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A climate change context for the decline of a foundation tree species in south-western Australia: insights from phylogeography and species distribution modelling

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- **Background and Aims** A worldwide increase in tree decline and mortality has been linked to climate change and, where these represent foundation species, this can have important implications for ecosystem functions. This study tests a combined approach of phylogeographic analysis and species distribution modelling to provide a climate change context for an observed decline in crown health and an increase in mortality in *Eucalyptus wandoo*, an endemic tree of south-western Australia.
- **Methods** Phylogeographic analyses were undertaken using restriction fragment length polymorphism analysis of chloroplast DNA in 26 populations across the species distribution. Parsimony analysis of haplotype relationships was conducted, a haplotype network was prepared, and haplotype and nucleotide diversity were calculated. Species distribution modelling was undertaken using Maxent models based on extant species occurrences and projected to climate models of the last glacial maximum (LGM).
- **Key Results** A structured pattern of diversity was identified, with the presence of two groups that followed a climatic gradient from mesic to semi-arid regions. Most populations were represented by a single haplotype, but many haplotypes were shared among populations, with some having widespread distributions. A putative refugial area with high haplotype diversity was identified at the centre of the species distribution. Species distribution modelling showed high climatic suitability at the LGM and high climatic stability in the central region where higher genetic diversity was found, and low suitability elsewhere, consistent with a pattern of range contraction.
- **Conclusions** Combination of phylogeography and paleo-distribution modelling can provide an evolutionary context for climate-driven tree decline, as both can be used to cross-validate evidence for refugia and contraction under harsh climatic conditions. This approach identified a central refugial area in the test species *E. wandoo*, with more recent expansion into peripheral areas from where it had contracted at the LGM. This signature of contraction from lower rainfall areas is consistent with current observations of decline on the semi-arid margin of the range, and indicates low capacity to tolerate forecast climatic change. Identification of a paleo-historical context for current tree decline enables conservation interventions to focus on maintaining genetic diversity, which provides the evolutionary potential for adaptation to climate change.

Key words: Climate change, *Eucalyptus wandoo*, Myrtaceae, evolution, forest decline, haplotypes, last glacial maximum, LGM, phylogeography, refugia, species distribution modelling, tree decline.

INTRODUCTION

In the last few decades, a worldwide increase in tree decline and mortality has been linked to climate change, including increasingly frequent extreme weather events, such as droughts and heatwaves (e.g. Breshears *et al.*, 2005; Allen *et al.*, 2010), and long-term global increases in temperature and decreases in rainfall (Carnicer *et al.*, 2011). Climate change interacts with, and amplifies, the impact of other disturbance factors affecting forest ecosystems, such as insect and pathogen outbreaks, landscape fragmentation and fire (Dale *et al.*, 2001; Breshears *et al.*, 2005). As such, and because climate change projections indicate an intensification of recent trends, it is thought that forest ecosystems could be increasingly vulnerable to climate-induced

decline (Anderegg *et al.*, 2013). Given that trees can be foundation species, with a major influence on other biota, community structure and composition, and ecosystem function (Manning *et al.*, 2006; Anderegg *et al.*, 2013), understanding their response to current and future climatic patterns is important for climate change adaptation strategies that maintain ecosystem function and facilitate species persistence (Moritz and Agudo, 2013).

Phylogeography can provide important insights into the effects of paleo-historical climate change on the current geographic distribution and genetic structure of species (Avice, 2000; Byrne, 2007), and this historical context is helpful to understand species responses to future climate change (Forester *et al.*, 2013). The effects of historical climatic oscillations on

biodiversity were particularly pronounced from the middle Pleistocene through to the last glacial maximum (LGM; approx. 21 000 years BP). While in the northern hemisphere significant areas were affected by glaciation, the southern hemisphere experienced cyclic conditions, varying from warm, wet environments during the interglacial periods to cold, dry environments during glacial periods. Responses to these climatic oscillations, such as repeated contraction and expansion, or persistence in localized refugia, have left signatures in the genetic composition of current populations that can be used to deduce the influence of the historical processes on their evolution (Avice, 2000; Hewitt, 2004; Byrne, 2008).

Species distribution modelling (SDM) is a useful approach to validate and/or complement biogeographic inferences derived from molecular markers (Scoble and Lowe, 2010; Svenning *et al.*, 2011; Forester *et al.*, 2013), and has been increasingly used to support evolutionary and phylogeographic studies (e.g. Schorr *et al.*, 2013). SDM describe the species ecological niches by quantifying the relationship between empirical observations of species distribution and environmental data across space and/or time (Guisan and Zimmermann, 2000). By assuming that the climatic niche of a species remains unchanged over a time period of interest (Wiens and Graham, 2005), its past distribution can be reconstructed by projecting SDM to earlier time periods using paleoclimatic data (Svenning *et al.*, 2011). Yet, a number of factors can affect SDM and result in poor projections, including uncertainty of climatic models, changes in ecological niche, decoupling of regional and local climates, and occurrence of non-analogue environments in the LGM (Worth *et al.*, 2014).

A combined approach using phylogeography and SDM can provide a helpful framework where current tree decline phenomena can be investigated. In this study, phylogeographic analysis of diversity in the chloroplast genome and SDM were used to assess the impacts of past and future climate changes on the genetic structure and geographic distribution of *Eucalyptus wandoo* (Myrtaceae), an endemic tree species of the inland woodlands of the south-western Australian global biodiversity hotspot (Hopper and Gioia, 2004). Recent and ongoing climate change has been implicated in the decline of *E. wandoo* crown health and increased mortality, which have been more pronounced in the low rainfall areas of the species distribution range (Hooper and Sivasithamparan, 2005; Brouwers *et al.*, 2013; Poot and Veneklaas, 2013).

Given these patterns of decline in *E. wandoo*, it is hypothesized that the species may have a long-term response to historical climate change that is different from that observed in phylogeographic studies of other widespread south-western Australian *Eucalyptus* species (e.g. *E. loxophleba*, *E. marginata*, *E. kochii*, *E. horistes* and *E. gomphocephala*; Byrne and Macdonald, 2000; Byrne *et al.*, 2003; Byrne and Hines, 2004; Wheeler and Byrne, 2006; Nevill *et al.*, 2014). These studies have identified a high diversity and differentiation in chloroplast DNA (cpDNA) and the presence of highly localized haplotypes throughout the species distribution ranges, which indicates persistence in localized refugia during the LGM. It is hypothesized that *E. wandoo* may have a different genetic signature in chloroplast diversity, with signals of contraction during times of aridity and expansion during more mesic conditions, and a geographic pattern that is consistent with modelling of the species distribution at the LGM.

Insight into the evolutionary history of *E. wandoo* will enable understanding of the species response to ongoing and future climate change and provide a context for the management of factors influencing its current decline.

MATERIALS AND METHODS

Study area and study species

Eucalyptus wandoo Blakely has a broad distribution in south-western Australia, from the high (800–1200 mm mean annual rainfall) to the transitional rainfall (300–800 mm) zones (Fig. 1). Nevertheless, the species has been largely cleared for agriculture, with <5 % of the pre-European extent (approximately the 1830s) of *E. wandoo* remaining, mostly in isolated remnants affected by multiple disturbance factors (e.g. fragmentation and salinity; Yates *et al.*, 2000). The species occurs in lowland areas across broad valleys and grows on loamy or sandy soils. Two subspecies are recognized; subspecies *pulverea* is restricted to the northern boundary of the species distribution and is distinguished from subspecies *wandoo* by its pruinose branchlets and powdery bark (Brooker and Hopper, 1991).

South-western Australia experiences a mediterranean climate, with 80 % of the rainfall occurring in winter and only 4 % in summer. The region has undergone significant climatic changes since the 1970s, with an increase of 0.6 °C in mean annual temperature and a decline of 17 % in mean winter rainfall (Bates *et al.*, 2008; CSIRO and Bureau of Meteorology, 2014). Further drying and warming is projected, with a forecast decrease of 5–60 % in annual rainfall and an increase of 1–5.5 °C in annual temperature by 2070 (Bates *et al.*, 2008).

Genotyping

Phylogeographic analyses were undertaken using restriction fragment length polymorphism (RFLP) technology, as this is known to detect intraspecific polymorphism and phylogeographic patterns in the chloroplast genome of Western Australian species, including eucalypts (Byrne, 2007, 2008), and enables comparison with other species from the study area previously analysed using the same method. A recent comparative study in *Calothamnus quadrifidus* has shown congruence between cpRFLP and sequence data sets, demonstrating the validity of RFLP methodology (Nistelberger *et al.*, 2014).

Previous studies on Western Australian eucalypts have shown that cpDNA diversity is generally maintained between populations (Byrne, 2007). Therefore, leaves were collected from five healthy and well-spaced (50–100 m) trees from 26 provenances covering the distribution range of *E. wandoo* (Fig. 1; Table 1). The majority of the populations sampled were from the subspecies *wandoo*, while two populations (Mt Lesueur and Dookanooka Nature Reserve) were from the restricted northern subspecies *pulverea*. A sample from each of three related species, *E. angustissima* (Point Malcolm), *E. loxophleba* (Lake King) and *E. loxophleba* (Bowgada), were included as outgroups. Although occasional interseries hybrids have been noted between *E. wandoo* and *E. loxophleba*, these

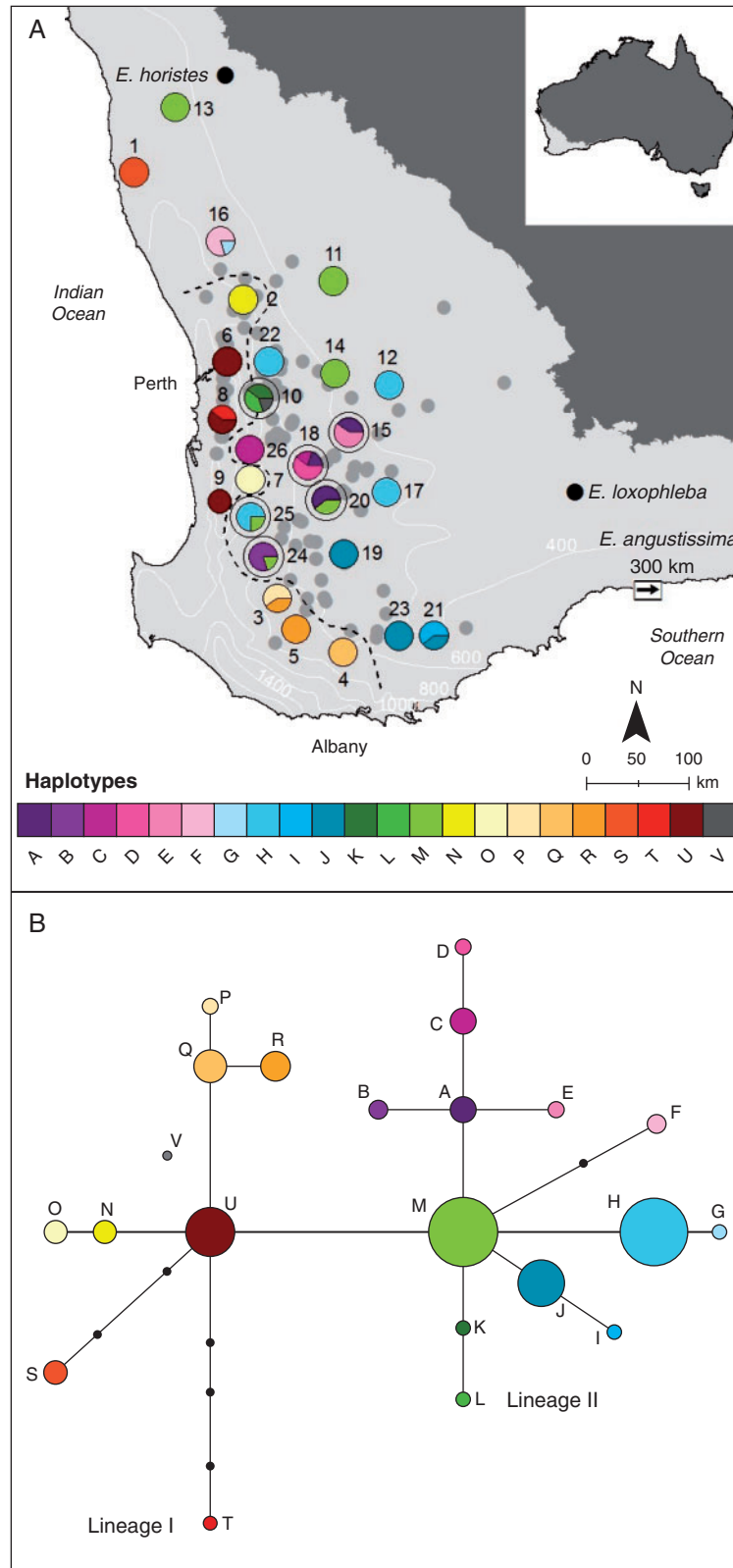


Fig. 1. Location of the sampled populations of *Eucalyptus wandoo* in south-western Australia and their haplotype composition. (A) Sampled populations of *E. wandoo* are represented with large circles that are coloured according to their haplotype composition. Populations with high haplotype diversity within the refugium area are marked with an outer circle. Species records obtained from NatureMap are represented by light grey circles. Locations of the outgroup species samples are represented with black dots (except for *E. angustissima*, which is located 300 km east from the arrow, on the coast). The dashed line represents the phylogeographic break between the two lineages. Rainfall isohyets lines (200 mm) are represented in white. (B) Haplotype network for *E. wandoo*. Haplotypes are identified with the letters A–V, according to Table 2. Interior haplotypes not detected in the samples are represented by small black circles. Each line represents a single polymorphism.

TABLE 1. Name, location, annual potential evapotranspiration (mm), annual rainfall (mm), aridity index and lineage identifier of the 26 *Eucalyptus wandoo* populations that were used in the chloroplast DNA analysis

Population name (ID)	Latitude (S)	Longitude (E)	Annual potential evapotranspiration (mm)	Annual rainfall (mm)	Aridity index	Lineage
<i>Mt Lesueur NP</i> (1)	30°09'47"	115°11'48"	1650	625	0.38	I
Bindoon east (2)	31°19'42"	116°15'47"	1605	654	0.41	I
Boyup Brook south (3)	33°59'16"	116°30'51"	1299	568	0.44	I
Rocky Gully east (4)	34°28'41"	117°11'56"	1217	575	0.47	I
Frankland west (5)	34°15'43"	116°42'16"	1251	611	0.49	I
John Forrest NP (6)	31°53'15"	116°04'35"	1605	830	0.52	I
Gorrie Rd (7)	32°53'49"	116°16'41"	1570	832	0.53	I
Serpentine NP (8)	32°22'55"	116°00'45"	1535	1016	0.66	I
Harvey Dam (9)	33°05'13"	115°56'47"	1454	963	0.66	I
Running Brook Rd (10)	32°11'47"	116°23'36"	1542	795	0.52	I and II
Namalcatchem NR (11)	31°11'18"	117°11'53"	1642	348	0.21	II
Boolanelling NR (12)	32°07'06"	117°44'55"	1525	333	0.22	II
<i>Dookanooka NR</i> (13)	29°36'04"	115°38'28"	1746	438	0.25	II
Quairading-York roadside (14)	32°00'26"	117°11'27"	1549	400	0.26	II
Tutanning NR (15)	32°31'47"	117°19'16"	1473	397	0.27	II
Moor south (16)	30°48'13"	116°02'60"	1670	463	0.28	II
Dongolocking NR (17)	33°03'56"	117°41'52"	1385	398	0.29	II
Dryandra woodlands NP (18)	32°49'11"	116°52'56"	1451	473	0.33	II
Wingedyne NR (19)	33°36'24"	117°14'12"	1342	442	0.33	II
Highbury SF (20)	33°07'34"	117°03'51"	1398	461	0.33	II
Stirling Range NP east (21)	34°20'39"	118°09'53"	1247	412	0.33	II
Talbot Block north (22)	31°53'04"	116°30'29"	1352	647	0.38	II
Stirling Range NP west (23)	34°20'36"	117°47'53"	1573	599	0.38	II
Boyup Brook north (24)	33°36'38"	116°23'04"	1223	470	0.48	II
Collie east (25)	33°14'19"	116°15'53"	1405	825	0.59	II
Boddington west (26)	32°43'45"	116°17'50"	1442	928	0.64	II

The two populations of subspecies *pulverea* are indicated in italic.

Populations are ordered by lineage (refer to Fig. 2) and aridity index (ratio between rainfall and evapotranspiration). Abbreviations in population names represent: Rd, Road; NP, National Park; NR, Nature Reserve; SF, State Forest.

are rare (Brooker and Hopper, 1991) due to the differences in the ecological niche of the two species. The aridity index of each provenance location (ratio between annual rainfall and annual potential evapotranspiration) was calculated, as it is regarded as a useful proxy for potential water availability (Gao and Giorgi, 2008).

Genomic DNA was extracted from 10–15 g of leaf material (Byrne *et al.*, 1993) with 0.1 M sodium sulphate (Byrne *et al.*, 2001) and 0.05 % bovine serum albumin (BSA) added to the extraction buffer. DNA of each sample was digested with six restriction enzymes (*BclI*, *BglII*, *DraI*, *EcoRI*, *EcoRV* and *XbaI*) and hybridized with heterologous probes covering most of the chloroplast genome, according to the protocol described in Byrne *et al.* (1993).

Genetic data analyses

Banding patterns obtained for each probe–enzyme polymorphism combination were interpreted as length or restriction site mutations. Mutations identified by consecutive cp probes were counted only once. Nucleotide diversity, the average number of nucleotide differences per site between two sequences (Nei, 1978), and haplotype diversity, Nei's gene diversity measure (Nei, 1973), were calculated using Arlequin v3.5 (Excoffier *et al.*, 2005), and partitioned within and between populations. The relationship between nucleotide and haplotype diversity

was estimated using PERMUT (Pons and Petit, 1996). A network of haplotype relationships was generated in Network v4.6.12 (<http://www.fluxus-engineering.com>) using a median-joining algorithm (Bandelt *et al.*, 1999). A parsimony analysis of haplotype relationships characterized by the presence or absence of each polymorphism was undertaken using Phylip v3.68 (Felsenstein, 2008). Regular bootstraps of 1000 data sets were produced (SEQBOOT), 100 trees were prepared using PARS and a majority rule (>50 %) consensus tree was created using CONSENSE (Felsenstein, 2008).

Nucleotide divergence can be used to estimate the time since separation, although there are caveats to the use of a molecular clock given the difficulties in calibration from independent evidence (Wheeler and Byrne, 2006). Nevertheless, similar levels of divergence between lineages in several south-western Australian species from different botanical families provide evidence for consistent influences of major historical events in the region (Wheeler and Byrne, 2006; Byrne, 2007). Estimation of the time of divergence between lineages was made as 0.1 % nucleotide divergence representing a separation of approx. 1 million years (Zurawski *et al.*, 1984), as this has been used for restriction site data from across the chloroplast genome.

Coalescent analyses using mismatch distributions and neutrality tests were completed for the entire data set, as well as within Lineages I and II separately using Arlequin v3.5. Mismatch distribution analyses were conducted by calculating the distribution of the total observed differences between pairs

of haplotypes. The parameters of instantaneous demographic expansion and spatial expansion were estimated using a least-squares approach with 1000 bootstrap replicates. Tajima's D (Tajima, 1989, 1996) and Fu's F_s (Fu, 1997) were calculated using 1000 coalescent simulations to test for departure from neutral evolution.

Species distribution modelling

A total of 182 presence-only records of *E. wandoo* were compiled from field work and from the online biodiversity database NatureMap (<http://naturemap.dec.wa.gov.au/>; accessed February 2014), which includes data from the Western Australian Herbarium. Data were scrutinized for misidentified, suspected inaccurate or duplicate records. The background environment was constrained to the focal biogeographic regions (IBRA v7, <http://www.environment.gov.au/topics/land/national-reserve-system/science-maps-and-data/australia-bioregions-ibra>) occupied by the species.

Modern climatic data (averaging period 1961–1990) at a spatial resolution of 0.0025° (approx. 250 m) were obtained from Yates *et al.* (2010), who derived 19 bioclimatic variables using minimum monthly temperature, average maximum monthly temperature, average monthly precipitation and average monthly areal potential evapotranspiration data layers provided by the Australian Bureau of Meteorology National Climate Centre. In this study, a sub-set of five bioclimatic variables was used. These variables have been suggested by Yates *et al.* (2010) as being most relevant to plant distributions in south-western Australia, and include annual precipitation, precipitation of the driest quarter, mean temperature of the warmest quarter, mean temperature of the wettest quarter and isothermality. Projections of these bioclimatic variables for 2070 were also obtained from Yates *et al.* (2010), using a medium severity climate change scenario that includes the moderate impact model MIROC-M combined with the A1B emission scenario and medium climate sensitivity.

The same five bioclimatic variables for the LGM were obtained at a spatial resolution of 0.05° (approx. 5 km) from the WorldClim database (Hijmans *et al.*, 2005), and resampled to a 0.0025° resolution via bilinear interpolation. Initially, two general circulation models (GCMs) were considered, CCSM3 (Collins *et al.*, 2006) and MIROC (Hasumi and Emori, 2004), both derived from the Paleoclimate Modelling Inter-comparison Project Phase II (Braconnot *et al.*, 2007). However, only CCSM was used, because MIROC predicts LGM annual mean precipitation values that are higher than modern values (Supplementary Data Table S1), which is highly inconsistent with the palynological evidence that the LGM climate in south Australia was drier than at present (see Dodson, 2001).

Maxent v3.3.3k (Phillips *et al.*, 2006; Phillips and Dudík, 2008) was used to model the distribution range of *E. wandoo* under modern climate conditions. This model was then used to project the species distribution at the LGM and in 2070. Maxent is one of the most widely used and effective methods for species distribution modelling using presence-only data (Elith *et al.*, 2011). The models were trained on 75 % randomly assigned occurrences, with the remaining 25 % used as an evaluation data set. Furthermore, the default Maxent settings were used, with the exception that model building was restricted to

hinge features (Phillips and Dudík, 2008). Because species occurrence data often exhibit a spatial bias in survey effort (Schulman *et al.*, 2007), a sampling effort bias layer (target group method; Phillips *et al.*, 2009) was created using the records density of all plant species belonging to the four main terrestrial botanical families (Myrtaceae, Proteaceae, Fabaceae and Cyperaceae). The variables response curves, their percentage contribution and individual importance on the Jackknife tests were used for selection of the best predictors.

The influence of niche suitability and stability on haplotype diversity was tested using generalized linear models (GLM) in R 3.1.2 (<http://www.R-project.org/>). Niche stability was calculated between the LGM and the present ($\text{NStab}_{\text{LGM-Present}} = 1 - |\text{NS}_{\text{LGM}} - \text{NS}_{\text{Present}}|$) and between the present and 2070 ($\text{NStab}_{\text{Present-2070}} = 1 - |\text{NS}_{\text{Present}} - \text{NS}_{2070}|$), using Maxent's niche suitability (NS) values (Gugger *et al.*, 2013). Five explanatory covariates were considered in the GLM: niche stability since the LGM, past and present niche suitability, latitude and longitude. These covariates have low Pearson's pairwise correlation coefficients, except $\text{NS}_{\text{Present}}$ and NS_{LGM} ($r = 0.63$). The Akaike information criterion (AIC) was used to select the most parsimonious model.

RESULTS

Polymorphism in cpDNA

The analysis of cpDNA variation in *Eucalyptus wandoo* revealed polymorphisms with all the enzymes used. In total, 27 polymorphisms were detected, 18 length mutations and nine restriction site mutations. The majority of the polymorphisms (18) were located in the large single-copy region of the cp genome, and the rest (9) in the small single-copy region. The arrangement of mutations in individuals resulted in the identification of 22 haplotypes. The most common haplotype (M) was present in six populations and represented 14.7 % of the samples (Table 2). Haplotype H was the second most common haplotype, present in four populations (14.0 % frequency). Haplotypes U, J and A were present in three populations with 10.1, 9.3 and 4.7 % frequencies, respectively. Haplotypes R and C were present in two populations, with 5.4 and 4.7 % frequencies, respectively. The remaining haplotypes (15) were only present in one population, with frequencies ranging from 0.8 to 3.9 %. Diversity within *E. wandoo* populations was mostly represented by a single haplotype (16 out of 26 populations; frequency = 61.5 %). Eight populations (30.8 %) were represented by two haplotypes, and two populations (7.7 %) by three haplotypes.

Haplotype relationships

The network of haplotype relationships showed a structured pattern of diversity, with haplotypes separated into two groups centred on haplotypes U and M (referred to as lineages for ease of identification). Lineage I consisted of nine haplotypes (N–V) representing ten populations from the western margin of the species distribution (Fig. 1). Lineage II was formed by 13 haplotypes (A–M) present in 17 populations in the eastern inland areas of the distribution (Fig. 1). Both lineages showed a star-shaped pattern of haplotype relationships, but the level of structure

TABLE 2. Frequency and distribution of haplotypes in *Eucalyptus wandoo*, *E. angustissima*, *E. loxophleba* and *E. horistes*

Haplotype	Frequency (%)	Mutations	Population ID (no. of individuals)
A	4.65	8, 23, 46, 47, 48	18 (1), 20 (3), 15 (2)
B	3.10	8, 15, 23, 46, 47, 48	24 (4)
C	4.65	4, 8, 23, 46, 47, 48	18 (1), 26 (5)
D	2.33	4, 8, 11, 23, 46, 47, 48	18 (3)
E	2.33	8, 10, 23, 46, 47, 48	15 (3)
F	3.10	1, 23, 28, 46, 47, 48	16 (4)
G	0.78	21, 23, 25, 46, 47, 48	16 (1)
H	13.95	21, 23, 46, 47, 48	12 (5), 17 (5), 22 (5), 25 (3)
I	2.33	13, 20, 23, 46, 47, 48	21 (3)
J	9.30	20, 23, 46, 47, 48	21 (2), 23 (5), 19 (5)
K	1.55	23, 46, 47, 48, 51	10 (2)
L	1.55	5, 23, 46, 47, 48, 51	10 (2)
M	14.73	23, 46, 47, 48	24 (1), 13 (5), 20 (2), 11 (5), 14 (5), 25 (1)
N	3.88	14, 46, 47, 48	2 (5)
O	3.88	14, 26, 46, 47, 48	7 (5)
P	2.33	10, 22, 46, 47, 48	3 (3)
Q	3.88	22, 46, 47, 48	4 (5)
R	5.43	7, 22, 46, 47, 48	3 (2), 5t (5)
S	3.88	16, 24, 27, 46, 47, 48	1 (5)
T	1.55	3, 6, 9, 12, 19, 46, 47, 48	8 (2)
U	10.08	46, 47, 48	6 (5), 9 (5), 8 (3)
V	0.78	50, 46, 47, 48	10 (1)
	–	31, 36, 40, 43, 49	<i>E. angustissima</i> (1)
	–	18, 29, 30, 33, 36, 40, 41, 45	<i>E. loxophleba</i> (1)
	–	2, 17, 32, 34, 35, 37, 38, 39, 42, 44	<i>E. horistes</i> (1)

Populations are identified by their ID number followed in parentheses by the number of samples for each population with that haplotype.

within the lineages differed. The haplotypes in Lineage I showed little structure, with only two sets of related haplotypes (N/O and P/Q/R) (Fig. 1). Lineage II showed greater structure, with several pairs of related haplotypes (G/H, I/J and K/L) and one group of five haplotypes (A/B/C/D/E) (Fig. 1). Lineage II contained three out of the four most widespread haplotypes present in *E. wandoo*. There was no genetic distinction between the two identified subspecies, as haplotypes in the two populations at Mt Lesueur and Dookanooka (S and M) were not closely related, and Dookanooka had the common haplotype (M) that was present in many of the subspecies *wandoo* populations.

The phylogenetic parsimony analysis produced a single tree with a length of 55 and a consistency index of 0.93, with only one case of homoplasy found (mutation 10; Fig. 2). The phylogeny clearly distinguished *E. wandoo* from the outgroup species *E. angustissima*, *E. loxophleba* and *E. horistes*. There were no shared haplotypes with the *E. loxophleba* sample. In addition, comparison of haplotypes identified here with those identified in a study across the range of *E. loxophleba* (Byrne and Hines, 2004) also showed no shared haplotypes, indicating that any introgression is minimal. Within *E. wandoo*, the phylogeny showed the same structured pattern of diversity as the network with haplotypes separated into two main groups, although group 1 was identified as a polytomy and group 2 as a derived lineage within the polytomy.

Geographic distribution of haplotypes

Populations with haplotypes in Lineage I were distributed in areas with a mean annual rainfall of 742 mm and an aridity index of 0.51, whereas populations with haplotypes in Lineage II

occupied areas with lower mean annual rainfall (502 mm) and aridity index (0.34).

The distribution of haplotypes in Lineage I was highly structured, with most haplotypes specific to populations. Only two haplotypes were present in more than one population. In Lineage II there were some haplotypes specific to populations, mainly in the centre of the distribution, but five of the haplotypes were present in multiple populations and had widespread distributions, particularly Haplotype M to the north, Haplotype H to the east and Haplotype J to the south. An area of high diversity was identified that included six populations (populations 10, 15, 18, 20, 24 and 25) located east of Perth, in the central western area of Lineage II (Fig. 1). All these populations had two or three haplotypes, and both widespread (M) and restricted haplotypes (H, A, C) were present (Fig. 1).

At the species level, tests of neutrality were negative, Fu's F_s was significant ($P = 0.014$) and Tajima's D was just non-significant ($P = 0.068$). Tests of mismatch distribution were not significantly different from that expected under a sudden expansion or a spatial expansion model (Table 3). Similarly, Fu's F_s and Tajima's D were negative but not significant for both lineages, and tests of mismatch distribution were not significant for either sudden expansion or spatial expansion models for both lineages (Table 3).

Nucleotide and haplotype diversity

Nucleotide diversity and haplotypes diversity are presented in Table 3. Nucleotide diversity was higher in Lineage I than in Lineage II, although haplotype diversity was similar between lineages. Nucleotide diversity among populations (N_{ST}) and

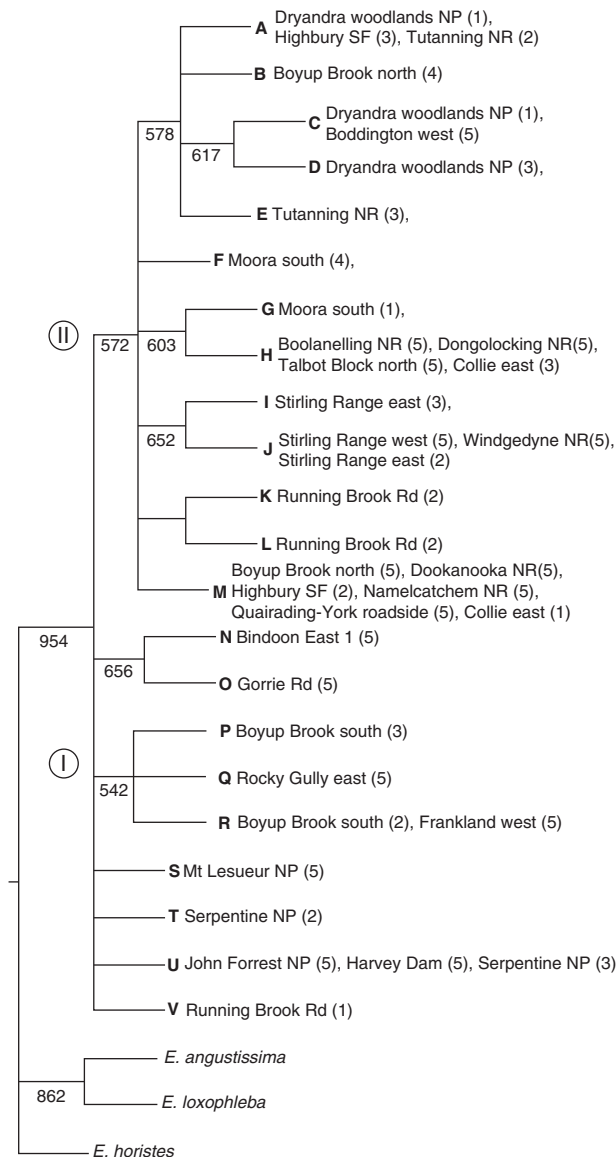


FIG. 2. Phylogenetic parsimony tree of haplotype relationships in *Eucalyptus wandoo*. The consensus tree is based on 1000 bootstrap replications. Haplotypes are identified with the letters A–V, according to Table 2. Numbers below lines represent bootstrap values. Numbers in parentheses indicate sample numbers of each provenance. Roman numerals refer to the lineage number.

haplotype diversity among populations (G_{ST}) were 0.830 and 0.765, respectively, and were higher for Lineage I than for Lineage II. Nucleotide diversity was not significantly higher than haplotype diversity overall or in either lineage. Nucleotide divergence between Lineage I and II was 0.053 %, and the two lineages are estimated to have been separated during the later Pleistocene, approx. 530 000 years ago, later than in other species where divergence was estimated during the middle Pleistocene or earlier (700 000 to 1 million years ago, Byrne *et al.*, 2002, 2003, Byrne and Hines, 2004; 1.76 mya, Nistelberger *et al.*, 2014).

Nucleotide diversity averaged 0.051 % over all pairs of individuals in the area of high diversity in Lineage II, whereas in

TABLE 3. Nucleotide and haplotype diversity, and parameters for tests of neutrality and mismatch distributions for *Eucalyptus wandoo* at the species level and for each lineage, including: haplotype diversity between populations (G_{ST}), nucleotide diversity between populations (N_{ST}), Harpenders raggedness index under a spatial expansion model (H_{spat}) and Harpenders raggedness index under a spatial expansion model (H_{sudd})

	All populations	Lineage I	Lineage II
Nucleotide diversity	0.091 (0.054)	0.081 (0.050)	0.063 (0.040)
Haplotype diversity	0.929 (0.009)	0.862 (0.027)	0.870 (0.019)
N_{ST}	0.830 (0.047)	0.857 (0.098)	0.811 (0.041)
G_{ST}	0.765 (0.060)	0.887 (0.077)	0.708 (0.086)
$P N_{ST} > G_{ST}$	0.525	0.619	0.478
Tajima's D	-1.315 (0.068)	-0.714 (0.262)	-0.747 (0.223)
Fu's F_s	-7.793 (0.014)	-0.560 (0.421)	-3.624 (0.08)
H_{spat}	0.029 (0.560)	0.023 (0.807)	0.051 (0.266)
H_{sudd}	0.029 (0.560)	0.023 (0.811)	0.051 (0.271)

Standard errors (for nucleotide diversity, haplotype diversity, G_{ST} and N_{ST}) and probability of significance (for D , F_s , H_{spat} , H_{sudd}) are presented in parentheses.

the rest of Lineage II distribution, nucleotide diversity averaged 0.021 %. In the high diversity area, total haplotype diversity was 0.896, haplotype diversity within populations was 0.601 and the proportion of haplotype diversity between populations was 0.329. For the rest of the Lineage II area, total haplotype diversity and diversity within populations was lower (0.788 and 0.091, respectively) and diversity between populations was higher (0.884).

Species distribution modelling

The model explaining the current distribution of *E. wandoo* (Fig. 3A) had high predictive performance [area under the curve (AUC) = 0.87] and included all five bioclimatic variables considered: mean temperature of the wettest (37.7 % variation explained) and warmest quarter (16.3 %), isothermality (22.0 %), annual precipitation (13.0 %) and precipitation of the driest quarter (11.0 %). Although mean temperature of the warmest quarter and precipitation of the driest quarter had a high Pearson pairwise correlation coefficient ($r = -0.82$), they were both introduced in the model because they were both important and not as strongly correlated during the LGM ($r = -0.73$). The realized current distribution of the species was generally well predicted by the model, with the exception of the far northern, eastern and southern range boundaries, which were scored with a low presence probability value, despite the presence of species records in those areas.

The distribution model of *E. wandoo* at the LGM was not strikingly different from the current distribution model, yet it showed a lower climatic suitability in the east (drier), and a higher suitability in the west (more mesic) of the range (Fig. 3B), suggesting range contraction. Most importantly, a central area of high climatic suitability with a north-west–south-east orientation was apparent in the LGM modelled distribution (Fig. 3B). The forecast distribution of *E. wandoo* for 2070 was consistent with the current and LGM distribution patterns, showing a pattern of decreased niche suitability from the

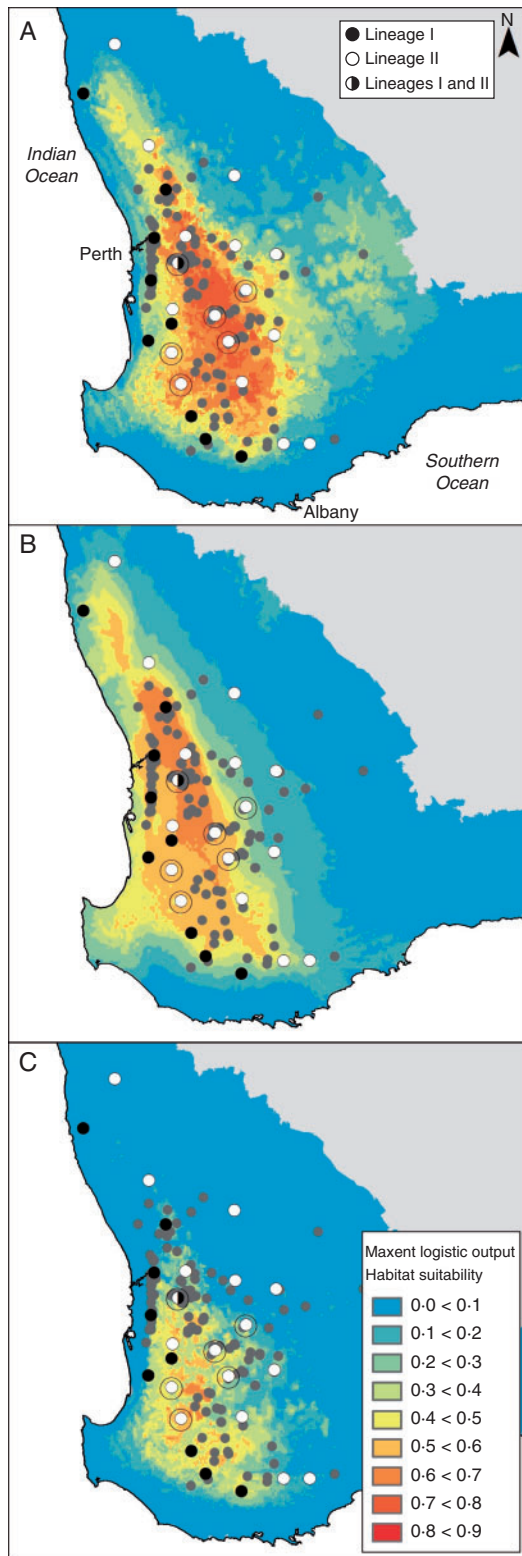


FIG. 3. Distribution models of *Eucalyptus wandoo* for (A) the present, (B) the last glacial maximum (approx. 21 000 years) and (C) 2070 periods. Warmer colours indicate a higher probability of species presence, whereas colder colours indicate a lower probability of species presence (see key). Records of *E. wandoo* obtained from NatureMap are represented by light grey circles, and sampled populations are represented by black/white circles as indicated in the key in (A). Populations with high haplotype diversity within the refugium area are marked with an outer circle.

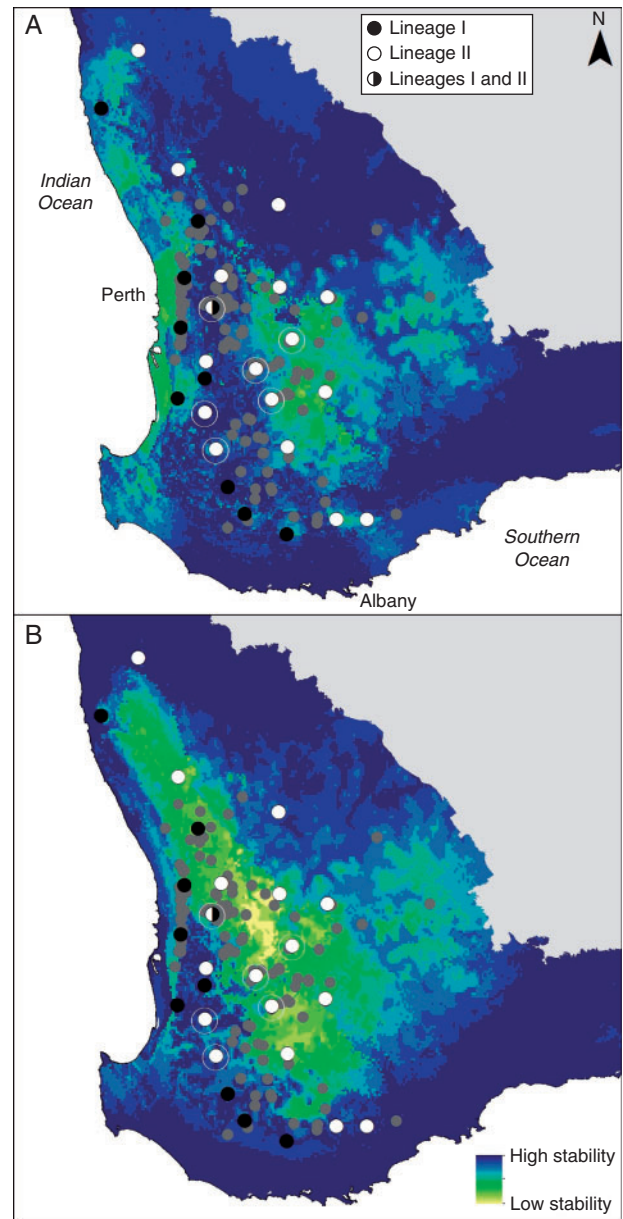


FIG. 4. Climatic stability for *E. wandoo* (A) in the past, between the LGM and present moment, and (B) in the future, projected for 2070. Records of *E. wandoo* obtained from NatureMap are represented by light grey circles, and sampled populations are represented by black/white circles as indicated in the key in (A). Populations with high haplotype diversity within the refugium area are marked with an outer circle.

eastern but also northern range boundaries. Under the medium severity climate change scenario, the model predicted the distribution of *E. wandoo* to become more restricted to the western and southern regions of the current distribution, with possible extirpations from the northern and eastern regions. Despite the location of the refugial populations in the area of high past climatic suitability and stability (Fig. 4A), the GLM did not show any significant effects of these variables, nor of latitude or longitude, on present haplotype diversity. Only current climatic suitability was positively related to haplotype diversity when

included in a model containing only this variable ($\beta = 0.57$; $P = 0.042$; $AIC = 11.23$).

DISCUSSION

This study revealed how a combined approach using phylogeographic analysis and SDM can provide a paleo-historical context to understand current climate-driven tree decline phenomena. In the case of *E. wandoo*, this approach showed a historical signature of long-term persistence in a central-western refugial area, contraction to this area during the LGM, and more recent expansion to the east but also north and south. SDM has also predicted that future climate change will lead to a similar range contraction from the drier eastern and northern regions of the species distribution range. While not evidence of causality, these patterns are consistent with the currently observed mortality and decline of *E. wandoo* crown health, which are more pronounced in the eastern low rainfall areas (e.g. Brouwers *et al.*, 2013), and consistent with other suggestions that the species is vulnerable to ongoing and future climate change (Hooper and Sivasithamparam, 2005; Poot and Veneklaas, 2013).

Phylogeography, SDM and climate change

While inference of population history from haplotype distributions can be complex (e.g. Ibrahim *et al.*, 1996; Excoffier and Ray, 2008; Worth *et al.*, 2014), the different patterns of haplotype distribution within the *E. wandoo* lineages suggest the influence of different evolutionary processes in different geographic regions. The highly structured distribution of haplotypes in the western lineage, with most haplotypes specific to populations, is consistent with persistence of the species through multiple climatic cycles. In contrast, the widespread distribution of several haplotypes with internal positions in the network in the northern, eastern and southern peripheral populations of the eastern lineage is consistent with contraction of the species from these areas during arid cycles, and expansion from a refugial area during mesic conditions (Crandall and Templeton, 1993; Templeton *et al.*, 1995). This latter pattern can occur when populations are in equilibrium and have been in their present range for a substantial period of time (Slatkin, 1993).

Coalescence analysis provides inferences of population history (Rosenberg and Nordborg, 2002) and indicated a scenario of both spatial and demographic expansion in *E. wandoo* following the LGM. The inference of refugia and contraction/expansion was supported by SDM, which showed maintenance of high past climatic suitability and stability in the same area where high haplotype diversity was observed, and lower climatic suitability in the inland peripheral areas at the LGM compared with the current period (although low climatic suitability does not necessarily mean that the species was not able to persist; see Worth *et al.*, 2014). While this pattern could also arise through contraction and persistence in very small populations and subsequent demographic expansion, such a scenario might be expected to lead to population-specific haplotypes (e.g. Byrne and Hines, 2004; Byrne, 2008), which were not observed here.

The *E. wandoo* western lineage is located along the Darling Scarp and the western edge of the Darling Plateau, which are areas of medium to low current climatic suitability, according to SDM. In this region, *E. wandoo* has a scattered distribution, occurring in small stands on sloping sides of valleys, in either lateritic or granitic substrates (Boland, 1984). This contrasts with the species main distribution range where it occurs as a dominant species, forming open forests on broad shallow valleys or low ridges, with clay or loam substrates (Boland, 1984). While climatic conditions along the Darling Scarp may not be optimal for *E. wandoo*, habitat heterogeneity and localized areas maintaining suitable microclimatic conditions may explain the long-term persistence of the species in the area.

The fact that past climate stability did not have a significant influence on haplotype diversity was unexpected; however, it may be explained by the low power of the analysis, due to the relatively low number of sampled populations and low haplotype diversity. Ideally, fossil evidence is required to determine historical distributions (Magri *et al.*, 2006). However, fossil evidence in south-western Australia is very limited and there are no fossil cores from the inland part of the distribution where contraction may have occurred at the LGM (Dodson, 2001).

Phylogeography and SDM can reveal new insights into species responses to climate change, as in this case where the central-western area had not been identified before as having characteristics of refugia. Refugial areas in south-western Australia have been identified in coastal areas, ranges and areas of high topographical complexity (including granite outcrops), and areas of high species richness and endemism (Hopper, 1979; Byrne, 2008; Nistelberger *et al.*, 2014). The putative refugium in *E. wandoo* does not show these characteristics, but does appear to have been a region of relative climatic stability during the LGM, based on SDM, and possibly during earlier arid cycles within the Pleistocene.

Despite the consistency between phylogeographic data and SDM, and the recognized usefulness of this combined approach (Scoble and Lowe, 2010; Forester *et al.*, 2013), more comprehensive analyses are required to draw more definitive conclusions about species responses to past and future climatic changes. Recognized issues of SDM-based predictions include the uncertainty of climatic models and climatic reconstructions, assumptions that the species fundamental niche do not change over time, difficulties in modelling the occurrence of non-analogue environments in the LGM, and implementing interspecific interaction, dispersal and migration scenarios (Collevatti *et al.*, 2013; Worth *et al.*, 2014).

Ideally, evidence from ecophysiological and genomic analyses, including field trial survival and growth responses and physiological data on drought tolerance thresholds, are required to understand fully species response to climate change. Combined ecophysiological and genomic approaches have identified both adaptation and plasticity across a climatic gradient in eastern Australia in *E. tricarpa* (McLean *et al.*, 2014; Steane *et al.*, 2014), and similar patterns of adaptation and plasticity identified in *E. loxophleba* in south-western Australia (D. A. Steane, unpubl. data) are consistent with previous phylogeographic inference of persistence in localized refugia throughout the range (Byrne and Hines, 2004; Byrne, 2008). In contrast, a similar approach identified cryptic lineages with variation not associated with climate in *E. salubris* (Steane *et al.*, 2015).

The recent decline observed in *E. wandoo* and an evolutionary pattern of contraction and expansion suggest that the adaptive and/or plastic capacity of this species may not be as strong as in other eucalypts.

Evolutionary history, tree decline and conservation

Insights derived from phylogeographic analysis and SDM, while not evidence of causality, provide evidence of species responses to climate change. In *E. wandoo* they provide additional lines of evidence consistent with recent reports of tree decline, such as a comparison of crown health that found a decline in dry areas and most sensitivity to climate change in the eastern dry, warm end of the range (Brouwers *et al.*, 2013). The observed phenomenon of decline in the last three decades as the climate became drier and warmer is consistent with the species' inferred response to historical climate change. This emphasizes the need for management and conservation strategies to focus on the western areas of the current distribution where the species has persisted with larger population size through Pleistocene climatic oscillations, since these areas maintain genetic diversity that provides the evolutionary potential for adaptation to climate change.

Other disturbance factors and local environmental conditions are likely to interact with climate change, and possibly exacerbate the impacts in *E. wandoo*. Effects of landscape fragmentation on remnant vegetation are greatest in regions with high maximum temperatures and declining rainfall (Mantyka-Pringle *et al.*, 2012). Thus, it is likely that the current decline of *E. wandoo* observed in the highly fragmented inland part of the distribution is impacted by this synergistic effect, as remnant woodlands with small size or a relative large perimeter/area ratio are more prone to crown decline (Brouwers *et al.*, 2013). Furthermore, species with shallower root systems may be affected by soil water deficits, and this has also been demonstrated in *E. wandoo*, as most water in clay sub-soils becomes unavailable as upper soil layers dry out (Poot and Veneklaas, 2013).

Climate-driven tree decline due to drought and heat stress has been observed worldwide and is expected to increase with future climate change (Anderegg *et al.*, 2013). This study has shown that a combined approach using phylogeography and SDM can provide an evolutionary context to climate-driven tree decline phenomena and a supportive framework where this can be investigated. Indeed, as both methods can cross-validate evidence for refugia and contraction under harsh climatic conditions, the combined approach offers insight into species response to past environmental changes, and sheds light on their trajectory into the future. Consistent patterns across genetic, spatial modelling, observational and/or ecophysiological data can trigger the development of tailored research questions to understand the patterns and mechanisms of tree mortality, and the development of targeted conservation measures and climate change adaptation strategies aiming at reducing and mitigating the ecological, societal and climatological impacts of forest decline. While a combined approach using phylogeography and SDM has been recognized as a useful tool to understand species response to past and future climate change (e.g. Scoble and Lowe, 2010), this

study is, to our knowledge, the first one to suggest its use in the context of the global issue of tree decline.

SUPPLEMENTARY DATA

Supplementary data are available online at www.aob.oxfordjournals.org and consist of Table S1: differences in annual mean temperature between current and LGM climate, and proportion of annual mean precipitation between LGM and current climate for the two global circulation models MIROC and CCSM.

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