 or 54	Carpenter et al. (1994)
B IV	Crown of *Banksia*	*B. achaeocarpa* and *B. longicarpa*	Min	40	Greenwood et al. (2001) and McNamara and Scott (1983)
Tribes Mirbelieae and Bossiaeeae (Fabaceae)					
P I	Stem of Faboideae	S	Root	58.5	Lavin et al. (2005)
P II	Arcoa stem	Florissant bed leaves	Min	34	Lavin et al. (2005)
P III	Stem of Mirbelieae plus Bossiaeeae	S	Min	54	Lavin et al. (2005)
Australian Restionaceae					
R I	Restionaceae crown	Arnot pipe pollen	Root	64	Linder et al. (2003)
Scaevola (Goodeniaceae)					
S I	Asteraceae vs Goodeniaceae	S	Root	60	Bremer et al. (2004)
Wurmbea (Colchicaceae)					
S I	Colchicaceae crown	S	Root	44	Janssen and Bremer (2004)
Xanthorrhoea (Xanthorrhoeaceae)					
X I	Alismatales vs rest of monocots	S	Root	131.5 or 120	Janssen and Bremer (2004) and Bell et al. (2005)
X II	*Spathiphyllum* vs *Arisaema* (Araceae)	Two pollens: *Mayoa* and an unnamed coprolite	Min	120	Friis et al. (2004)
X III	Crown of palms (Arecaceae)	*Sabalites*	Min	83.5	Savolainen et al. (2006)
X IV	*Dianella* vs *Phormium* (Hemerocallidaceae)	*Dianellophyllum* and *Phormium* pollen	Min	45	Greenwood and Conran (2000)

[a] Indicates the node in the phylogeny where the constraint was placed; 'vs' indicates the divergence point between two taxa.
[b] The fossil(s) on which the constraint is based, unless the constraint is secondary (S) and derived from the cited dating analysis.
[c] It was necessary to fix the age of the root for NPRS analyses. In the Bayesian dating, these same calibration points were used as priors for the root. All calibration points above the root were treated as minimum ages.

B included all divergences between taxa in sclerophyll communities in Australia and the southern hemisphere, except those across the Nullarbor ($n = 241$). This latter data set was used as a 'null' distribution for comparison with data set A. Sampling for data set B was restricted to the last 30 Myr because this is the period represented by the SW–SE divergences. Two categories of divergences were not included in data set B: divergences in lineages occurring in non-sclerophyll biomes (such as rainforest and temperate northern hemisphere biomes) and sparsely sampled outgroups, which were likely to under-represent more recent divergences; and the SW–SE divergences comprising data set A.

The mean divergence times in each data set were compared using the *t* test in Excel and both data sets were tested for normality (at http://www.physics.csbsju.edu/

stats/KS-test.html). Data set B failed the normality test, so comparisons between A and B were also made using the following non-parametric tests (Siegel, 1956; Sokal and Rohlf, 1969), implemented as indicated: χ^2 test (Excel), median test (http://www.fon.hum.uva.nl/Service/Statistics.html), Mann–Whitney U test (http://eatworms.swmed.edu/~leon/stats/utest.html) and Kolmogorov–Smirnov (KS) test (http://www.physics.csbsju.edu/stats/KS-test.html). For the χ^2 test, it was necessary to lump the data into three bins to achieve a minimum of 80% of expected frequencies greater than or equal to five (Siegel, 1956). All tests were two-tailed.

2.6. Edaphic barrier

Much of the sclerophyll flora of the SW and SE is adapted to acidic siliceous soils and does not tolerate calcareous soils (Hopper and Gioa, 2004; Marchant, 1973; Nelson, 1974). To test whether the calcareous karst surface of the Nullarbor Plain is an edaphic barrier, we examined the ranges of 112 species occurring on siliceous sand patches at the western edge of the Plain (Nelson, 1974). The species were partitioned according to whether they are restricted to siliceous soils or tolerate both siliceous and calcareous soils, and according to whether they are endemic to SW Australia or are distributed in both SW and SE Australia. The resulting 2×2 contingency table was evaluated using a χ^2 test with one degree of freedom.

3. Results

3.1. Sequences, phylogenetic analyses and divergence times

Seven independent data sets (details in Table 1) were identified that comprised sufficient sampling to apply relaxed molecular-clock dating to the question of divergence times across the Nullarbor Plain. These contained a total of 881 terminal taxa (represented by 915 sequences) and incorporated 596 nodes (divergences) of which 23 defined SW–SE disjunctions (Supplementary Figs. 1–7). All the saturation plots showed a strong fit to a linear regression (r^2 values all >0.85), though in most there was a slight tendency to saturation at the deeper divergences. It is assumed that the fitted models corrected for this effect in estimating branch lengths.

The mean estimates of SW–SE divergence times, derived using both methods (Bayesian and NPRS), were strongly clustered around 13–14 Myr ago (Figs. 2c, and 3a). Each of the seven plant families sampled included at least one SW–SE divergence in this cluster. Within the errors of dating, this timing coincides with both the inundation of the Eucla Basin about 16 Myr ago and severe aridification (Chill III) about 14 Myr ago (Fig. 2). The 95% confidence intervals around most date estimates rule out a link with either the earlier (Chill II, about 34 Myr ago) or later (Chill IV, about 3 Myr ago) cooling and drying events.

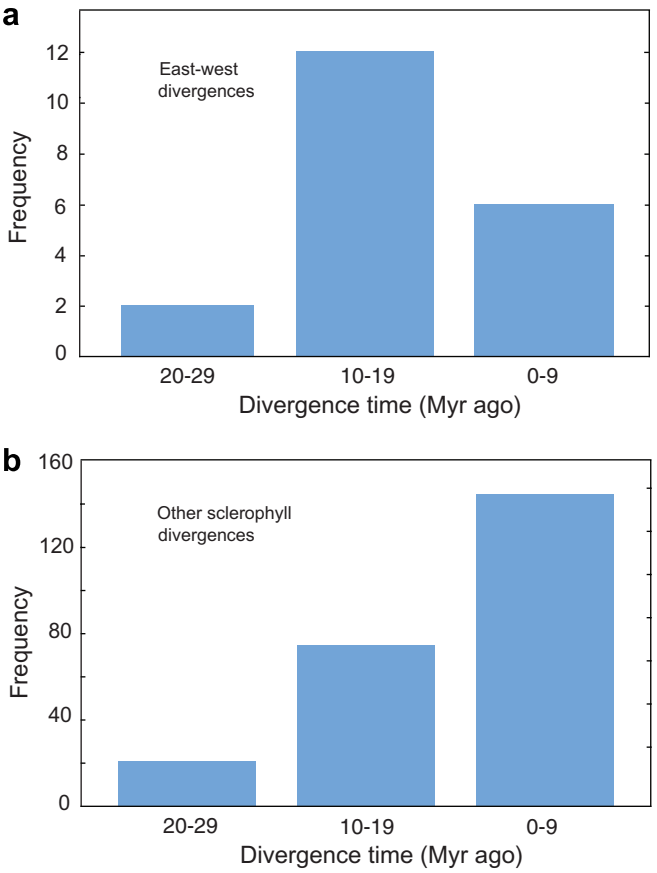

Fig. 3. Distribution of divergences between taxa in the SW and SE biomes (a) and in related sclerophyll taxa (b). These distributions, in three 10 Myr bins, are significantly different ($P = 0.023$, $n = 261$, 5 df, χ^2 test).

We found significant differences between the distribution through time of SW–SE divergences (data set A) and that of other divergences in sclerophyll taxa (data set B): t test ($P = 0.04$), χ^2 test ($P = 0.02$; Fig. 3), median test ($P = 0.0101$), Mann–Whitney U test ($P = 0.05$) and Kolmogorov–Smirnov (KS) test ($P = 0.012$; Fig. 4). The SW–SE divergences were normally distributed (KS test, $P = 0.56$), indicating a central tendency of these divergence times around a mean of 12.9 Myr (SD = 6.1) and a median of 13.0 Myr. By contrast, divergence times in the other sclerophyll taxa were not normally distributed (KS test, $P = 0.00$) and instead were highly consistent with an exponential distribution (Fig. 4), which is the null expectation under a constant-rate stochastic speciation model (Nee et al., 1994).

3.2. Edaphic barrier

The cluster of SW–SE divergence times also coincided with the elevation of limestone sediments to form the karst surface of the Nullarbor Plain (Fig. 2), which occurred about 14 Myr ago (Li et al., 2004). The Plain has remained exposed through to the present (Sheard and Smith, 1995). The karst is a strong edaphic barrier,

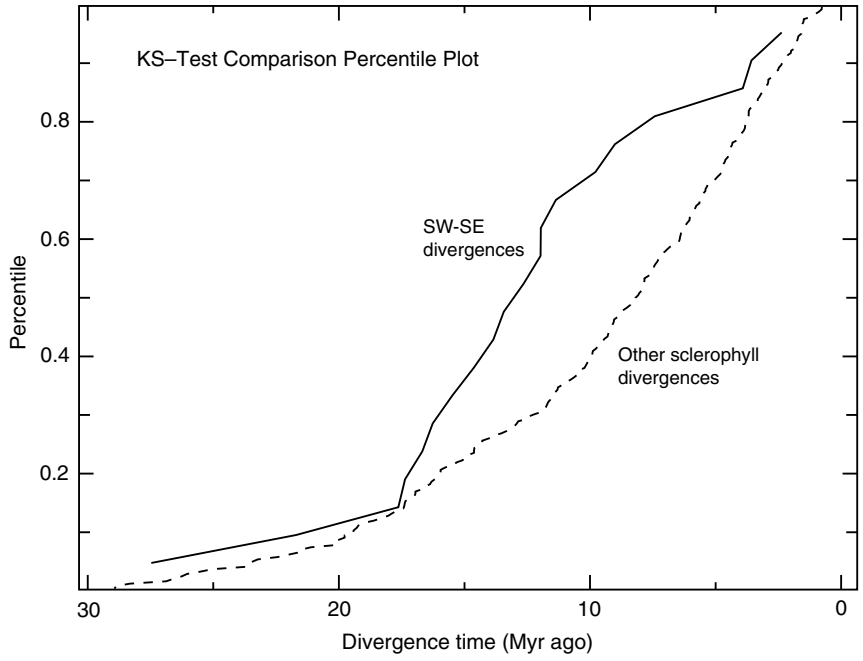

Fig. 4. Cumulative plots of divergences between taxa in the SW and SE biomes and in related sclerophyll taxa. The shapes of these curves are significantly different ($P = 0.012$, $n = 261$, Kolmogorov–Smirnov test).

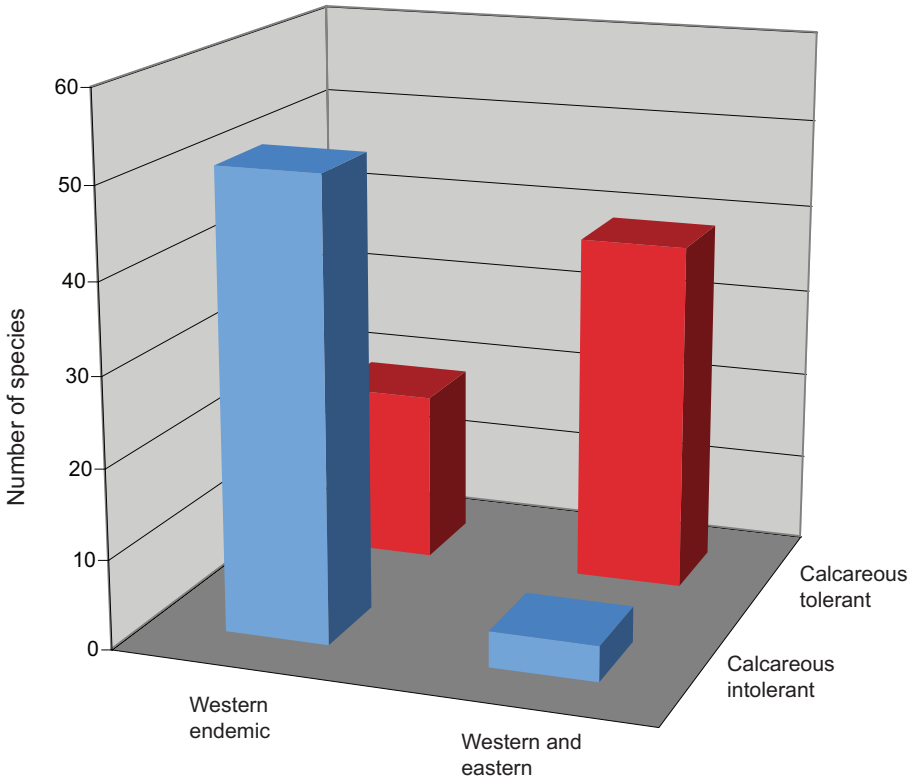

Fig. 5. Disjunctions between plant populations in the SW and SE biomes in relation to soils. This comparison shows that the karst surface of the Nullarbor Plain is a significant barrier to migration. Of calcareous-soil-intolerant plant species occurring on siliceous sand dunes at the western edge of the Nullarbor Plain (Nelson, 1974), 93% do not occur any farther to the east, whereas 67% of calcareous-soil-tolerant species occur on both sides (SE and SW) of the Nullarbor Plain ($P = 10^{-11}$, $n = 112$, 1 df, χ^2 test).

with 93% of the limestone-intolerant plant species on siliceous sand dunes at the western edge of the Nullarbor Plain not occurring any farther to the east. In counterpoint, 67% of limestone-tolerant species occur on both sides (SW and SE) of the Nullarbor Plain ($P = 10^{-11}$, $n = 112$, 1 df, χ^2 test) (Fig. 5).

3.3. Estimates of error

Mean SW–SE divergence time estimates were similar between the Bayesian and NPRS methods (Fig. 2), though differing by 6–8 Myr for taxa Pea 4 and Pea 7 (Table 3). In the Bayesian analyses, posterior and prior estimates of the ages of the root nodes differed by less than 5 Myr in all data sets except monocots (which include the SW–SE divergence of *Xanthorrhoea*). In monocots, the posterior age of the root (196 Myr) was considerably older than the prior estimate of 131.5 Myr. Using the alternative prior age of 120 Myr (Table 2) yielded a posterior estimate of 153 Myr for the root (Supplementary Fig. 7). Omitting the Araceae calibration point (X II, 120 Myr; Table 2) reduced the posterior age of the root to 172 Myr and omitting all three internal calibration points (X II–IV; Table 2) reduced the estimate to 128 Myr. Therefore, the estimate of the age of the root in monocots is very sensitive to the calibration points used, indicating a need for further investigation of the fossils and their phylogenetic placement. Despite the uncertainty in this data set, point estimates of the SW–SE divergence time in *Xanthorrhoea* varied between 8 and 17 Myr ago, spanning the 13 Myr mean for all SW–SE divergences. This is a much narrower range of variance than at the root, possibly because the age of the *Xanthorrhoea* node is more strongly influenced by a closer calibration point (cf. Won and Renner, 2006), located in its sister group at the divergence between *Dianella* and *Phormium*.

Table 3
Estimated ages of SW–SE divergences (Myr ago)

Divergence[a]	Bayesian			NPRS	
	Estimate	SD[b]	CI[c]	Estimate	CI[c]
Allocasuarina 1	21.7	8.2	8.4–39.8	20.5	[d]
Allocasuarina 2	11.4	6.8	1.7–27.6	10.8	[d]
Allocasuarina 3	12.6	7.2	2.1–29.5	13.9	13.0–14.8
Allocasuarina 4	17.6	8.3	4.5–36.2	14.9	13.7–15.6
Banksia 1	16.3	7.0	4.3–31.3	15.7	14.9–16.3
Banksia 2	27.5	7.5	13.7–42.8	27.1	26.6–27.7
Pea 1	3.9	2.3	0.6–9.4	4.1	3.9–4.3
Pea 2	9.8	4.4	2.8–19.8	10.0	9.7–10.3
Pea 3	13.4	4.8	5.6–24.3	14.6	14.2–14.9
Pea 4	12.0	4.7	4.6–22.9	18.2	17.5–18.7
Pea 5	3.6	2.5	0.3–9.9	4.9	4.4–5.3
Pea 6	9.0	3.6	3.3–17.2	9.4	9.0–9.8
Pea 7	14.6	4.8	6.3–25.0	22.1	21.7–22.6
Pea 8	13.8	4.2	7.0–23.4	13.7	13.2–14.0
Pea 9	15.5	4.7	7.8–26.1	17.0	16.5–17.3
Restio 1	—	—	—	14.4	7.4–23.9
Restio 2	—	—	—	16.3	8.7–27.4
Restio 3	—	—	—	24.6	8.5–35.9
Scaevola 1	12.0	4.8	4.5–23.0	12.7	9.1–15.8
Scaevola 2	2.4	2.1	0.1–7.8	2.8	[d]
Scaevola 3	17.4	6.1	7.5–30.9	17.9	14.2–20.9
Wurmbea	7.4	2.7	3.4–13.7	7.1	7.0–7.2
Xanthorrhoea	17.0	8.4	4.3–37.4	15.3	7.7–31.6

[a] Node definitions for divergences (SW, SE): *Allocasuarina* 1 (*Allocasuarina campestris* and *Allocasuarina littoralis*); *Allocasuarina* 2 (*Allocasuarina huegeliana* and *Allocasuarina verticillata*); *Allocasuarina* 3 (*Allocasuarina trichodon* and *Allocasuarina torulosa*); *Allocasuarina* 4 (*Allocasuarina scleroclada* and *Allocasuarina luehmannii*); *Banksia* 1 (*Banksia benthamiana* and *Banksia serrata*); *Banksia* 2 (*Banksia grandis* and *Banksia ericifolia*); Pea 1 (*Aotus* sp. aff. *procumbens* and *Aotus subspinescens*); Pea 2 (*Dillwynia dillwynioides* and *Dillwynia cinerascens*); Pea 3 (*Mirbelia microphylla* and *Mirbelia rubiifolia*); Pea 4 (*Callistachys lanceolata and Podolobium alpestre*); Pea 5 (*Chorizema genistoides* and *Chorizema parviflorum*); Pea 6 (*Bossiaea praetermissa* and *Bossiaea* sp. 4117); Pea 7 (*Bossiaea dentata* and *Bossiaea cinerea*); Pea 8 (*Daviesia uniflora* and *Daviesia corymbosa*); Pea 9 (*Daviesia angulata* and *Daviesia genistifolia*); *Restio* 1 (*Lepidobolus chaetocephalus* and *Coleocarya gracilis*); *Restio* 2 (*Chordifex stenandrus* and *Acion hookeri*); *Restio* 3 (*Taraxis grossa* and *Winifreda sola*); *Scaevola* 1 (*Scaevola phlebopetala* and *Scaevola albida*); *Scaevola* 2 (*Scaevola virgata* and *Scaevola calendulacea*); *Scaevola* 3 (*Diaspasis filifolia* and *Scaevola ramosissima*); *Xanthorrhoea* (*Xanthorrhoea acaulis* and *Xanthorrhoea preissii*).

[b] Standard deviation.

[c] Lower-upper 95% credibility (Bayesian) or confidence (NPRS) limits.

[d] Indicates that r8s was unable to estimate values.

Uncertainty in other calibration points made little difference to estimates of SW–SE divergence times. With the two alternative placements of *Normapolles* (A I) in the Cucurbitales–Fagales phylogeny, the four SW–SE divergences within *Allocasuarina* varied by only 1–2 Myr. Similarly, these estimates varied 0–3 Myr by omitting a minimum constraint of 55 Myr ago (A VI) on the crown of Casuarinaceae. With the alternative ages of 85 and 70 Myr for the backbone of Proteaceae subfam. Grevilleoideae (B I), the two SW–SE divergence estimates within *Banksia* varied by 2–3 Myr.

4. Discussion

Our finding of significant clustering of SW–SE divergence events around 13–14 Myr ago strongly suggests that the rapid succession of marine incursion, aridification and the origin of the Nullarbor Plain as an edaphic barrier had a strong concerted effect on the flora of southern Australia. Any combination of these three events might have been responsible for the cluster of divergences, depending upon the ecological tolerances of the taxa. However, marine inundation seems least likely to have disrupted populations, despite previous suggestions to this effect (Heatwole, 1987; Mast and Givnish, 2002, in part). Populations could have remained linked via the extensive siliceous soils of central Australia (north of the Eucla Basin) which, at those times, experienced a relatively warmer and wetter climate (the Miocene climatic optimum; Alley, 1998; Li et al., 2004). Moreover, this climatic amelioration could have favoured the spread (dispersal) of species around the Basin from refugia in the SW or SE, possibly exposing populations to subsequent disruption (vicariance) by the aridification and limestone elevation that followed.

Our results corroborate a longstanding hypothesis that the SW and SE biotas were isolated by the mid-Miocene aridification and/or elevation of the Nullarbor Plain as an edaphic barrier (Burbidge, 1960; Cracraft, 1991; Marchant, 1973; Mast and Givnish, 2002; Nelson, 1981; Unmack, 2001). Some (but not all) previously published SW–SE divergence times for animal taxa, derived using a

molecular clock, coincide with the cluster found by this study: 12–16 and 17–23 Myr ago in pygopodid lizards (Jennings et al., 2003); 10–13 Myr ago in *Litorea* frogs (Burns and Crayn, 2006); and about 10–19 Myr ago in the freshwater crayfish *Cherax* (Munasinghe et al., 2004).

The three youngest SW–SE divergences (Peas 1 and 5, and *Scaevola* 2) coincide with the onset of severe aridification 2–4 Myr ago (Fujioka et al., 2005) and their error bars rule out divergence during the earlier periods of aridification (13–14 and 34 Myr ago) (Fig. 2). Therefore, these taxa must have been in more recent contact or underwent more recent long-distance dispersal than taxa of the other SE–SW divergences examined. Taxa of each of the three youngest divergences display ecological tolerances that might favour an explanation of recent contact. *Aotus* and *Chorizema* (Peas 1 and 5, and Fig. 2, respectively) are dry-tolerant. Even today, the eastern species of *Aotus* (Peas 1) occurs at the eastern edge of the Nullarbor Plain and one of the western species of *Chorizema* (Peas 5) extends to the margins of the Central Australian desert. The SW–SE sister taxa in *Scaevola* 2 tolerate calcareous soil and, in the east, extend along the coast to the margins of the Nullarbor Plain. Populations in each of these three taxa could have maintained contact across or around the Nullarbor Plain until the onset of severe aridification about 3 Myr ago.

The older divergences in Restionaceae (*Restio* 3), *Banksia* (*Banksia* 2) and *Allocasuarina* (Allocasuarina 1 and possibly also 4) do not coincide with any of the drying events illustrated in Fig. 2. The error bars of these divergence times overlap both Chill II (34 Myr ago) and Chill III (14–13 Myr ago). The sister taxa in *Restio* 3 currently occur in very wet habitats in the extreme SW and SE corners of Australia. The SW and SE taxa of *Banksia* 2 (Phanerostomata clade) are typically coastal and less drought-adapted than *Banksia* 1 taxa (Cryptostomata clade), which appear to have diverged more recently, coincident with aridification and uplift of the Nullarbor Plain (Mast and Givnish, 2002) (Fig. 2). Both taxa are less tolerant of dry conditions than other groups represented in the analyses and their ancestors might have vicariated in response to the mild aridification at Chill II, about 34 Myr ago (Fig. 2).

It should be noted that the phylogenetic signatures of some older vicariance events are likely to have been obscured by subsequent re-expansion of populations across the barrier when conditions ameliorated, such as during the Miocene climatic optimum. The approach used here has been applied only to the most recent SW–SE divergences events evident within each taxon. Older potential SE–SW divergences within these taxa were not examined because they no longer appear in phylogenies as clear SW–SE disjunctions.

Several sources of variance and error were identified and quantified in this study, including uncertainty in branch lengths, uncertainty in identification and placement of calibration points, and variation between rate smoothing methods. Despite these uncertainties, the significant clustering we have found in a narrow window of time is compelling evidence that events during this period had a strongly congruent effect on taxa occurring across southern temperate Australia. The corollary of the disruption of populations across the Nullarbor Plain during this period was an increased speciation rate, contributing to the current species richness, and subsequent endemism, of one of the world's most biodiverse hot-spots—the SW of WA. Teasing apart the interwoven traces of vicariance and long-distance dispersal, and explaining their respective roles in speciation and the distribution of taxa, remains one of the greatest challenges of historical biogeography.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ympev.2007.02.030.

References

Alley, N.F., 1998. Cainozoic stratigraphy, palaeoenvironments and geological evolution of the Lake Eyre Basin. Palaeogeogr. Palaeocl. 144, 239–263.

Bell, C.D., Soltis, D.E., Soltis, P.S., 2005. The age of the angiosperms: a molecular timescale without a clock. Evolution 59, 1245–1258.

Benbow, M.C., Lindsay, J.M., Alley, N.F., 1995. Eucla basin and palaeodrainage. In: Drexel, J.F., Preiss, W.V. (Eds.), The Geology of South Australia, The Phanerozoic, vol. 2. Department of Mines and Energy, Adelaide, pp. 178–186.

Bremer, K., Friis, E.M., Bremer, B., 2004. Molecular phylogenetic dating of Asterid flowering plants shows early Cretaceous diversification. Syst. Biol. 53, 496–505.

Briggs, B.G., Marchant, A.D., Gilmore, S., Porter, C.L., 2000. A molecular phylogeny of Restionaceae and allies. In: Wilson, K.L., Morrison, D.A. (Eds.), Monocots: Systematics and Evolution. CSIRO, Melbourne, pp. 661–671.

Burbidge, N.T., 1960. The phytogeography of the Australian region. Aust. J. Bot. 8, 75–209.

Burns, E.L., Crayn, D.M., 2006. Phylogenetics and evolution of bell frogs (*Litoria aurea* species-group, Anura: Hylidae) based on mitochondrial ND4 sequences. Mol. Phylogenet. Evol. 39, 573–579.

Calsbeek, R., Thompson, J.N., Richardson, J.E., 2003. Patterns of molecular evolution and diversification in a biodiversity hotspot: the California Floristic Province. Mol. Ecol. 12, 1021–1029.

Carpenter, R.J., Jordan, G.J., Hill, R.S., 1994. *Banksieaephyllum taylorii* (Proteaceae) from the Late Paleocene of New South Wales and its relevance to the origin of Australia's scleromorphic flora. Aust. Syst. Bot. 7, 385–392.

Chase, M.W., 2004. Monocot relationships: an overview. Am. J. Bot. 91, 1645–1655.

Cook, L.G., Crisp, M.D., 2005a. Directional asymmetry of long-distance dispersal and colonisation could mislead reconstructions of biogeography. J. Biogeogr. 32, 741–754.

Cook, L.G., Crisp, M.D., 2005b. Not so ancient: the extant crown group of *Nothofagus* represents a post-Gondwanan radiation. Proc. R. Soc. London B. 272, 2535–2544.

Cracraft, J., 1991. Patterns of diversification within continental biotas: hierarchical congruence among the areas of endemism of Australian vertebrates. Aust. Syst. Bot. 4, 211–227.

Crisp, M.D., Cook, L.G., Steane, D.A., 2004. Radiation of the Australian flora: what can comparisons of molecular phylogenies across multiple taxa tell us about the evolution of diversity in present-day communities? Philos. Trans. R. Soc. B. 359, 1551–1571.

de Queiroz, A., 2005. The resurrection of oceanic dispersal in historical biogeography. Trends Ecol. Evol. 20, 68–73.

Dettmann, M.E., 1994. Cretaceous vegetation: the microfossil record. In: Hill, R.S. (Ed.), History of the Australian vegetation: Cretaceous to Recent. Cambridge University Press, Cambridge, UK, pp. 143–170.

Donoghue, M.J., Moore, B.R., 2003. Toward an integrative historical biogeography. Integr. Compar. Biol. 43, 261–270.

Donoghue, M.J., Smith, S.A., 2004. Patterns in the assembly of temperate forests around the Northern Hemisphere. Philos. Trans. R. Soc. B. 359, 1633–1644.

Doyle, J.A., Donoghue, M.J., 1993. Phylogenies and angiosperm diversification. Paleobiology 19, 141–167.

Feary, D.A., James, N.P., 1998. Seismic stratigraphy and geological evolution of the Cenozoic, cool-water Eucla platform, Great Australian Bight. AAPG Bull. Am. Assoc. Petrol. Geol. 82, 792–816.

Forest, F., Savolainen, V., Chase, M.W., Lupia, R., Bruneau, A., Crane, P.R., 2005. Teasing apart molecular-versus fossil-based error estimates when dating phylogenetic trees: a case study in the birch family (Betulaceae). Syst. Bot. 30, 118–133.

Friis, E.M., Pedersen, K.R., Crane, P.R., 2004. Araceae from the Early Cretaceous of Portugal: evidence on the emergence of monocotyledons. Proc. Natl. Acad. Sci. USA 101, 16565–16570.

Friis, E.M., Pedersen, K.R., Crane, P.R., 2006. Cretaceous angiosperm flowers: innovation and evolution in plant reproduction. Palaeogeogr. Palaeocl. 232, 251–293.

Friis, E.M., Pedersen, K.R., Schonenberger, J., 2003. *Endressianthus*, a new Normapolles-producing plant genus of Fagalean affinity from the Late Cretaceous of Portugal. Int. J. Plant Sci. 164, S201–S223.

Fujioka, T., Chappell, J., Honda, M., Yatsevich, I., Fifield, K., Fabel, D., 2005. Global cooling initiated stony deserts in central Australia 2–4 Ma, dated by cosmogenic Ne-21–Be-10. Geology 33, 993–996.

Galley, C., Linder, H.P., 2006. Geographical affinities of the Cape flora, South Africa. J. Biogeogr. 33, 236–250.

Givnish, T.J., Pires, J.C., Graham, S.W., McPherson, M.A., Prince, L.M., Patterson, T.B., Rai, H.S., Roalson, E.H., Evans, T.M., Hahn, W.J., Millam, K.C., Meerow, A.W., Molvray, M., Kores, P.J., O'Brien, H.E., Hall, J.C., Kress, W.J., Sytsma, K.J., 2005. Repeated evolution of net venation and fleshy fruits among monocots in shaded habitats confirms *a priori* predictions: evidence from an *ndhF* phylogeny. Proc. R. Soc. B. 272, 1481–1490.

Gradstein, F.M., Ogg, J.G., 2004. Geologic Time Scale 2004—why, how, and where next! Lethaia 37, 175–181.

Greenwood, D.R., Conran, J.G., 2000. The Australian Cretaceous and Tertiary monocot fossil record. In: Wilson, K.L., Morrison, D.A. (Eds.), Monocots: Systematics and Evolution. CSIRO Publishing, Collingwood, Victoria, pp. 52–59.

Greenwood, D.R., Haines, P.W., Steart, D.C., 2001. New species of *Banksieaeformis* and a *Banksia* 'cone' (Proteaceae) from the Tertiary of central Australia. Aust. Syst. Bot. 14, 871–890.

Heatwole, H., 1987. Major components and distributions of the terrestrial fauna. In: Dyne, G.R. (Ed.), Fauna of Australia. Australian Government Publishing Service, Canberra, pp. 101–135.

Hill, R.S., Scriven, L.J., Jordan, G.J., 1995. The fossil record of Australian Proteaceae. In: McCarthy, P. (Ed.), Flora of Australia, vol. 16, Eleagnaceae, Proteaceae 1. CSIRO Australia, Melbourne, pp. 21–30.

Hopper, S.D., Gioa, P., 2004. The south–west Australian floristic region: evolution and conservation of a global hotspot of biodiversity. Annu. Rev. Ecol. Evol. Syst. 35, 623–650.

Howarth, D.G., Gustafsson, M.H.G., Baum, D.A., Motley, T.J., 2003. Phylogenetics of the genus *Scaevola* (Goodeniaceae): implication for dispersal patterns across the Pacific Basin and colonization of the Hawaiian Islands. Am. J. Bot. 90, 915–923.

Humphries, C.J., Parenti, L., 1999. Cladistic Biogeography. Oxford University Press, Oxford, UK.

Janssen, T., Bremer, K., 2004. The age of major monocot groups inferred from 800+ *rbcL* sequences. Bot. J. Linn. Soc. 146, 385–398.

Jennings, W.B., Pianka, E.R., Donnellan, S., 2003. Systematics of the lizard family Pygopodidae with implications for the diversification of Australian temperate biotas. Syst. Biol. 52, 757–780.

Jordan, G.J., Carpenter, R.J., Hill, R.S., 1998. The macrofossil record of Proteaceae in Tasmania: a review with new species. Aust. Syst. Bot. 11, 465–501.

Jordan, G.J., Dillon, R.A., Weston, P.H., 2005. Solar radiation as a factor in the evolution of scleromorphic leaf anatomy in Proteaceae. Am. J. Bot. 92, 789–796.

Joseph, L., Moritz, C., Hugall, A., 1995. Molecular support for vicariance as a source of diversity in rainforest. Proc. R. Soc. London B. 260, 177–182.

Knapp, M., Stöckler, K., Havell, D., Delsuc, F., Sebastiani, F., Lockhart, P.J., 2005. Relaxed molecular clock provides evidence for long-distance dispersal of *Nothofagus* (Southern Beech). PLoS Biol. 3, 38–43.

Lavin, M., Herendeen, P.S., Wojciechowski, M.F., 2005. Evolutionary rates analysis of Leguminosae implicates a rapid diversification of lineages during the tertiary. Syst. Biol. 54, 575–594.

Li, Q., Simo, J.A., McGowran, B., Holbourn, A., 2004. The eustatic and tectonic origin of Neogene unconformities from the Great Australian Bight. Mar. Geol. 203, 57–81.

Linder, H.P., Eldenas, P.K., Briggs, B.G., 2003. Contrasting patterns of radiation in African and Australian Restionaceae. Evolution 57, 2688–2702.

Linder, H.P., Hardy, C.R., Rutschmann, F., 2005. Taxon sampling effects in molecular clock dating: an example from the African Restionaceae. Mol. Phylogenet. Evol. 35, 569–582.

Macphail, M.K., Alley, N.F., Truswell, E.M., Sluiter, I.R.K., 1994. Early Tertiary vegetation: evidence from spores and pollen. In: Hill, R.S. (Ed.), History of the Australian vegetation: Cretaceous to Recent. Cambridge University Press, Cambridge, UK, pp. 189–261.

Magallon, S., Sanderson, M.J., 2001. Absolute diversification rates in angiosperm clades. Evolution 55, 1762–1780.

Marchant, N.G., 1973. Species diversity in the southwestern flora. J. R. Soc. Western Aust. 56, 23–30.

Martin, H.A., 1994. Australian Tertiary phytogeography: evidence from palynology. In: Hill, R.S. (Ed.), History of the Australian vegetation: Cretaceous to Recent. Cambridge University Press, Cambridge, pp. 104–142.

Mast, A.R., Givnish, T.J., 2002. Historical biogeography and the origin of stomatal distributions in *Banksia* and *Dryandra* (Proteaceae) based on their cpDNA phylogeny. Am. J. Bot. 89, 1311–1323.

McGlone, M.S., 2005. Goodbye Gondwana. J. Biogeogr. 32, 739–740.

McGowran, B., Holdgate, G.R., Li, Q., Gallagher, S.J., 2004. Cenozoic stratigraphic succession in southeastern Australia. Aust. J. Earth Sci. 51, 459–496.

McNamara, K.J., Scott, J.K., 1983. A new species of *Banksia* (Proteaceae) from the Eocene Merlinleigh Sandstone of the Kennedy Range, Western Australia. Alcheringa 7, 185–193.

Munasinghe, D.H.N., Burridge, C.P., Austin, C.M., 2004. Molecular phylogeny and zoogeography of the freshwater crayfish genus *Cherax* Erichson (Decapoda: Parastacidae) in Australia. Biol. J. Linn. Soc. 81, 553–563.

Myers, N., Mittermeier, R.A., Mittermeier, C.G., da Fonseca, G.A.B., Kent, J., 2006. Biodiversity hotspots for conservation priorities. Nature 403, 853–858.

Nee, S., May, R.M., Harvey, P.H., 1994. The reconstructed evolutionary process. Philos. Trans. R. Soc. B. 344, 305–311.

Nelson, E.C., 1974. Disjunct plant distributions on the south-western Nullarbor Plain, Western Australia. J. R. Soc. Western Aust. 57, 105–116.
Nelson, E.C., 1981. Phytogeography of southern Australia. In: Keast, A. (Ed.), Ecological biogeography of Australia. Dr. W. Junk, The Hague, pp. 734–759.
Nelson, G., Platnick, N., 1981. Systematics and biogeography: cladistics and vicariance. Columbia University Press, New York.
Orthia, L.A., Crisp, M.D., Cook, L.G., de Kok, R.P.J., 2005. Bush peas: a rapid radiation with no support for monophyly of *Pultenaea* (Fabaceae: Mirbelieae). Aust. Syst. Bot. 18, 133–147.
Penny, D., Phillips, M.J., 2004. The rise of birds and mammals: are microevolutionary processes sufficient for macroevolution? Trends Ecol. Evol. 19, 516–522.
Posada, D., Crandall, K.A., 1998. MODELTEST: testing the model of DNA substitution. Bioinformatics 14, 817–818.
Poux, C., Madsen, O., Marquard, E., Vieites, D.R., Jong, W.W.D., Vences, M., 2005. Asynchronous colonization of Madagascar by the four endemic clades of primates, tenrecs, carnivores, and rodents as inferred from nuclear genes. Syst. Biol. 54, 719–730.
Rambaut, A., 1999. TreeThief: a tool for manual phylogenetic tree entry from scanned images. Department of Zoology, University of Oxford, Oxford.
Renner, S., 2004. Plant dispersal across the tropical Atlantic by wind and sea currents. Int. J. Plant Sci. 165, S23–S33.
Ronquist, F., Huelsenbeck, J.P., 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19, 1572–1574.
Rosen, D.E., 1978. Vicariant patterns and historical explanation in biogeography. Syst. Zool. 27, 159–188.
Rutschmann, F., 2006. Molecular dating of phylogenetic trees: a brief review of current methods that estimate divergence times. Divers. Distr. 12, 35–48.
Sanderson, M.J., 2003. r8s: inferring absolute rates of molecular evolution and divergence times in the absence of a molecular clock. Bioinformatics 19, 301–302.
Sanderson, M.J., Thorne, J.L., Wikstrom, N., Bremer, K., 2004. Molecular evidence on plant divergence times. Am. J. Bot. 91, 1656–1665.
Sanmartín, I., Ronquist, F., 2004. Southern Hemisphere biogeography inferred by event-based models: plant versus animal patterns. Syst. Biol. 53, 216–243.
Savolainen, V., Anstett, M.-C., Lexer, C., Hutton, I., Clarkson, J.J., Norup, M.V., Powell, M.P., Springate, D., Salamin, N., Baker, W.J., 2006. Sympatric speciation in palms on an oceanic island. Nature 441, 210–213.
Scriven, L.J., Hill, R.S., 1995. Macrofossil Casuarinaceae: their identification and the oldest macrofossil record, *Gymnostoma antiquum* sp. nov., from the late paleocene of New South Wales, Australia. Aust. Syst. Bot. 8, 1035–1053.
Sheard, M.J., Smith, P.C., 1995. Karst and mound spring deposits. In: Drexel, J.F., Preiss, W.V. (Eds.), The Geology of South Australia, The Phanerozoic, vol. 2. Department of Mines and Energy, Adelaide, pp. 257–261.
Siegel, S., 1956. Nonparametric Statistics for the Behavioural Sciences. McGraw-Hill, New York.
Sokal, R.R., Rohlf, F.J., 1969. Biometry. W.H. Freeman & Co, San Francisco.
Steane, D.A., Wilson, K.L., Hill, R.S., 2003. Using *matK* sequence data to unravel the phylogeny of Casuarinaceae. Mol. Phylogenet. Evol. 28, 47–59.
Swofford, D.L., 2002. PAUP*, Phylogenetic Analysis Using Parsimony (*and Other Methods). Sinauer Associates, Sunderland, Massachusetts.
Tam, S.M., Boyce, P.C., Upson, T.M., Barabe, D., Bruneau, A., Forest, F., Parker, J.S., 2004. Intergeneric and infrafamilial phylogeny of subfamily Monsteroideae (Araceae) revealed by chloroplast *trnL-F* sequences. Am. J. Bot. 91, 490–498.
Thorne, J.L., Kishino, H., 2002. Divergence time and evolutionary rate estimation with multilocus data. Syst. Biol. 51, 689–702.
Unmack, P.J., 2001. Biogeography of Australian freshwater fishes. J. Biogeogr. 28, 1053–1089.
Vinnersten, A., Reeves, G., 2003. Phylogenetic relationships within Colchicaceae. Am. J. Bot. 90, 1455–1462.
Waters, J.M., Craw, D., 2006. Goodbye Gondwana? New Zealand biogeography, geology, and the problem of circularity. Syst. Biol. 55, 351–356.
Weston, P.H., Barker, N.P., 2006. A new suprageneric classification of the Proteaceae, with an annotated checklist of genera. Telopea 11, 314–344.
Winkworth, R.C., Wagstaff, S.J., Glenny, D., Lockhart, P.J., 2005. Evolution of the New Zealand mountain flora: origins, diversification and dispersal. Organisms Divers. Evol. 5, 237–247.
Won, H., Renner, S.S., 2006. Dating dispersal and radiation in the gymnosperm *Gnetum* (Gnetales): clock calibration when outgroup relationships are uncertain. Syst. Biol. 55, 610–622.
Yoder, A.D., Nowak, M.D., 2006. Has vicariance or dispersal been the predominant biogeographic force in Madagascar? Only time will tell. Annu. Rev. Ecol. Evol. Syst. 37, 405–431.