



Fire is a major driver of patterns of genetic diversity in two co-occurring Tasmanian palaeoendemic conifers

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ABSTRACT

Aim The impacts of Holocene fires on the genetic architecture of fire-intolerant species have largely been overlooked. Here, we investigate the relative impacts of the last glacial climate versus Holocene fires on the genetic diversity of two co-occurring, fire-intolerant conifers using a comparative population genetic study.

Location The palaeoendemic plant-rich montane rain forests and alpine coniferous heath of Tasmania, Australia.

Methods The Tasmanian endemic conifers *Athrotaxis cupressoides* D. Don (461 samples from 20 populations) and *Diselma archeri* Hook.f. (576 samples from 23 populations, 16 of which were for sites sampled for *A. cupressoides*), were genotyped using eight and nine EST nuclear microsatellites respectively. Genetic diversity and structure was compared between the two species and the factors underlying genetic patterns in both species were investigated by examining isolation by distance, correlations with Last Glacial Maximum modelled distributions and the fossil record, and a fire history index of the sampled stands.

Results The range-wide genetic structure of the two species was similar ($F_{st} = 0.09$ and $F'_{st} = 0.21$ for *A. cupressoides* versus *D. archeri*; $F_{st} = 0.06$ and $F'_{st} = 0.24$), and there were significant correlations between species for population-based expected heterozygosity, allelic richness, private allelic richness and pairwise genetic divergences. Furthermore, genetic diversity metrics decreased significantly with an index of fire history. Given fossil evidence and modelling evidence that both species occurred near their current ranges during the last glaciation and a lack of evidence for isolation by distance in either species, the plausible explanation for the patterns of diversity is genetic decline resulting from repeated Holocene fires.

Main conclusions Our study suggests that fire can have substantial impacts on genetic structure and diversity of plant species, particularly those without fire-tolerant traits, and that any increases in fire resulting from climate change may impose substantial threats to such species. In Tasmania, the observed increase in dry lightning in recent years, combined with periods of abnormally dry conditions, may therefore further degrade the range and genetic diversity of fire-intolerant palaeoendemic species.

Keywords

alpine coniferous heath, *Athrotaxis cupressoides*, comparative population genetics, *Diselma archeri*, fire history, genetic diversity, Holocene, Last Glacial Maximum, montane rain forest

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INTRODUCTION

Most studies of species range-wide genetic variation have focussed on the impacts of glacial climates, especially during the Last Glacial Maximum (LGM; *c.* 21,000 years ago). Indeed, there is abundant evidence for major vegetation changes during the last glacial, with cold climate taxa expanding as warm climate or mesic taxa became restricted in glacial refugia (Williams *et al.*, 1997). Phylogeographical studies demonstrate the significant legacy of LGM species distributions on both current species ranges and patterns of genetic variation within species (Petit *et al.*, 2003; Svenning & Skov, 2007). Although fire is a major factor in determining species distributions and vegetation structure (Bowman *et al.*, 2009) little is known about its long-term impacts on genetic diversity and structure of species. While studies have revealed strong impacts of recent human-induced habitat fragmentation on species range-wide genetic diversity (Finger *et al.*, 2012) there are few studies that have investigated the genetic legacy of environmental change over the Holocene (*i.e.* the last 11,700 years), such as changes in fire regime, on the genetic diversity and structure of species.

The western half of Tasmania, Australia, is an ideal system to investigate the relative importance of last glacial and Holocene environmental changes on extant genetic diversity. The vegetation consists of a mosaic of fire tolerant sedgeland and *Eucalyptus* forests mixed with fire-intolerant rain forests and alpine vegetation. Palaeoendemic Tasmanian species, notably Nothofagaceae, Cupressaceae and Podocarpaceae species, are dominants of many of these fire-sensitive vegetation types (Jordan *et al.*, 2016). Palaeoendemic species are eliminated by moderate to severe fire events and poorly regenerate after low-intensity fires by seed or asexual growth (Kirkpatrick, 1977). Many of these species are long-lived enabling reconstruction

of past climate variation (Allen *et al.*, 2014a,b) and dating of past fire events using fire scars (Holz *et al.*, 2015). The region also has numerous fossil pollen records that have enabled an understanding of the distributional changes of plant species in response to environmental change since deglaciation, and in some cases, the LGM (Colhoun & Shimeld, 2012). In summary, this region is an ideal study system to investigate the impact of fire on species genetic diversity.

Fossil records from western Tasmania indicate that some of the palaeoendemic conifers (*Athrotaxis*, *Diselma*, *Microcachrys* and *Pherosphaera*) were more abundant and widespread during the last and previous glacial periods than at present (Van Der Geer *et al.*, 1994). The transition from the last glacial to the Holocene involved an increase in mean annual temperature of *c.* 6° and the spread of highly flammable sclerophyll vegetation. Tasmanian palaeoendemic species are therefore likely to have been impacted by the warmer climate and increased fire frequency characterizing the Holocene environment. For example, at the end of the last glacial, *Pherosphaera hookeriana* disappeared entirely from a former stronghold in the West Coast Range (Colhoun *et al.*, 1999).

This study investigates the genetic diversity and structure of two key conifers of the palaeoendemic fire-intolerant Tasmanian flora, *Athrotaxis cupressoides* D. Don and *Diselma archeri* Hook.f. These species currently have similar distributions in perhumid mountainous areas of western Tasmania (Fig. 1) and often grow together. Fire events resulted in the contraction of both species in the late Holocene (Kirkpatrick & Dickinson, 1984). It remains unclear if these fires were set by Aboriginal people or lightning. After European settlement in the early 19th century, there has been an increase in anthropogenic ignitions exacerbated by periods of lower than average rainfall resulting in substantial contraction of fire-

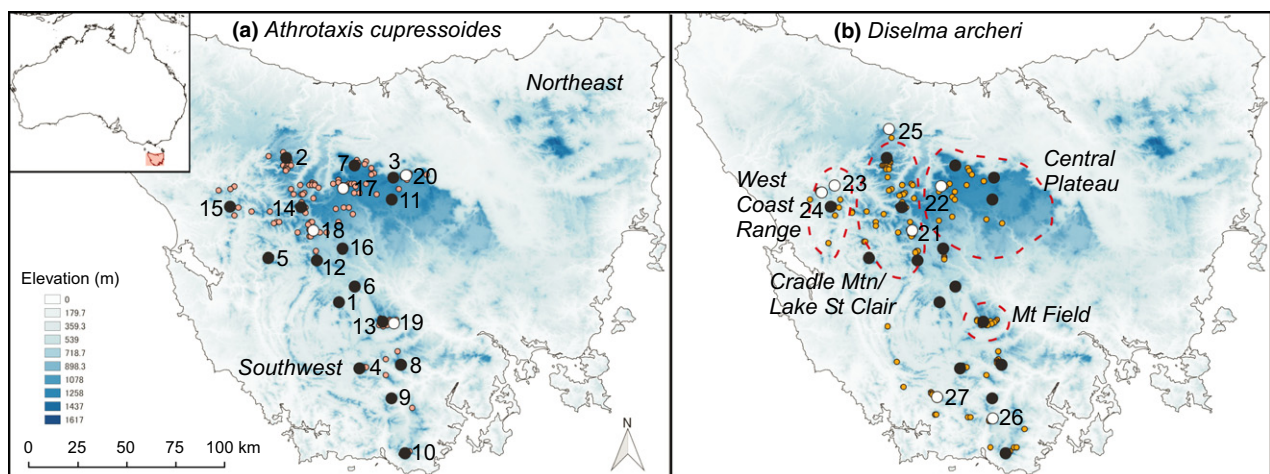


Figure 1 The location of (a) the 20 populations of *Athrotaxis cupressoides* and (b) the 23 populations of *Diselma archeri* sampled for this study. Population numbers for all 16 populations where both species were collected (black circles) are given in panel (a) while numbers for those populations where only one species was collected (white circles) are given in either panel (a) or (b) respectively. Population numbering follows Table S1 in Appendix S2. Distribution records of each species are shown as small circles (pink for *A. cupressoides* and orange for *D. archeri*). The location of regions mentioned in the text are encircled by red dashed lines in panel (b).

sensitive vegetation (Mariani & Fletcher, 2016). Since the end of the 20th century, more frequent and longer durations of droughts coupled with increased dry lightning storms (Allen *et al.*, 2014a,b) has led to more landscape fires (Parks and Wildlife Service 2015). Collectively, these factors have resulted in the loss of *c.* 33% of *A. cupressoides* stands since European colonization (Cullen, 1989), and substantial but poorly documented contraction of *D. archeri*.

This study aims to assess the relative importance of glacial refugia, genetic isolation by distance and/or Holocene burning in shaping the extant range-wide genetic diversity of two fire-intolerant conifers, *A. cupressoides* and *D. archeri*. The sampling strategy aimed to sample almost the entire geographical range of each species including range-edge populations and targeted 16 sites where both species grew together. Specifically, we aimed to: (1) determine if these two species have similar genetic structure and diversity; and (2) examine the correspondence between genetic diversity with evidence for (1) glacial refugia from the fossil record and species distribution modelling and (2) fire impact on the sampled populations.

MATERIALS AND METHODS

The study species

Athrotaxis cupressoides and *Diselma archeri* are evergreen, wind-pollinated, long-lived conifers with individual stem ages reaching over 1000 (Ogden 1978) and 550 years (Gibson *et al.*, 1995) respectively. The species have overlapping discontinuous distributions in western, central and southern Tasmania (Fig. 1) usually between 800–1400 m in elevation. Both species are capable of root suckering and can form clonal patches (Eckenwalder, 2009), but seed dispersal is generally over very short distances (Kirkpatrick & Dickinson, 1984). Both species are intolerant of fire (Kirkpatrick & Dickinson, 1984) with, for example, no recovery after 55 years in 209 stands of *A. cupressoides* killed by a fire in the Central Plateau in the 1960s (Holz *et al.*, 2015). However, some individuals of both species recover after low intensity burns via root sprouts albeit extremely slowly (Fitzgerald, 2011).

The species differ in habit and ecology. *Athrotaxis cupressoides* ranges from single-stemmed 30-m tree in subalpine forests to a multiple-stemmed shrub under 50-cm high in exposed alpine areas. *Diselma archeri* is usually a shrub, 0.05–3-m tall, although it occasionally reaches 10-m high. Two pieces of evidence emphasise the strong role that fire, rather than physiology or competition, has in limiting both species to their current ranges. First, the only occurrences of these species below 800 m above sea level are in fire refugia that have remain unburnt for thousands of years (Kirkpatrick, 1975). Second, *D. archeri* has been found to be drought tolerant in contrast to *A. cupressoides* (Brodrigg *et al.*, 2014) but their similar distributions are consistent with fire restricting them to the same low fire frequency habitats.

Sampling

Populations of both species were collected from across most of their geographical distributions. For *A. cupressoides*, 461 samples (average 23 per population) were collected from 20 populations (Fig. 1a) while for *D. archeri* 576 samples (average of 25 per population) were collected from 23 populations (Fig. 1b). Both species were collected from the same locality in 16 sites (Table S1 in Appendix S2 in Supporting Information). Thirty genetically identical individuals of *A. cupressoides* and two of *D. archeri* (evaluated using GIMLET 1.3.3 (Valière, 2002)) were excluded from further analyses (Table S1 in Appendix S2). Three other samples of *A. cupressoides* were excluded because they were identified as backcrosses with the only other species of the genus, *A. selaginoides* (see Worth *et al.*, 2016).

Molecular methods

Eight expressed sequence tag (EST) nuclear microsatellite loci and a single mitochondrial locus (Sakaguchi *et al.*, 2014a) were used to genotype all *A. cupressoides* samples. For *D. archeri*, nine nuclear EST loci (Sakaguchi *et al.*, 2014b) were used (See Appendix S1 for further details on molecular methods and data integrity). Such genetic markers are suitable for investigating the past impacts of Late Quaternary (i.e. the LGM and Holocene) environmental change on genetic diversity and structure of these species given their high diversity and the susceptibility of rare alleles to genetic drift and bottlenecks. Especially in long-lived trees including conifers, the impacts of the LGM refugia and post-glacial dispersal are evident in range-wide genetic studies (e.g. Kimura *et al.*, 2014; Souto *et al.*, 2015).

Genetic structure and gene flow

Global, population and locus level F_{st} , F_{st}^2 and Jost's D were calculated using GENODIVE 2.0b23 (Meirmans & Van Tienenderen, 2004) for both *A. cupressoides* and *D. archeri* using all nuclear loci. The genetic relationships of populations were investigated using neighbour net unrooted trees based on Nei's genetic distance (D_a) (Nei *et al.*, 1983) computed in MSANALYZER 4.05 (Dieringer & Schlotterer, 2003). Global- and population-level genetic statistics were also calculated for the single mtDNA microsatellite in *A. cupressoides* in GENODIVE 2.0b23.

To assess geographical structuring of genetic variation for both species, Bayesian cluster analysis was undertaken in the program BAPS 6 (Corander *et al.*, 2003). BAPS minimizes the Hardy–Weinberg and linkage disequilibrium that would result if individuals from different randomly mating populations were grouped into one population and performs well when population differentiation is relatively low (i.e. F_{st} is between 0.03–0.1) (Latch *et al.*, 2006). Mantel tests were conducted for each species in GENALEX 6.5 (Peakall & Smouse, 2012) to assess whether genetic differentiation was correlated with geographical distance using a population-based pairwise matrix of $F_{st}/(1 - F_{st})$ calculated in SPAGED1 1.5a (Hardy & Vekemans, 1999).

Genetic diversity and bottlenecks

For all nuclear loci, population-based observed heterozygosity (H_o), expected heterozygosity (H_e) and inbreeding co-efficients (F_{is}) were calculated in GENODIVE 2.0b23 (Meirmans & Van Tienderen, 2004) in both species. Rarefied allelic richness (Ar) and rarefied private allelic richness (PAR) was calculated in HPRARE 1.1 (Kalinowski, 2005) using the minimum sample size minus one of the smallest populations ($N = 14$ for *A. cupressoides* and $N = 19$ for *D. archeri*). For the analysis of allelic richness, the small *A. cupressoides* population at Mt Picton ($N = 4$) was excluded because this parameter is particularly sensitive to effective population size (Petit *et al.*, 1998). The average number of rare alleles per individual (under 5% frequency in a population) was also calculated for each population. Population-based genetic diversity statistics were mapped using the inverse distance weighted interpolation method in QUANTUM GIS LYON 2.12 (Quantum GIS Development Team, 2014). Population-based haplotype richness (Hr) for the single mtDNA microsatellite in *A. cupressoides* was calculated using the minimum population size of 14-1 in ADZE 1.0 (Szpiech *et al.*, 2008).

Associations between the species population-based genetic diversity statistics (Number of alleles, H_e , H_o , Ar , PAR , F_{is} and rare alleles) were assessed using the Pearson correlation coefficient based on 15 sites where both *A. cupressoides* and *D. archeri* were sampled (Table S1 in Appendix S2), excluding Mt Picton. For this analysis, Ar and PAR values were calculated using a minimum population size of $N = 14-1$ for both species. Pairwise genetic distance based on F_{st}^2 (Hedrick, 2005) was used to assess correlations between population pairwise genetic distance because, unlike F_{st} , it is independent of within species diversity (Meirmans *et al.*, 2011) and is suitable for comparing species with different population sizes (Hedrick, 2005).

Two methods were employed to detect past reductions in population size (i.e. bottlenecks). Firstly, allelic frequencies at nuclear loci were evaluated for each population to assess whether allele distributions followed a non-L-shaped curve pattern expected for bottlenecked populations (Luikart *et al.*, 1998). Secondly, we used the M -ratio method of Garza & Williamson (2001). Due to the sensitivity of this test to individual loci diversity, the analysis for *A. cupressoides* was undertaken with and without two low diversity loci (Ath_0110469 and Ath_10673).

Species distribution modelling and the fossil record

Presence data for *Athrotaxis cupressoides* and *Diselma archeri* was sourced from the Tasmanian Natural Values Atlas (Department of Primary Industries, Parks, Water and Environment) (177 point locations for *A. cupressoides* and 192 for *D. archeri*). Modelling was undertaken using the maximum entropy algorithm implemented in MAXENT 3.3.3a (Phillips *et al.* 2006) in the present and using four LGM general circulation models (FGOALS, CCSM, MIROC and

CNRM-CM) following the procedure of Worth *et al.* (2014) (see Appendix S1 for further details). Fossil records of Tasmania, from both published and unpublished sources (see Appendix S1), covering the last 50 kya were examined for pollen and/or macrofossil presence of *Athrotaxis* and *Diselma*. *Athrotaxis* and *Diselma* pollen is poorly dispersed with levels over 2% likely to present local presence (Fletcher & Thomas, 2007). When available, maximum charcoal abundance values were recorded within the following time periods within each core, pre-LGM (50–24 kyr), LGM (24–19 kyr), post-glacial (19–11 kyr), Early/Middle Holocene (11–4 kyr) and the Late Holocene (4–1 kyr).

Correlation of genetic diversity with fire or glacial history

To investigate possible factors underlying the nuclear genetic diversity in the two species correlations between the population-based genetic diversity of all populations (H_e , H_o , Ar , PAR , F_{is} and rare alleles) versus LGM habitat suitability and the Holocene fire legacy at each site was assessed. For the fire analysis, observations in the field were used to assess the correlation between past fires and extant genetic diversity. This was preferred to maps of fire boundaries (e.g. Marsden-Smedley, 1998) which do not give accurate information at the local level and do not exist for fires older than 200 years. This assessment was aided by the fact that the dead stems of both species are easy to identify, are highly rot resistant and can remain in place for possibly hundreds of years after a fire event. We calculated a fire legacy index which is the sum of five variables: (1) the presence or absence of fire-killed *A. cupressoides*/*D. archeri* within stands (1 = yes, 0 = no); (2) the presence or absence of fire-killed trees of any species adjacent to stand (1 = yes, 0 = no); (3) *A. cupressoides*/*D. archeri* mostly confined to river/lake edge and/or fire protected topographic or rocky sites (1 = yes, 0 = no); (4) no evidence of expansion of young plants of *A. cupressoides* or *D. archeri* via either root sucker and/or seed which is an indicator of the time since the last fire (0 = yes, 1 = no); and (5) the abundance of the highly fire-sensitive *Nothofagus gunnii* within each sampled stand (1 = absent, 0.5 = rare, 0 = common). This species has a similar widespread distribution to *A. cupressoides* and *D. archeri* and is known to have become locally extinct due to past fires (Fletcher *et al.*, 2014). The fire legacy index was calculated for each site (Table S4) by summing the scores for each category. Associations between variables were assessed using Pearson correlation coefficient.

RESULTS

Data integrity

There was no evidence for the microsatellite loci of either species having been affected by null alleles. In addition, there was no indication of loci being under either balancing or divergent selection based on Jeffreys' scale of evidence

(Jeffreys, 1961) (Table S5 & S6) with posterior probability values all below 0.5.

Genetic diversity

For *A. cupressoides*, 71 alleles were found (Table 1) with between 2 and 24 alleles per locus (average = 8.88). H_o averaged 0.50 (varying from 0.20 to 0.73), H_e averaged 0.57 (0.24–0.83) and F_{is} averaged 0.131 (0.08–0.19). Lowest H_o values (< 0.45) were observed at Crooked Lake (population 1), Lake Laurel (6) and Lake Skinner (8). The Labyrinth (14), Dixons Kingdom (17) and Eliza Plateau (4) had the highest Ar values (> 4.5) while lowest values were observed at Crooked Lake (2.67), Lake Laurel (3.06), Lake Skinner (3.11) and Lady Barron Creek (19; 3.41) (Table S2; Fig. S1). Sixteen alleles (22.5% of all alleles) were private, with three each found at Lake McKenzie (7), The Labyrinth (14) and Tyndall Range (15); two at Dunnings Rivulet (3) and Eliza Plateau (4); and one each in populations Ouse River (11), Tarn Shelf (13) and at Crooked Lake (1). For the single mitochondrial locus, the genetic diversity patterns were similar to the nuclear SSR results (Fig. S2) with The Labyrinth having the highest haplotype richness of 8.05 (average over all populations = 5.46) while Lake Laurel, Crooked Lake and Lady Barron Creek had the lowest haplotype richness values of 2.56, 2.94 and 3.00 respectively.

Diselma archeri had more alleles (127) than *A. cupressoides* and averaged 14 alleles per locus (varying from 8 to 21) (Table 2). Observed heterozygosity and expected heterozygosity averaged 0.75 (0.62–0.84) and 0.76 (0.62–0.85) respectively. F_{is} values were lower on average than *A. cupressoides* ranging from –0.04–0.05 (Table 2). The Labyrinth (14), Tyndall Range (15) and Western Arthurs (27) had the highest allelic richness (> 8) while lowest values (< 5.1) were observed at Paddys Lake (25), Pine Lake Moor (26) and at Crooked Lake (1) (Table S3 & Fig. S1). Pine Lake Moor had

Table 1 Genetic statistics for the eight nuclear loci and single mitochondrial locus (marked with an asterisk) for *Athrotaxis cupressoides*. The genetic statistics are as follows: The number of genotypes observed (Num), observed heterozygosity (H_o), expected heterozygosity (H_e), the inbreeding coefficient (F_{is}) and three measures of genetic differentiation F_{st} , F'_{st} (Hedrick, 2005) and Jost's D (Jost 2008).

Locus	Num	H_o	H_e	F_{is}	F_{st}	F'_{st}	Jost's D
Ath_0007271	6	0.47	0.51	0.09	0.08	0.17	0.10
Ath_0107243	6	0.56	0.66	0.15	0.10	0.30	0.23
Ath_10577	21	0.66	0.74	0.11	0.12	0.48	0.41
Ath_0110469	2	0.20	0.24	0.18	0.11	0.14	0.04
Ath_10673	2	0.38	0.44	0.14	0.09	0.16	0.08
Ath_0012547	4	0.40	0.44	0.08	0.04	0.07	0.03
Ath_0004711	7	0.59	0.73	0.19	0.07	0.26	0.21
Ath_10333	23	0.73	0.83	0.12	0.09	0.53	0.48
Ath_0007753*	12	–	–	–	0.10	0.11	0.09
Nuclear loci average	8.88	0.50	0.57	0.13	0.09	0.21	0.14

Table 2 Genetic statistics for the nine nuclear loci for *Diselma archeri*. The genetic statistics are as follows: The number of genotypes observed (Num), observed heterozygosity (H_o), expected heterozygosity (H_e), the inbreeding coefficient (F_{is}) and three measures of genetic differentiation F_{st} , F'_{st} (Hedrick, 2005) and Jost's D (Jost 2008).

Locus	Num	H_o	H_e	F_{is}	F_{st}	F'_{st}	Jost's D
Dis_2015338	9	0.69	0.72	0.05	0.05	0.18	0.14
Dis_2011621	18	0.82	0.81	–0.01	0.06	0.34	0.30
Dis_2007758	8	0.67	0.67	0.01	0.07	0.21	0.15
Dis_2059421	21	0.80	0.82	0.02	0.04	0.23	0.20
Dis_2010620	16	0.82	0.80	–0.02	0.04	0.19	0.16
Dis_2013526	11	0.62	0.62	0.01	0.03	0.09	0.06
Dis_2015338	16	0.82	0.79	–0.04	0.07	0.34	0.29
Dis_2005261	17	0.84	0.85	0.00	0.05	0.35	0.32
Dis_2007543	11	0.69	0.73	0.05	0.09	0.33	0.26
Average	14.1	0.75	0.76	0.01	0.06	0.24	0.19

three private alleles, two each were found at Lake McKenzie (7) and Western Arthurs (27), while numerous populations had single private alleles.

Genetic structure and genetic relatedness

Geographical structuring in *A. cupressoides* was observed with an F_{st} of 0.09, F'_{st} of 0.21 and Jost's D of 0.14 (Table 1). The neighbour net based on Nei's Da showed that populations in the main distributional area of the species range (populations 3, 7, 12, 14, 17, 20) and the western mountains (5 and 15) were closely related while, apart from populations 4 and 13, populations further south were more diverged (Fig. 2a). The most diverged populations were Lake Laurel (6) and Crooked Lake (1). BAPS found 10 clusters with probabilities of $K = 10$ being 0.991 versus 0.0092 for the next best value of $K = 9$ (Fig. S3). One BAPS cluster was widespread and contained 11 populations. The other nine clusters each consisted of one population. Six of these were range-edge populations (2, 8, 10, 11, 15, 19), but Lake Laurel, Crooked Lake and Shadow Lake (populations 1, 6 and 18) occurred in the central part of the range.

Overall genetic structure for *Diselma archeri* was quite similar to *A. cupressoides* with an F_{st} of 0.06, F'_{st} of 0.24 and Jost's D of 0.19. The neighbour net showed the most diverged populations to be the northernmost population (pop. 25), the lowest elevation population (pop. 26) and Lake Laurel and Crooked Lake populations in central Tasmania (Fig. 2b). Most populations in the core of the range in the Central Plateau and Lake St Clair/Cradle Mountain area were closely related. BAPS found six clusters with a probability of $K = 6$ being 1.0. Eighteen populations from across the species' range shared the same cluster (Fig. S4). The remaining five clusters consisted of one population each and matched the most diverged populations according to the neighbour net analysis.

There was no significant association between genetic distance and geographical distance of populations for either species ($R^2 = 0.0166$ and $P = 0.136$ for *A. cupressoides* and

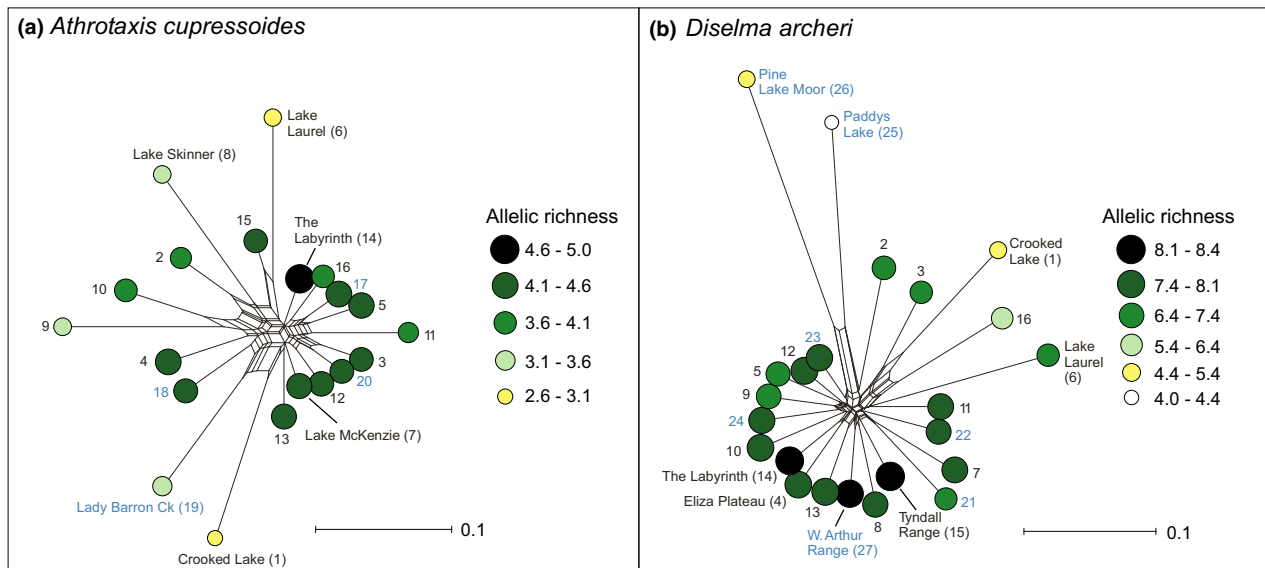


Figure 2 Unrooted neighbour net based on Nei's genetic distance (D_a) (Nei *et al.*, 1983) for 20 populations of *Athrotaxis cupressoides* (a) and 23 populations of *Diselma archeri* (b). Populations where both species were sampled are labelled in black while populations where only one species was sampled are in blue text. Population numbering follows Table S1 in Appendix S2.

$R^2 = 0.0003$ and $P = 0.402$ for *D. archeri*; Fig. S5). This remained the case when the most diverged populations in central Tasmania were excluded or when southern populations and northern ones were analysed separately (data not shown).

Comparative genetic diversity and structure

When only the 15 populations where both species were collected are considered (excluding the small population nine), the two species showed similar genetic structure: $F_{st} = 0.09$, $F_{st}^2 = 0.21$ and Jost's $D = 0.14$ for *Athrotaxis cupressoides*; and $F_{st} = 0.04$, $F_{st}^2 = 0.19$ and Jost's $D = 0.16$ for *Diselma archeri*. There were significant correlations between the species for H_e , Ar and PAr but not for rare alleles (Fig. 3) or H_o and F_{is} (data not shown). Low diversity at Crooked Lake and Lake Laurel (1 and 6) and high diversity in the core of both species' ranges contributed to the significant correlations for expected heterozygosity (H_e) ($P = 0.014$) and allelic richness (Ar) ($P = 0.005$). Lake Skinner (8) was an outlier with low Ar and H_e in *A. cupressoides* but relatively high values for *D. archeri*. A Mantel test showed significant ($P = 0.035$) association between the pairwise genetic distance matrices of the 15 paired populations based on the standardized F_{st} (Fig. 4).

Bottlenecks

Most populations of *Athrotaxis cupressoides* had L-shaped allele frequency histograms due to an abundance of alleles in the lowest frequency class (under 10%) (Fig. S6) consistent with them being stable populations retaining rare alleles. However, three populations (Crooked Lake, Lake Laurel

and Lake Skinner) showed non-L-shaped histograms. For *Diselma archeri*, which had more alleles per locus on average, the allele histograms were mostly L-shaped and with steeper trend line slopes than *A. cupressoides*. Evidence for bottlenecks in *D. archeri* was most evident in three populations (Crooked Lake, Paddys Lake and Pine Lake Moor) with the most distorted allele frequency histograms and the only populations with alleles in the 0.9 frequency class (Fig. S7). M -ratios below the 0.68 value, which is considered to provide definitive evidence of a past bottleneck (Garza & Williamson, 2001), for *A. cupressoides* were observed for Crooked Lake (0.71 and 0.61 with and without the two lowest diversity loci respectively) and Dunning's Rivulet (3) (0.73 and 0.64). Lake Laurel (0.78 and 0.71) and Ouse River (0.78 and 0.70) were the closest to the critical value of 0.68 in all other populations. For *D. archeri*, no populations had M -ratio values under 0.68 with the lowest three values for populations at populations 25 (0.71), 26 (0.71) and 3 (0.74).

Species distribution models and the fossil record

The species distribution models for the present predicted all known records of both species under the most stringent threshold (10th percentile training presence) apart for records along the Ouse River where both species are confined close to the river edge (Fig. 5). The predicted habitat suitability maps were highly similar for both species with the largest area of contiguous suitable habitat in the north of the species' ranges in the high elevation areas of central Tasmania while suitable habitat was confined to the higher mountain ranges in southern Tasmania. *Athrotaxis cupressoides* is absent from suitable habitat in some mountain ranges of

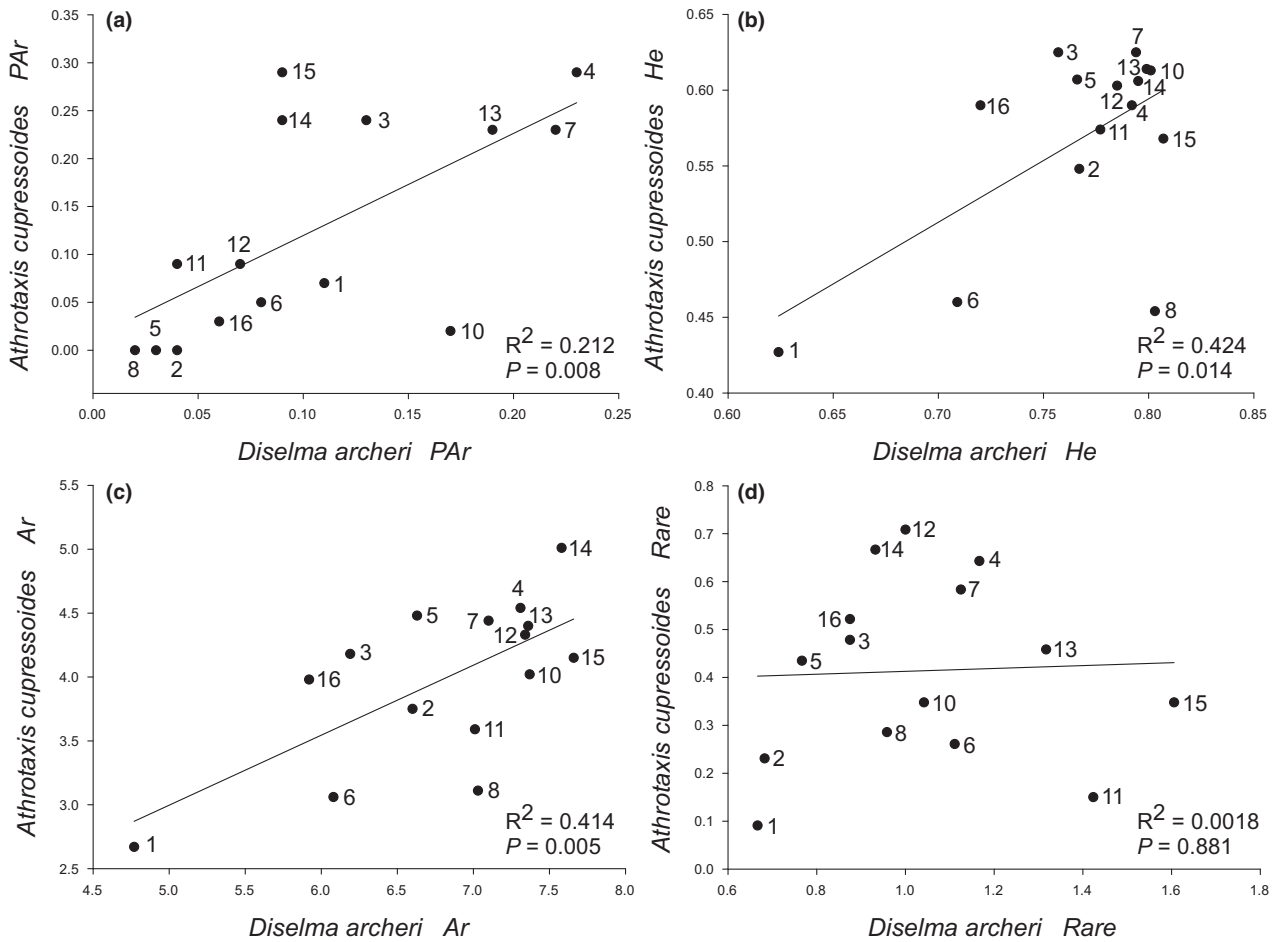


Figure 3 The correlations of genetic diversity ((a) private allelic richness (PAR), (b) expected heterozygosity (He), (c) rarefied allelic richness (Ar) (when both species were rarefied to a sample size of 13 individuals) and (d) the number of rare alleles per individual (Rare) for the 15 populations) where both *Athrotaxis cupressoides* and *Diselma archeri* were sampled, excluding Mt Picton (9). Population numbering follows Table S1 in Appendix S2.

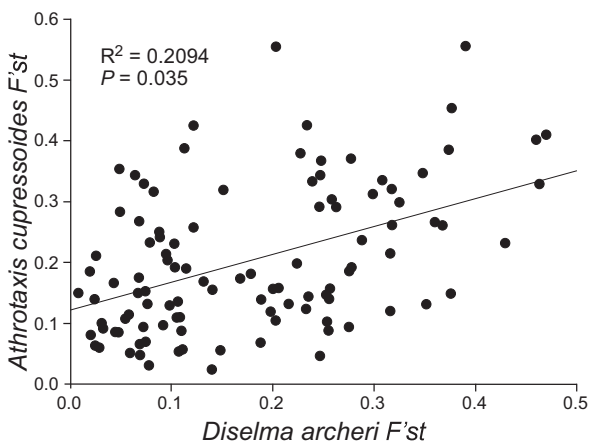


Figure 4 Pairwise genetic distances (standardized F_{st} ; Hedrick, 2005) between the 15 populations where both *Athrotaxis cupressoides* and *Diselma archeri* were collected, excluding Mt Picton. A Mantel test of the correlation between the two was statistically significant ($P = 0.035$) after 9999 permutations.

southwestern Tasmania while both species are absent from suitable habitat in northeast Tasmania.

Based on the average of the four LGM general circulation models (GCM) and agreement between the models large areas of the current range were suitable for both species (Fig. 5) with strongest habitat suitability values in the West Coast Range and southern Tasmanian mountain ranges. The 10th percentile training presence (10%) threshold (0.436 for *D. archeri* and 0.449 for *A. cupressoides*), which tends to slightly underpredict a species' range, predicted suitable habitat in these two regions. On the other hand, the equate entropy of thresholded and original distributions (ENT) (0.117 for *D. archeri* and 0.1009 for *A. cupressoides*), which tends to over predict a species' range, predicted the both species to be widespread.

The fossil record shows that Cupressaceae species are likely to have occurred in both southern and northern Tasmania before the LGM within the current ranges of *A. cupressoides* and *D. archeri* (Fig. 6). Although most LGM fossil records are confined to the West Coast Range area, the immediate presence of Cupressaceae pollen and macrofossils at the start of cores well

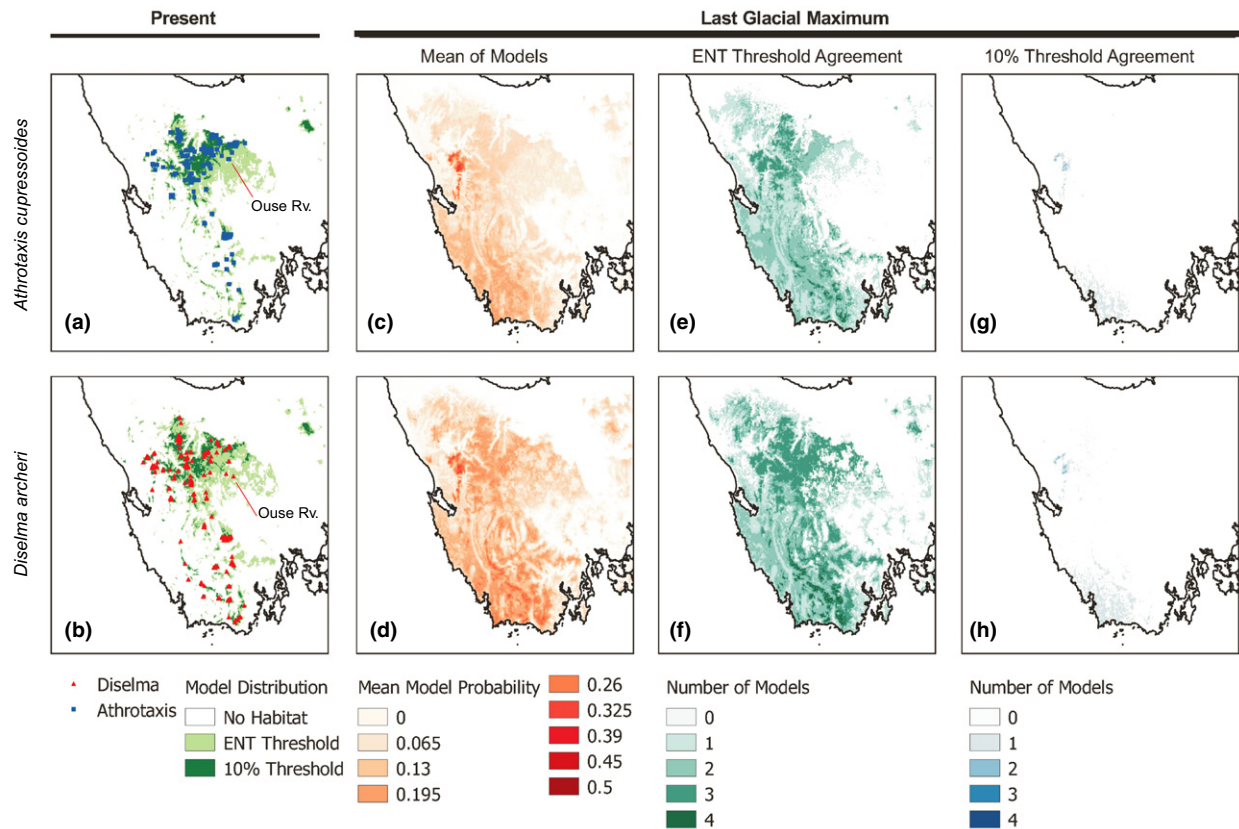


Figure 5 Results of the species distribution modelling for *Athrotaxis cupressoides* and *Diselma archeri* in the present and Last Glacial Maximum (LGM) climates. The mean of four General Circulation Models (GCMs) (FGOALS, CCSM, MIROC and CNRM-CM) and the agreement between the LGM models based on these four GCMs is shown using two thresholds of species presence. The 10th percentile training presence (10%) threshold was 0.436 for *D. archeri* and 0.449 for *A. cupressoides* while the equate entropy of thresholded and original distributions (ENT) for *D. archeri* was 0.117 and 0.1009 for *A. cupressoides*.

outside this region in the post-glacial period support the hypothesis that both *A. cupressoides* and *D. archeri* dispersed from local glacial populations spread across the species current ranges. Although there are fewer LGM and post-glacial charcoal records than the Holocene it is evident that charcoal values increased after the post-glacial period in Tasmania (Fig. 6).

Correlation tests

For *A. cupressoides* none of the correlations between LGM habitat suitability and genetic diversity measures were significant (Table S7). For *D. archeri*, habitat suitability based on the CCSM GCM was significant for F_{IS} , H_o and PAr but not for Ar or rare alleles. For the fire legacy index, *A. cupressoides* showed significant correlations for H_o , F_{IS} , Ar , PAr and rare alleles while for *D. archeri* significant correlations were only observed for PAr and rare alleles (Table S7 & Fig. 7).

DISCUSSION

The similar genetic patterns of both *Athrotaxis cupressoides* and *Diselma archeri* observed in this study suggest that a common factor has shaped the genetic architecture of these

species. The lack of significant association between genetic divergence and geographical distance for either species makes it unlikely that genetic isolation contributes substantially to the patterns of genetic diversity. Reduced genetic diversity arising from population isolation at the edge of species' ranges (Eckert *et al.*, 2008) may have played a minor role in shaping patterns of genetic diversity of the two species. For instance, the northernmost populations of *A. cupressoides* and *Diselma archeri* (populations 2 and 25) and the populations at the dry end of the species' range (population 11 for both species) all have relatively low diversity.

A striking finding was that for both species there is similar pairwise population-level genetic divergence of the same sampled locations. The major driver of this population genetic divergence is distorted allele frequency associated with low genetic diversity most likely arising from bottlenecks over the last tens of generations. This result suggests that both species have undergone parallel population declines in response to a common factor. We argue that Holocene fire is likely the cause of this covariance in genetic diversity and structure of these fire-sensitive species with smaller contributions from other factors such as the legacies of glacial climate and genetic isolation of populations.

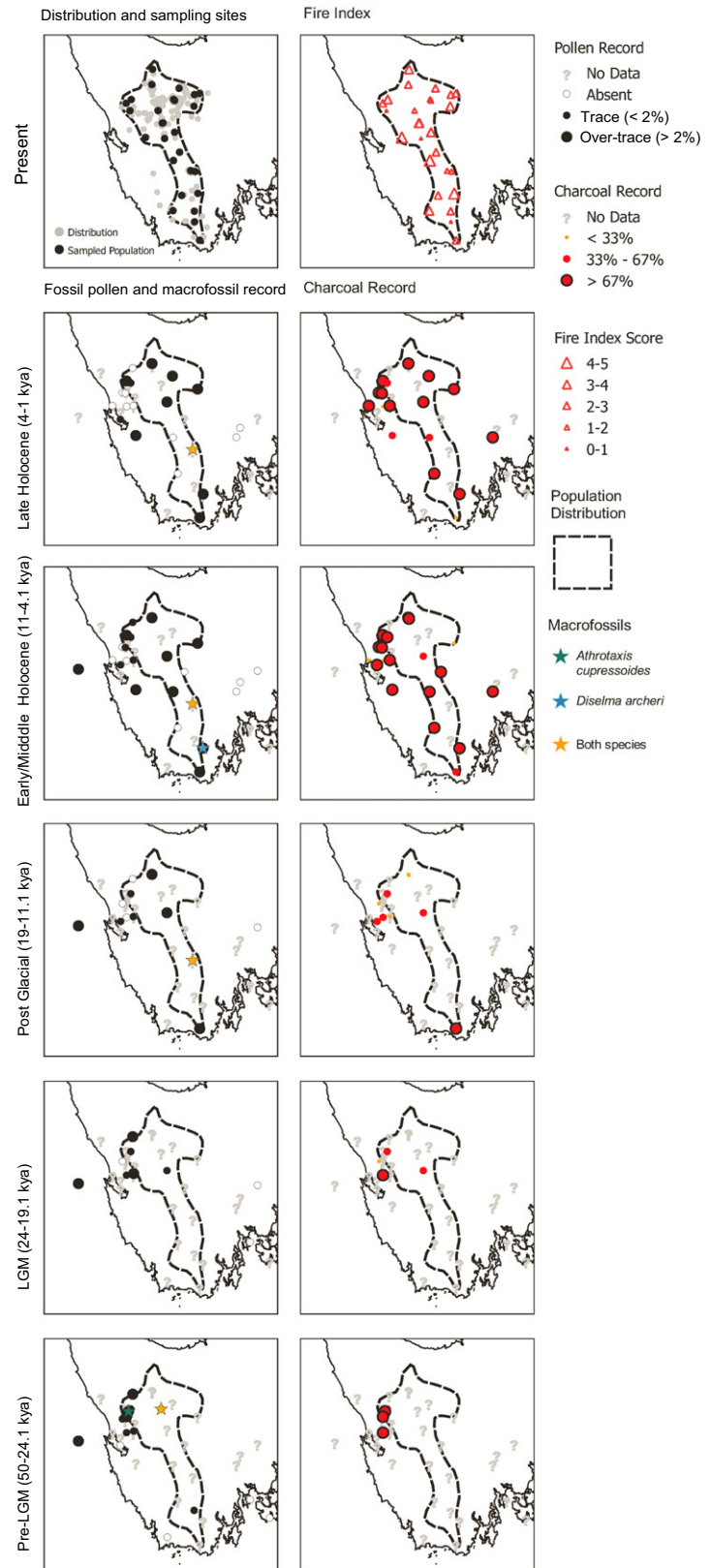


Figure 6 The Cupressaceae fossil and charcoal records from the pre-Last Glacial Maximum to the Late Holocene periods. Pollen is not differentiated into either *Athrotaxis cupressoides* or *Diselma archeri* due to the fact that the pollen of these two species and *A. selaginoides* are so similar that they have not been separated in previous studies. Pollen percentages of over 2% (i.e. ‘over-trace’) is considered to represent local occurrence. For the pollen record ‘No data’ indicates that the core did not extend to the time period while ‘Absent’ means that Cupressaceae pollen were not recorded. For the charcoal record ‘No data’ means that the core did not extend to that time period or charcoal data were not presented. Charcoal percentages represent the percentage of the maximum charcoal quantity found across all time periods in that core, with the period having maximum charcoal concentration being assigned a value of 100%. The modern distribution of both *Athrotaxis cupressoides* and *Diselma archeri* are shown in grey in the top left panel while the fire index for each site is shown in the top right panel. The fire index represents the impacts of fires or a single fire event on the local vegetation over the last 200 years and possibly longer with an index of 5 indicating the most evidence for past fire and 0 the least.

We found a significant correlation between observed fire legacies in each stand and genetic diversity of *A. cupressoides* and *D. archeri*. For instance, the most fire-impacted

population sampled was at Crooked Lake where a fire in the last 100 years had penetrated the stand killing *c.* 50% of all individuals and restricting the species to lake/stream edges and

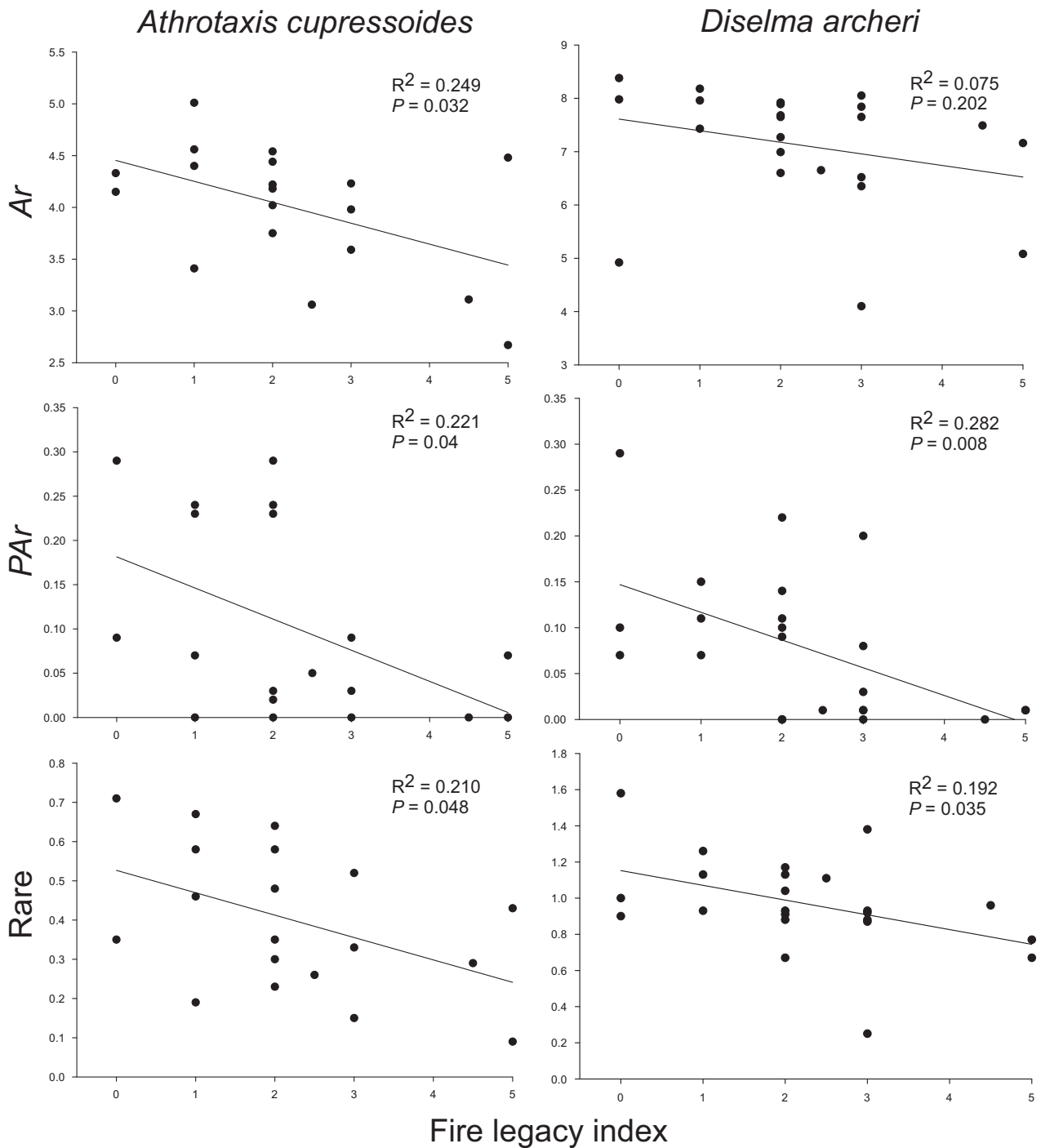


Figure 7 The relationship between the fire legacy index and three genetic diversity indices, allelic richness (*Ar*), private allelic richness (*PAr*) and rare alleles per individual (*Rare*).

topographic-based fire refugia. This population occurs in an area that fossil pollen and charcoal data indicate was also heavily impacted by successive fires from the middle to late Holocene (Macphail, 1981). Fire is also likely to have contributed to the low genetic diversity of other *A. cupressoides* populations such as Lake Skinner (population 8), and *D. archeri* at Paddys Lake (25) both of which are likely to have been impacted by past fires given that surviving individuals are confined to only

the most fire protected sites in the local area. One population, Frenchmans Cap (5), has been heavily impacted by fire, but retains relatively high genetic diversity, although this is mostly due to the high genetic diversity of the largest subpopulation which is likely to have escaped successive past fires.

Environmental factors other than fire are unlikely to explain the genetic diversity patterns in both species. While insect and pathogens have caused population declines in

other conifers (e.g. Bhiry & Filion, 1996), no such impacts are known for *A. cupressoides* or *D. archeri*. The *A. cupressoides* population at Pine Lake (20) which was infected by a *Phytophthora* species in 1994 (Whinam *et al.*, 2001) did not suffer from major dieback and genetic diversity was relatively high in this population.

Pollen and macrofossil data suggest that glacial refugia and recolonization history are unlikely to explain the observed patterns of genetic diversity and structure in the two species. The fossil pollen record from a sea-core off the coast of western Tasmania spanning 75,000 to 5000 years ago shows that the Cupressaceae pollen-type (represented in the western half of Tasmania only by two *Athrotaxis* species and *D. archeri*) was more abundant in the regional pollen rain during the LGM than present, suggesting that this suite of species was more common during this period than present (Van Der Geer *et al.*, 1994). This is consistent with high physiological cold tolerance of these species (Sakai *et al.*, 1981). Indeed, despite the paucity of pollen and macrofossil records during the LGM (Fig. 6), *Athrotaxis* and *Diselma* were likely to have remained close to their current ranges during this time, although at lower elevation (Colhoun *et al.*, 1993). However, macrofossil evidence from Lake Dobson at Mt Field in central Tasmania shows that both species likely persisted at 1000 m in elevation through the LGM within their current ranges, being immediately present at the beginning of the macrofossil record starting at 15,500 years ago (Astorga, 2016).

Modelling for both species under four GCMs showed that suitable habitat was likely widespread across most of the species ranges during the LGM especially under the least stringent threshold (ENT). In the current environment, *A. cupressoides* and *D. archeri* occur within areas predicted by only the ENT threshold in fire protected mesic microsites (Fig. 5) but the species may have been more widespread within this threshold under the colder climate and reduced fire regime of the LGM. Although, under the most stringent threshold (the 10% threshold), modelled LGM ranges match some patterns of genetic diversity, for example, the high values for rare alleles and private alleles in the West Coast Range (Fig. S1), these modelled refugial locations are not consistent with the fossil evidence for the LGM occurrence of the species elsewhere, the species' widespread fossil presence in the post-glacial or the high number of private and/or rare alleles in the centre of the species' ranges at The Labyrinth, Slatters Peak and in the eastern Central Plateau.

Glacial refugia and post-glacial dispersal is also unlikely to explain the low diversity observed in both species in the central part of their ranges at Crooked Lake and Lake Laurel. Without invoking fire, low diversity of these populations would be puzzling given that fossil pollen evidence shows the long occupation of the Crooked Lake area by one or both species throughout at least the last 11,000 years (Macphail, 1981). Crooked Lake and Lake Laurel are also < 20 km from some of the most genetically diverse populations of both species (populations 12 and 16). For glacial refugia to explain the low genetic diversity observed it would require that the two species

underwent bottlenecks via genetic drift in small glacial populations while diversity was maintained in populations on adjacent mountains with similar past and present climate suitability.

Implications for conservation management

The results of our data highlight the vulnerability of *A. cupressoides* and *D. archeri* to genetic decline in response to the synergistic effect of anthropogenic climate change and fire. The species are close to their environmental limits with few refugia available for them to migrate to given they already occur on the highest plateaus and mountain peaks in Tasmania (Porfirio *et al.*, 2014). Fires in the Holocene due to natural and anthropogenic causes have left major legacies in the current distribution and genetic structure of the populations. More frequent fires under climate change have the capacity to cause abrupt range contractions, which may be potentially counteracted with replanting of burnt sites. However, the utility of this approach is dubious given the slow growth of these species and the potentially rapid rate of climate change. The creation of fire breaks around surviving stands is a possible response yet this interferes with landscape aesthetic and wilderness values. A prudent option may be translocation of as much genetic diversity to artificially created refugia. This study helps identify where such genetic material should be collected.

CONCLUSIONS

This study has utilized an extensive sampling regime extending across nearly the entire ranges of two fire-sensitive, palaeoendemic conifer species. This comprehensive snapshot of genetic diversity has suggested a legacy of fire during the Holocene on the genetic diversity of both species. These findings imply that the future of these species is imperilled because anthropogenic climate change is resulting in increasing frequency of warm and dry meteorological conditions that favour extensive fires ignited during dry lightning storms (Marris, 2016). Both *A. cupressoides* and *D. archeri* exhibit poor post-fire recovery, and fire events are likely lead to further erosion of genetic diversity by restricting them to only the most fire-protected places in the landscape, possibly endangering the long-term viability of these species.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Supplementary materials and methods and fossil and charcoal references.

Appendix S2 Supplementary tables.

Appendix S3 Supplementary figures.

BIOSKETCH

James Raymond Peter Worth is interested in improving predictions of the impacts of environmental change due to human activities and climate change on forest biomes by undertaking multidisciplinary research integrating information from genetics, ecology, palaeobotany and species distribution modelling.

Author contributions: J.R.P.W., S.S. and M.I. conceived the original idea; J.R.P.W. and J.R.M. collected the data; J.R.P.W., S.S., G.J.W., and E.A.C. analysed the data; and J.R.P.W., G.J.J., D.M.J.S.B. and E.A.C. led the writing.

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