Last Glacial and Holocene dynamics override post-colonial disturbance in shaping genetic diversity of a heavily exploited palaeoendemic conifer, *Lagarostrobos franklinii*

James Worth

jrpw2326@affrc.go.jp

Forestry and Forest Products Research Institute

James Marthick

Royal Hobart Hospital

Yoshihisa Suyama

Tohoku University https://orcid.org/0000-0002-3136-5489

Gregory Jordan

University of Tasmania https://orcid.org/0000-0002-6033-2766

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Abstract

The impact of past anthropogenic disturbance on the amount and distribution of genetic diversity is a key factor in determining the resilience of tree species to environmental change. This is particularly the case for narrowly distributed species where this disturbance has impacted most of the species' range. Here we examine the legacy of post-colonial logging and fire on patterns of genetic diversity in the Tasmanian palaeoendemic conifer Lagarostrobos franklinii (Podocarpaceae), a fire sensitive and slow growing rainforest tree valued for its durable timber. Thirty-three populations (12 of which represent primary stands) from across the species range were genotyped using 8 nuclear SSRs (871 samples) and MIG-seq-based single nucleotide polymorphisms (254 samples). Genetic differentiation was relatively high for conifers (Fst of 0.113 and 0.143 for nuclear SSR and MIG-seq, respectively) with the most diverged populations near the species northern and southern range limits and cryptic divergence between populations geographically close but in differing river catchments likely reflecting postglacial dispersal from distinct Last Glacial refugia and low levels of gene flow. Population level genetic diversity was greatest in the core of the range with no significant correlation with the history of post-colonial human disturbance (i.e. primary vs. non primary stands) and, unexpectedly, given the greater impact of logging at lower elevations, a significant decline in allelic richness with elevation. Overall, this study shows that L. franklinii has been resilient to past timber exploitation and uncovers previously undetected genetic patterns that will help guide the conservation of this important conifer into the future.

Introduction

Most of the world's forests have been impacted by agriculture, timber extraction and fire incursion, with only around 30% of the world's forests considered to be primary and even lower percentages remaining in the northern hemisphere temperate zone (Parviainen, 2005; Pelz *et al.*, 2023). Human disturbance has mostly had negative impacts on tree species genetic diversity (Ledig, 1992), with potential deleterious implications for these species' capacity to tolerate and/ or adapt to environmental change (Schaberg *et al.*, 2008). In recognition of the need to protect forests and their intrinsic biodiversity and ecosystem services, the area of conserved forests has risen across the globe since the 1990s (Morales-Hidalgo *et al.*, 2015). However, while direct disturbance by humans including timber extraction has largely ceased in these protected areas, the legacy of past human impacts on the amount and distribution of genetic diversity in forest tree species is poorly understood for most species.

The cool temperate island of Tasmania is the most forested state in Australia and retains 73.1% of its original native vegetation cover (Tasmanian Planning Commission, 2024). The western half of the island includes one of the few intact forest landscapes designated in southeastern Australia (Potapov *et al.*, 2008), with extensive areas of intact native vegetation comprised of a mosaic of moorland, eucalyptus forests and cool temperate rainforests. However, during the early 19th to mid-20th centuries, cool temperate rainforests were selectively logged by post-colonial Europeans for the decay resistant, high-quality timber of Tasmanian endemic Podocarpaceae and Cupressaceae conifers. The extensive river systems of the region allowed access to conifer stands across their ranges and also a means of transporting logs downstream. The chief species targeted for logging was *Lagarostrobos franklinii* (Hook. f.) Quinn (Podocarpaceae), or Huon pine, a slow growing, evergreen conifer distributed in western and southern Tasmania (Figure 1). It primarily occurs as a dominant tree of cool temperate rainforests along lowland rivers and the shores of sheltered sea inlets where it reaches heights of 10 – 38 m (Eckenwalder, 2009) but also occurs at higher elevations near lakes and streams in the upper watershed of catchments. A few stands not associated with water bodies, including some near the subalpine zone (Gibson *et al.*, 1991), are found in long unburnt areas (fire refugia) (Gibson, 1988). Of the estimated 10,500 ha of pre-colonial *L. franklinii* forest, 15% has been lost to dam construction and fire (Farjon and Page, 1999) while only approximately 10% remains primary (Kerr and McDermott, 1999). Selective logging occurred across the species' range including in the core of its range, the rivers draining into Macquarie Harbour (Millington *et al.*, 1979), and in other important occurrences along the Huon and Davey Rivers in the south and the Pieman catchment at the northern

edge of its range. Logging of *L. franklinii* declined as accessible trees were depleted, and concerns about the sustainability of the practice led to a ban in cutting green wood in the 1970s (Holbeck, 2022). Apart from the direct loss of trees, past logging may have resulted in an increase in burning of the landscape (Adeleye *et al.*, 2023) due to the use of fire to open up the dense vegetation to ease land access to *L. franklinii* stands. With no capacity to recover from fire *L. franklinii* is likely to have been impacted with the complete loss of stands documented in some areas (Peterson, 1990; Gibson *et al.*, 1991; Buckley *et al.*, 1997) although its mostly riverine habitat has resulted in fewer losses than for non-riverine fire-sensitive Tasmanian conifers such as *Athrotaxis* spp. (Holz *et al.*, 2015, 2020). Most of the range of *L. franklinii* is now protected in national parks.

Given the species slow growth, fire sensitivity and high proportion of the species range that was selectively logged, impacts on genetic diversity and structure of *L. franklinii* forests may have been severe. However, this species remains an important part of the rainforest canopy and whether a legacy of post-colonial human impacts is evident at the genetic level is unknown. In other forest trees, including conifers, genetic analyses have found that primary forests can harbour a greater diversity of rare alleles, which are crucial in determining species adaptive capacity (Petit *et al.*, 1998), than logged forests (Mosseler *et al.*, 2003; Widiyatno *et al.*, 2016; Roque *et al.*, 2023). This loss of rare alleles is due to fragmentation of stands and reduction in density of individuals reducing effective population size and, in the case of selective logging, the loss of older age classes. In Tasmania, range-wide genetic studies of fire-sensitive palaeoendemic conifers, *Athrotaxis cupressoides* and *Diselma archeri*, found significant reductions in stand-level genetic diversity with increasing past fire severity (Worth *et al.*, 2017).

This study investigates the genetic diversity, and its spatial distribution, within and between 33 stands of *L. franklinii* from across the species' range. Twenty-one of the stands are recorded as having been selectively logged while 12 have remained untouched by past fires and selective logging based on Peterson (1990) and Kerr and McDermott (1999). Two genetic markers with contrasting mutational mechanisms were used: nuclear simple sequence repeats (SSRs) (Marthick *et al.*, 2020) and genome wide single nucleotide polymorphisms (SNPs) obtained using multiplexed ISSR genotyping by sequencing (MIG-seq) (Suyama and Matsuki, 2015). Here we aim to examine the impact of post-colonial human disturbance on *L. franklinii* including past logging on the species by comparing genetic diversity of: (1) primary stands untouched by past logging and fire versus non-primary stands; (2) stands at low elevation areas, i.e. more readily accessible to loggers, versus stands at less accessible higher elevations; and (3) more accessible riverine stands versus non-riverine ones. In addition, we assess whether past human disturbance may have differentially impacted the species genetic diversity across the major catchments containing the species. Lastly, we aim to identify populations whose loss would have the greatest impact on the species overall genetic diversity. Protecting remaining stands has become an increasing priority for *L. franklinii* because the frequency and severity of lightning ignited fires (Kirkpatrick *et al.*, 2018; Fletcher *et al.*, 2024) have increased within the distribution of this highly fire-sensitive species.

Materials and Methods

The species

Lagarostrobos franklinii is a wind-pollinated, mostly dioecious conifer (Shapcott, 1993). Its seed are dispersed poorly by gravity, but may be dispersed over longer distances via water (Shapcott, 1997). The species is notable for being one of the longest lived trees in the world with individual stems reaching ages of more than 2500 years (Buckley, 1997) with clonal reproduction extending the lifespan of individual genets to potentially tens of millennia (Anker *et al.*, 2001). *Lagarostrobos* is an ancient Podocarpaceae genus (Biffin *et al.*, 2011) that was widespread across the southern hemisphere in the Cretaceous and Palaeogene periods (Brown *et al.*, 2021). The closest extant relative is the New Zealand endemic *Manoao colensoi* (Hook. f.) Molloy, a monotypic genus erected from *Lagarostrobos* (Molloy 1995),

with the two genera diverging 64 mya to 84 mya (Biffin *et al.*, 2011; Khan *et al.*, 2023). The earliest definitive occurrence of fossils of the extant species is from the Early Pleistocene in western Tasmania (Hill and MacPhail, 1985).

Sampling

A total of 890 samples of *L. franklinii* were collected from 33 populations (an average of 26.4 samples per population) spanning most of the species' range with the exception of the Spero/ Wanderer River area (Figure 1). The 33 populations represented the elevational range of the species, with the exception of the highest elevation stand at Mt Read which has been shown to consist of 4 clonal genotypes (Marthick *et al.*, submitted) and is not considered in this study. Adult trees were collected at least 20 m apart to avoid sampling closely related individuals.

Laboratory work and scoring

DNA was extracted using the DNeasy 96 PlantKit (Qiagen) and eight dinucleotide repeat microsatellite loci used to genotype all 890 samples. These included six loci from (Marthick *et al.*, 2020) (Huon_23318, Huon_50967, Huon_23863, Huon_18442, Huon_3945 and Huon_13112) and two developed for this study (Huon_7288: Forward primer 5 $\top GC \top TGA \top CTA \top TGACGC- 3$ and R primer 5 $\forall GGC \top \ \top CTGG \forall CACGA- 3$ and Huon_50224: Forward primer 5$CATGTGCA\mathbb{C}TAG\mathbb{C}AGAGA- 3$ and R primer 5 $\mathbb{C}T\mathbb{C}CT\mathbb{C} \forall CATAGCAGA- 3$). The PCR thermocycle followed Marthick *et al.* (2020). The length of the amplified products was analysed using an ABI 3130xl Genetic Analyzer and fragments scored using the Microsatellite Plugin in Geneious 9.1.5 (https://www.geneious.com).

A subset of samples (254 samples from the 33 populations– an average of 7.7 samples per population) representing the within-stand spatial extent of samples used for the nSSR analyses and excluding samples identified as clonal in the nSSR dataset were selected for genotyping using MIG-seq (Suyama and Matsuki, 2015). The MIG-seq library was prepared using the protocol of Suyama *et al.*, (2022) with a two-step PCR amplification process, after which all the amplicons larger than 250 bp were purified and sequenced on an Illumina MiSeq platform using a MiSeq Reagent Kit v3 (150 cycles, Illumina, San Diego, CA, USA). Trimmomatic 0.39 was used to eliminate low-quality and extremely short reads that contained adapter sequences (Bolger *et al.*, 2014) after the first 17 bases of reads 1 and 2 (SSR primer regions and anchors) were skipped using "DarkCycle". *De novo* assembly was performed from the 53,467 – 304, 541 MIG-seq reads using Stacks 2.4 pipeline software (Catchen *et al.*, 2013; Rochette *et al.*, 2019) with default parameters. Next, loci filtering in the Stacks POPULATIONS program was undertaken as follows: the minimum percentage of shared loci to detect SNPs was set to 90%, the minimum number of minor alleles was set to 2, and the maximum allowable observed value of heterozygosity was set to 60%. The resulting 983 SNPs data set was then filtered to exclude loci with linkage disequilibrium coefficients below 0.6 in PLINK ver. 1.90 (Purcell *et al.*, 2007), producing a final dataset consisting of 845 SNPs.

Data integrity

For all eight nuclear SSR loci, allelic correlations among loci (indicating linkage disequilibrium) were tested at the population level in Genepop v 4.2 (Raymond and Rousset, 1995) with sequential Bonferroni correction ($p < 0.05$) (Holm, 1979). In addition, FreeNA (Chapuis and Estoup, 2007) was used to test the potential for null alleles impacting estimates of genetic diversity and differentiation. This program estimates the null allele frequency at each locus and also calculates global *Fst* with and without null allele correction (Chapuis and Estoup, 2007). To detect if any nuclear SSR and MIG-seq SNP loci were potentially subject to selection the *Fst* outlier method, BayeScan v2.1 (Foll and Gaggiotti, 2008), was used with each run repeated three times. Existing genetic structuring can increase the number of false positives (Excoffier *et al.*, 2009), therefore, the analyses were undertaken using genetic clusters detected in Bayesian Analysis of Population Structure (BAPS) version 6 (Corander *et al.*, 2003) implementing 'clustering of groups of individuals' with a

maximum *K* of 20. BAPS identified 13 clusters with probability of 0.79 for the nSSR dataset and 3 clusters for the MIG-seq SNP data set with a probability of 1 (Figure S3). The false discovery rate of BayeScan can be impacted by demographic history (e.g. island model, isolation by distance or expansion from refugia), therefore, we tested more realistic prior odds of 100 and 1,000 following (Lotterhos and Whitlock, 2014) and assessed each locus under Jeffreys' scale of evidence (Jeffreys, 1998).

Genetic diversity and structure analyses

For the nuclear SSR dataset the extent of clonality was investigated in GenAlEx 6.5 (Peakall and Smouse, 2006) using the 'find clones' function. If clonality was detected, subsequent analyses included only one individual from each clone. Genetic diversity indices (*Na*, *Ne*, *Ho*, *He*, *uHe* and percent of polymorphic loci) were analysed in GenAlEx 6.5 at the species, population and locus level for both nuclear SSR and MIG-seq SNP datasets. Rarefied allelic richness (*Ar*), which is strongly influenced by the abundance of rare alleles (El Mousadik and Petit, 1996), and private allelic richness (*PAr*) were calculated using a samples size of the smallest population minus one in HP Rare 1.1 (Kalinowski, 2005) for the nSSR dataset and Metapop2 v2.4.2 (López-Cortegano *et al.*, 2019) for the MIG-seq dataset.

Three measures of genetic differentiation, genetic differentiation among populations (*Fst*), Hedrick's standardized (*G'stH*) (Hedrick, 2005) and Jost's D (Jost, 2008) were calculated in GenAlEx 6.5 and the mmod package (Winter, 2012) in R (R Core Team, 2022). Neighbour joining trees were constructed in SplitsTree 4.14.4 (Huson and Bryant, 2006) using a matrix of Nei's D_A (Nei *et al.*, 1983) calculated in Populations 1.2.32 (Langella, 2011) for the nuclear SSR dataset and a matrix of Nei's distance calculated in the R program adegenet (Jombart, 2008) for the SNP dataset. For both datasets, isolation by distance (IBD) was tested for using the mantel.randtest command in adegenet package (Jombart, 2008) in R using 9999 permutations. For this purpose, decimal coordinates were converted to geodesic distances in pegas (Paradis, 2010) and a distance matrix calculated using Nei's distance in adegenet. In addition, correlation between elevation and *Ar* were assessed in Jamovi 2.6.17 (The jamovi project, 2025) using the Pearson correlation coefficient for both datasets.

One-way analysis of variance (ANOVA) undertaken in Jamovi 2.6.17 was used to determine if there were statistically significant differences between the means of genetic diversity indices (*Ne*, *Na*, *Ho*, *He*, *uHe*, *Ar*, *Par* and *Fis*) grouped according to catchment, stand type (riverine/creek, lake and not associated with water body) and human disturbance history (disturbed, primary). Here we treat the Franklin River and rivers/streams that feed it, a large tributary of the Gordon River, as a separate catchment because of the genetic distinctiveness of populations there (see Results).

Population prioritization for conservation

The contribution of each population to the overall species level allelic diversity, including its within- and between-population components, were calculated for both the nuclear microsatellite and MIG-seq datasets using Metapop2 v2.4.2 (López-Cortegano *et al.*, 2019). This program assesses the contribution of each population to overall species-level allelic diversity by assessing the impact of the removal of a given population on the global allelic diversity of the remaining populations (as a percentage of change in diversity).

Results

Data integrity of nuclear SSR loci

Nineteen individuals were excluded to avoid analysing ramets of the same clone as detected by GenAlEx. Clonal individuals were found in 14 populations with between 1-3 individuals removed from those populations (Table 1). In total, 3 of the 924 population by locus-pairs were found to have significant allelic associations (results not shown) indicating there were no consistent linkage disequilibrium between loci across populations. The estimates of null allele

frequency were small, averaging 0.016 across all loci (between 0.007 to 0.028) (Table S1). Null alleles had negligible effect on overall genetic differentiation with global *Fst* including null alleles 0.100 compared to 0.099 when excluding null alleles (Table S1). Posterior probabilities were low for all nuclear SSR loci with an average across the three runs of 0.014 under prior odds of 100 and 0.00072 under prior odds of 1000 (Table S2) suggesting no evidence for either balancing or diversifying selection. Similarly, for MIG-seq loci posterior probabilities were low for all loci with an average of 0.0098 across the three runs under prior odds of 100 and 0.00099 under prior odds of 1000 (Table S3).

Nuclear SSR genetic diversity and structure

For the nuclear SSR dataset, a total of 80 alleles were observed with the number of alleles per locus ranging from 4 to 18 (Table S4). The amount of missing data was 0.13%. The mean number of different alleles (*Na*) and the number of effective alleles averaged over all loci (*Ne*) was 4.08 and 2.12, respectively (Table S4). Observed heterozygosity (*Ho*) and expected heterozygosity (*He*) across loci ranged between 0.38-0.62 (average = 0.49) and 0.36-0.61 (average = 0.48), respectively (Table S4). The inbreeding coefficient (*Fis*) values for each locus were all negative, varying between -0.05 to -0.01, except for Huon_7288 (0.01). *Fst*, *G'stH* and Jost's D values across loci ranged between 0.08-0.14, 0.11-0.29 and 0.06-0.21, respectively (Table S4).

Genetic diversity was distributed unevenly across the species range with highest *Ho*, *He* and *Ar* in the King, Bird, Franklin and Gordon/ Denison catchments (Figure 2 and Table 2). All but two populations with *Ar* above the overall population-level average were from these catchments, with highest *Ar* on the Franklin River at Kutikina Cave, Denison River and Jane River (Figure 2, Table S5). Lowest *Ar* values were observed in the northern and southern extremities of the range in the Pieman and Huon catchments including Yellow Creek, Stanley River and Lake Judd. However, some populations in these catchments had notably high genetic diversity including the Harman River (highest *Ho*) and above average *Ar* for Corrina and Southwood (Figure 2, Table S5).

Private alleles were found in 12 populations across all catchments with a maximum of 2 per population. Eight populations had one private allele while 2 were observed at Corrina, Newell Creek, Lake Marilyn and Denison River (Figure 3).

Geographic structure was moderate with an average *Fst* of 0.113, *GstH* of 0.190 and Jost's D of 0.105. Generally, geographically close populations were more closely related especially in the isolated Pieman and Davey catchments and the Franklin and King/ Bird catchments which formed distinct clusters in the neighbour joining (NJ) tree (Figure 4). On the other hand, populations from the Huon catchment were not all grouped together in the NJ tree with some closer to populations in the Gordon/ Denison catchment in the core of the species range. Genetic differentiation of populations was significantly associated with geographic distance (r= 0.2156, p = 0.0186 - Mantel test, Figure S1). A significant correlation between elevation (m asl) and *Ar* was evident (r= -0.391, P = 0.024) due to a decline in *Ar* with elevation (Figure 5).

Apart from *Na*, *Ar*, *PAr* and *Fis*, all genetic diversity indices were higher on average for disturbed stands compared to primary stands (Table S7) but no differences were statistically significant (Figure 6 and Table S8). Similarly, for stand type no differences were statistically significant (Table S9-S10). In contrast, statistically significant differences (P < 0.05) were observed between catchment for mean *Ar* and *He* (Table S11-S12).

MIG-seq genetic diversity and structure

The overall amount of missing data was 4.03%. The genetic diversity at MIG-seq SNP loci was similar to the nSSR dataset with both *Ar* and *Ho* population-level values significantly correlated using the Pearson correlation coefficient (for *Ar* r = 0.686 and P = 0.00001 and for *Ho* r = 0.374 and P = 0.0316). Highest *Ho*, *He* and *Ar* were observed in the Bird, Franklin, Gordon/ Denison catchments and, unlike the nSSR dataset, the Davey catchment (Figure 2 and Table 3). Above

average *Ar* were observed in all but one population from these catchments. Four populations in the Gordon/ Denison catchment, especially Gordon River - 15 km upstream and Sir John Falls, were notable for their consistently high diversity in terms of *Na*, *Ho*, *He* and *Ar* (Figure 2, Table S6). Identical to the nSSR dataset, lowest *Ar* levels were observed at Yellow Creek and Stanley River (Pieman catchment) and the isolated Lake Judd in the Huon catchment. Tahune and Southwood were found to have above average *Ar* for population outside the core range catchments (Figure 2).

Private alleles were found in 22 of the 33 populations from across all catchments with a maximum of 7 per population (Figure 3). The highest numbers of private alleles were observed in the Pieman and Huon/ Picton catchments with 18 and 14, respectively (Figure 3, Table S5). Populations with the most private alleles were mostly restricted to these catchments with 4 in Wilson River, Stanley River (both Pieman), Lake Judd (Huon/ Picton) and Erebus/ Jane River Confluence (Franklin), 5 at Harman River and Corrina (Pieman) and 7 at Newell Creek (King).

Geographic structure was moderate with an average *Fst* of 0.143, *GstH* of 0.118 and Josts' D of 0.0197. The same geographic based genetic clusters as identified in the nSSR dataset were recovered but with greater resolution in the internal nodes of the neighbour joining tree (Figure 4). The MIG-seq dataset-based NJ tree also found greater clustering of Gordon/ Denison and Huon/ Picton catchment populations and resolved the position of the low diversity Lake Judd population as closest to the Davey, Gordon/ Denison and Huon/ Picton catchment populations rather being most closely related to the Franklin cluster as resolved using nSSRs. A significant association between genetic differentiation of populations with geographic distance, stronger than the nSSR dataset, was also found in the MIG-seq dataset (r= 0.4547, p = 0.0001 Mantel test) (Figure S2). Similar to the nSSR dataset, there was a significant correlation between elevation (masl) and *Ar* (r= -0.491, *P* = 0.005) due to a decline in *Ar* with elevation (Figure 5).

One-way ANOVA tests showed that differences in genetic diversity between catchments (Table S11) were statistically significant (*P* < 0.05) (Table S12) for all genetic diversity indices except *PAr* and *Fis*. No other statistically significant differences were observed for the other groupings (i.e. disturbance history and stand type, Figure 6) except for *Ne* and *Ho* for comparisons between stand types.

Population prioritization for conservation

Similar patterns were observed for both nSSR and MIG-seq datasets for the Gordon/ Denison catchment populations and populations in the lower Franklin catchment (Figure 7) whereby removal of these population resulted in losses of overall within-population allelic diversity. On the other hand, results for the other catchments were mostly inconsistent between nSSR and MIG-seq datasets. All populations in the Pieman catchment were notable for high losses after removal of overall between-population allelic diversity in the MIG-seq dataset (Figure 7).

Discussion

Nuclear SSRs and MIG-seq datasets revealed concordant patterns but MIG-seq provided greater resolution of internal relationships between genetic clusters and more reliable estimates of population level genetic diversity. This is likely due to the increased number of loci (Sunde *et al.*, 2020), the potential for convergence of allele size in SSRs (homoplasy) (Coates *et al.*, 2009; Munoz *et al.*, 2017) and the bi-allelic nature of SNPs, whose genetic differentiation between populations is more impacted by genetic drift than multi-allelic microsatellites (Haasl and Payseur, 2011). Overall, despite sampling nearly all known primary stands of *L. franklinii* this study found no significant differences in genetic diversity between primary and disturbed stands. In addition, contrary to expectations that stands more accessible to past logging at lower elevations would show reduced genetic diversity, non-riverine stands showed no difference to riverine stands in terms of rarefied allelic richness and rarefied allelic richness decreased with increasing elevation of sampled stands.

Rather than post-colonial human impact, this study of the range-wide genetic diversity of *L. franklinii*, indicate that factors pre-dating European colonization have had the most impact on the species' genetic diversity. These factors include (1) the role of limited realised gene flow in shaping genetic patterns in the species as evidenced by the significant isolation by geographic distance across the species range; (2) a core-edge pattern of genetic diversity whereby populations in the core of the species range, particularly the lower Gordon and Franklin Rivers, had the greatest genetic diversity, contrasting with populations in catchments at the edge of the species range which mostly had lower diversity forming unique genetic clusters with high numbers of unique alleles; and (3) the presence of distinct genetic lineages restricted to catchments that were previously undetected using lower resolution isozymes (Shapcott, 1997) and Sanger sequence based chloroplast haplotypes (Clark and Carbone, 2008), that is most likely a legacy of long term geographic isolation probably since the Last Glacial. In comparison to other Tasmanian fire sensitive conifers with range-wide nuclear SSR based studies available (*Athrotaxis cupressoides*, *Diselma archeri* and *Pherosphaera hookeriana*; Worth *et al.*, 2017, 2021), *L. franklinii* has the lowest mean *Ne* and mean *Ho* suggesting that the species may have undergone greater past reductions in effective population size. However, more robust conclusions could be gained by comparing species level genetic diversity using a marker with a greater number of loci, such as MIG-seq.

Maintenance of genetic diversity in disturbed stands

Although we found no evidence for loss of genetic diversity due to post-colonisation human impacts, some losses are likely to have occurred that could not be detected in this study, given the high number of population specific alleles and the fact that some large stands have been completely lost during dam construction and inundation, including one of the most extensive areas of primary forest in the upper Gordon River (Kerr and McDermott, 1999). The maintenance of high genetic diversity in disturbed stands, including the highest diversity stands found in the lower Gordon and Franklin catchments, may be due to the nature of past logging of the species targeting intermediate size classes and restricted to areas within reach of the riverbank, so that most logged areas remained mostly intact (Farjon and Page, 1999). Misshapen trees were also left standing including some of the oldest individuals (Kerr and McDermott, 1999). The selective nature of past logging is well demonstrated by stumps at a site in northwest Tasmania (Sunday Creek) probably from the early 20th century which demonstrates how trees of 600-900 mm basal diameter and particularly the >900 mm class were cut but many trees <600 mm were left standing (Millington *et al.*, 1979).

Origin of the core-edge pattern of genetic diversity

The high diversity of the core range is most likely associated with the formation of the species' modern range. As evidenced by fossil pollen evidence, *L. franklinii* was more common during the Last Glacial than at present (Van De Geer *et al.*, 1994; De Deckker *et al.*, 2019) and may have had a wider ecological niche occurring not only in riverine sites but as a shrub in non-riverine sites into the subalpine zone (Macphail and Colhoun, 1985; Colhoun *et al.*, 1999; Colhoun and Shimeld, 2012). The species expanded to occupy its current distribution along lowland rivers in the mid to late Holocene as indicated by the rapid increase in its pollen during this time (Colhoun *et al.*, 1991; Van De Geer *et al.*, 1991; Colhoun *et al.*, 1993; Fletcher and Thomas, 2007a; Fletcher *et al.*, 2021) expanding into already established closed *Nothofagus cunninghamii* rainforests (Macphail, 1981). This expansion most likely occurred from scattered Last Glacial populations that occurred from upland areas, where the species occurred as a rare shrub in the subalpine zone, down to the now submerged coastline where, even below the Last Glacial depressed tree line, tall closed canopy rainforests probably did not occur (Colhoun, 1985).

Given the widespread occurrence of the species prior to and during the Last Glacial (Colhoun and Goede, 1979; Colhoun, 1980; Colhoun and van de Geer, 1986; Jordan *et al.*, 1991; Colhoun *et al.*, 1999; Cooley *et al.*, 2024), most of the species current range was likely shaped by fragmentation during the Holocene. The mid-late Holocene expansion of open fire-dependent vegetation communities such dry forest and moorland (Jackson, 1999; Fletcher and Thomas, 2010) within the distribution of *L. franklinii*, likely occurred due to an increasingly variable and dry climate (Fletcher *et al.*, 2018) and

an increase in fire frequency across the landscape via the arrival of Aboriginal people in the Last Glacial resulting in a new ignition source (Henríquez *et al.*, 2023). Despite Aboriginal people's skillful application of fire use for promoting growth of fodder for marsupial herbivore game, burning of fire sensitive species such as *L. franklinii* may have occurred during extreme climatic events (Fletcher *et al.*, 2014) or when non-moorland vegetation was burnt for track construction (Colhoun, 1996). The increasingly dry and fire-prone landscape excluded *L. franklinii* from a large proportion of its potential climatic range (Read and Busby, 1990) almost confining the species to the fire protection afforded by waterbodies such as rivers and lakes. At this time, the Gordon and Franklin areas may have become the core of the species range due to a suitable climate and a lack of firing relative to other areas.

Cryptic genetic divergence in the species core range

The existence of a distinct genetic cluster apparent in two independently evolving nuclear markers in the Franklin River catchment was unexpected due to its geographical proximity to the Gordon/ Denison catchment. The origin of this cryptic divergence is uncertain but is most likely a result of postglacial migration from distinct refugial populations within these catchments to occupy its current range. Alternative explanations could involve historical logging or postglacial range contraction due to fire reducing genetic diversity leading to the differentiation of populations in these river systems. However, this is unlikely because these impacts were spread over the whole species' range and the lower levels of diversity in the Huon/ Picton versus the Franklin catchment has not led to a unique genetic cluster in the former catchment. The high capacity for dispersal downstream by water due to the high flotation capacity of seed but extremely limited lateral seed dispersal by wind away from water (Shapcott, 1991) has probably prevented the two lineages from mixing. In addition, similar to the Tasmanian endemic conifer *Pherosphaera hookeriana* (Podocarpaceae) which has a similarly high level of genetic structuring across its narrow range (Worth *et al.* 2021), *L. franklinii* is under-represented in pollen rain (Fletcher and Thomas, 2007b) indicating potential for restricted pollen-mediated gene flow further limiting mixing of gene pools.

Conservation implications

Overall, this study has shown that the extensive and mostly unregulated selective logging across the range of *L. franklinii* from the early 1800s to late-1900s did not result in any detectable decline in genetic diversity. Therefore, in terms of genetic diversity alone both logged and primary forests have similar conservation value. In the present day, the most pressing threat to the species is the increase in frequency and severity of dry lightning induced wildfires over the last two decades within the distribution of *L. franklinii* (Styger *et al.*, 2018; Bowman *et al.*, 2022). Other palaeoendemic species have undergone significant losses including the destruction of approximately 1% of the range of the fire sensitive conifer *Athrotaxis cupressoides* in 2016 wildfires (Bowman *et al.*, 2021). Fires in February 2025 burnt the edges *L. franklinii* forests including the Harman River primary stand sampled in this study but did not penetrate far across the rainforest edge only scorching a few trees outside the main stand (Department of Natural Resources and Environment Tasmania, Tasmanian Government, 2025). Conserving the distinct genetic clusters identified in this study should be a priority for management that could involve physical protection during fire events and collection of seed for *in situ*/ *ex situ* plantings. The Pieman catchment in particular is of special conservation significance consisting of mostly primary stands and forming a unique genetic cluster with consistently high level of unique alleles.

Declarations

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Author contributions

JRPW, JM and YS contributed to the study concept and design. Sample collection was performed by JRPW, JM and GJJ. Analyses were performed by JRPW with advice from JM, GJJ and YS. The first draft of the manuscript was written by JRPW with contributions from GJJ and JM. All authors read and approved the final manuscript.

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Data availability

The nuclear SSR and MIG-seq SNP data files used for analyses during the current study are available as supplementary files.

Competing interest

The authors declare no competing interests.

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Tables

Table 1 Details of the 33 populations of *Lagarostrobos franklinii* sampled including geographical location, stand type (either occurring along rivers/creeks, at the edge of lakes or non-riverine), the water catchment named after the largest river(s) (for the non-riverine stand this corresponded with catchment of the closest creeks), disturbance history (i.e. disturbed by fire/logging or primary) and the number of samples used for nuclear SSR and MIG-seq genotyping.

No.	Population	Latitude	Longitude	Elevation (masl)	Stand Type	Catchment	Disturbance history	n for nSSR*	n for MIG-seq
1	Yellow Ck.	-41.589	145.357	524	River/ Creek	Pieman	primary	29 (1)	8
2	Harman Rv.	-41.635	145.338	467	River/ Creek	Pieman	primary	29 (0)	8
3	Wilsons Rv.	-41.647	145.382	203	River/ Creek	Pieman	primary	27 (0)	8
4	Stanley Rv.	-41.704	145.291	243	River/ Creek	Pieman	disturbed	24 (0)	8
5	Pieman Rv. Corrina	-41.654	145.091	14	River/ Creek	Pieman	disturbed	28 (1)	8
6	Newell Ck.	-42.159	145.537	66	River/ Creek	King	disturbed	31 (2)	8
7	Teepookana Plateau	-42.21	145.433	270	Non-riverine	King	disturbed	25 (0)	5
8	Bird Rv.	-42.349	145.586	80	River/ Creek	Bird	disturbed	24 (0)	7
9	Loddon-Franklin conflu.	-42.228	145.893	327	River/ Creek	Franklin	primary	19 (1)	7
10	Lake Vera	-42.274	145.878	585	Lake	Franklin	primary	32 (1)	8
11	Lake Marilyn	-42.284	145.875	710	Lake	Franklin	primary	31 (0)	8
12	Buckley's Chance	-42.271	145.848	890	Non-riverine	Franklin	primary	28 (2)	8
13	Erebus- Jane conflu.	-42.402	145.982	320	River/ Creek	Franklin	disturbed	29 (1)	7
14	Newlands Cascade	-42.426	145.77	61	River/ Creek	Franklin	disturbed	20 (0)	8
15	Jane Rv.	-42.435	145.805	87	River/ Creek	Franklin	disturbed	30 (0)	7
16	Kutikina Cave	-42.527	145.768	40	River/ Creek	Franklin	disturbed	10 (0)	8
17	Gordon Rv. - 15km upstream	-42.483	145.672	22	River/ Creek	Gordon/ Denison	disturbed	31 (0)	8
18	Sir John Falls	-42.57	145.693	26	River/ Creek	Gordon/ Denison	disturbed	24 (3)	7
19	Denison-Gordon conflu.	-42.717	145.829	44	River/ Creek	Gordon/ Denison	disturbed	18 (0)	8
20	Denison Rv.	-42.611	145.992	147	River/ Creek	Gordon/ Denison	primary	29 (1)	6
21	Serpentine Gorge	-42.761	145.969	256	River/ Creek	Gordon/ Denison	primary	29 (0)	8

22	Gilbert Leitch HPR	-42.733	146.085	389	River/ Creek	Gordon/ Denison	primary	29 (0)	8
23	Davey Rv.	-43.123	145.983	37	River/ Creek	Davey	disturbed	29 (0)	8
24	Badgers Ck.	-43.1	146.034	311	River/ Creek	Davey	primary	13 (0)	8
25	Crossing Rv.	-43.139	146.07	95	River/ Creek	Davey	disturbed	29 (1)	8
26	Condominium Ck.	-42.954	146.369	383	River/ Creek	Huon/ Picton	primary	29 (1)	8
27	Lake Judd	-42.971	146.43	609	Lake	Huon/ Picton	disturbed	28 (0)	8
28	Scotts Peak Dam	-43.056	146.289	230	River/ Creek	Huon/ Picton	disturbed	29 (1)	8
29	Huon Rv. Gorge	-43.104	146.503	157	River/ Creek	Huon/ Picton	disturbed	26 (1)	8
30	Farmhouse Ck.	-43.232	146.668	174	River/ Creek	Huon/ Picton	disturbed	30 (0)	8
31	Riveaux Ck.	-43.187	146.673	148	River/ Creek	Huon/ Picton	disturbed	19 (0)	8
32	Tahune	-43.096	146.727	67	River/ Creek	Huon/ Picton	disturbed	33 (2)	8
33	Southwood	-43.052	146.821	48	River/ Creek	Huon/ Picton	disturbed	30 (0)	8

* Denotes the number of clonal samples excluded from the nSSR analyses

Table 2 Average genetic diversity statistics at the catchment level based on the nuclear SSR dataset.

Catchment*	*Polymorphic loci (%)*	*Ne*	*Ho*	*He*	*uHe*	*Fis*	*Ar*	*PAr*
Pieman (5)	100	2.084	0.489	0.473	0.482	-0.035	2.946	0.056
King (2)	100	2.209	0.503	0.507	0.517	0.019	3.610	0.080
Bird (1)	100	1.950	0.521	0.470	0.480	-0.098	2.940	0.050
Franklin (8)	100	2.274	0.515	0.509	0.521	-0.019	3.460	0.043
Gordon/Denison (6)	100	2.089	0.506	0.489	0.499	-0.029	3.565	0.055
Davey (3)	100	2.134	0.487	0.465	0.477	-0.049	3.100	0.060
Huon/Picton (8)	98.4	2.005	0.460	0.457	0.465	-0.005	2.983	0.029
Overall Average	99.8	2.106	0.497	0.481	0.492	-0.031	3.229	0.053

*The numbers in brackets indicate the number of populations sampled in each catchment

Table 3 Average genetic diversity statistics at the catchment level based on the MIG-seq SNP dataset.

Catchment	*Polymorphic loci (%)*	*Ne*	*Ho*	*He*	*uHe*	*Fis*	*Ar*	*PAr*
Pieman (5)	39.57	1.205	0.126	0.125	0.133	-0.022	1.327	0.0042
King (2)	41.60	1.213	0.136	0.130	0.143	-0.053	1.359	0.0053
Bird (1)	45.33	1.235	0.150	0.142	0.154	-0.058	1.381	0.0024
Franklin (8)	47.32	1.238	0.147	0.145	0.155	-0.028	1.386	0.0011
Gordon/Denison (6)	50.10	1.242	0.155	0.150	0.161	-0.038	1.409	0.0012
Davey (3)	48.92	1.231	0.146	0.143	0.153	-0.022	1.389	0.0016
Huon/Picton (8)	44.62	1.220	0.138	0.135	0.145	-0.031	1.360	0.0021
Overall Average	45.35	1.227	0.143	0.139	0.149	-0.036	1.373	0.003

Figures

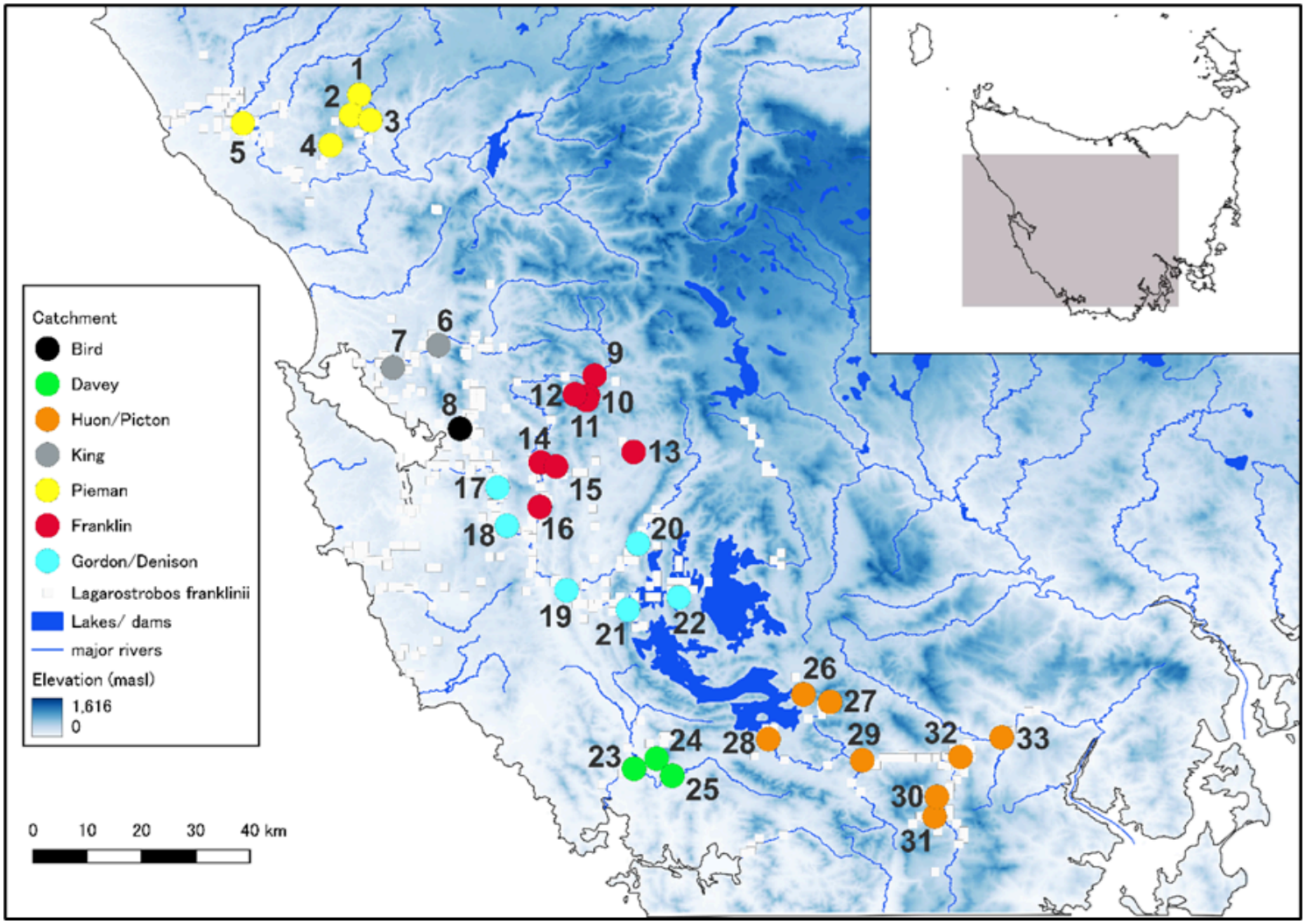

Figure 1

Distribution of the 33 sampled *Lagarostrobos franklinii* populations with the known distribution of the species indicated by small grey squares (sourced from Petersen 1990, the Natural Values Atlas of Tasmania and personal records). The

populations are coloured according to the major river catchment they occur in. Populations are numbered according to Table 1 from north to south. Major rivers and waterbodies are shown along with blue shading indicating elevation.

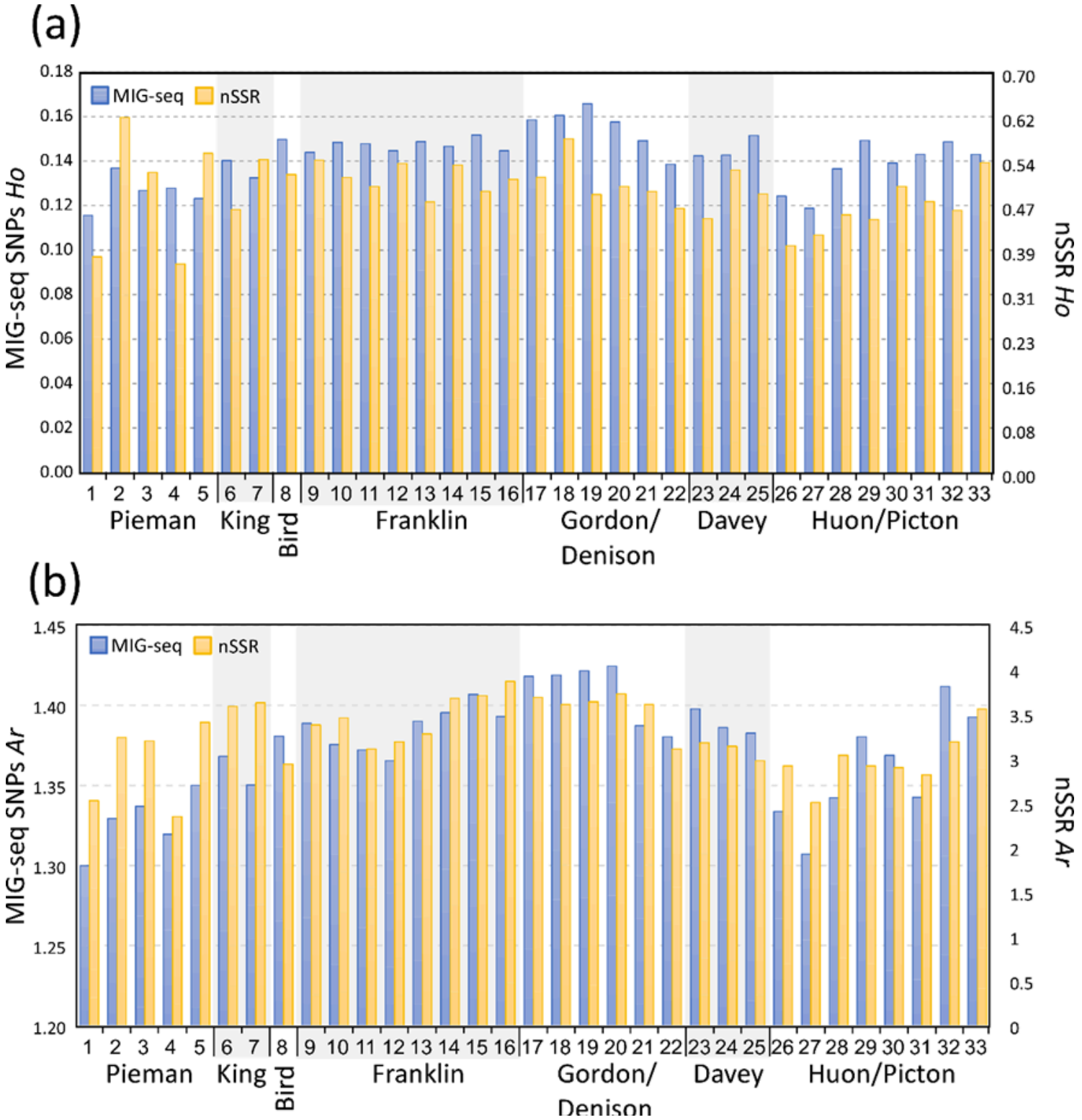

Figure 2

Genetic diversity according to observed heterozygosity (*Ho*) (a) and rarefied allelic richness (*Ar*) (b) for all populations of *Lagarostrobos franklinii* based on the eight nuclear SSR loci and 845 MIG-seq SNPs. The shaded areas delimit the seven catchments. Population numbers follow the order in Table 1.

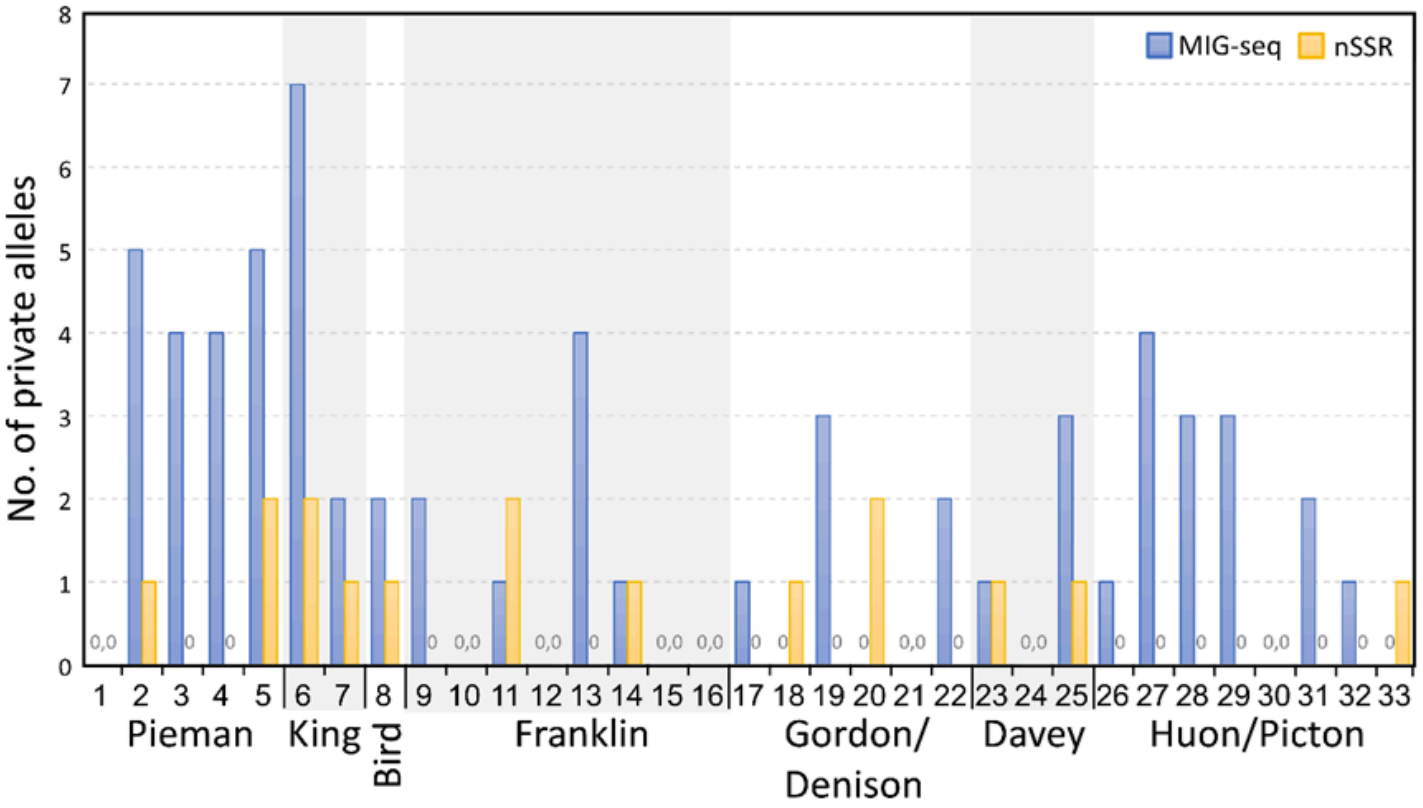

Figure 3

The number of private alleles for all populations of *Lagarostrobos franklinii*identified at the eight nuclear SSR loci and 845 MIG-seq SNP datasets. The shaded areas delimit the seven catchments. Population numbers follow the order in Table 1.

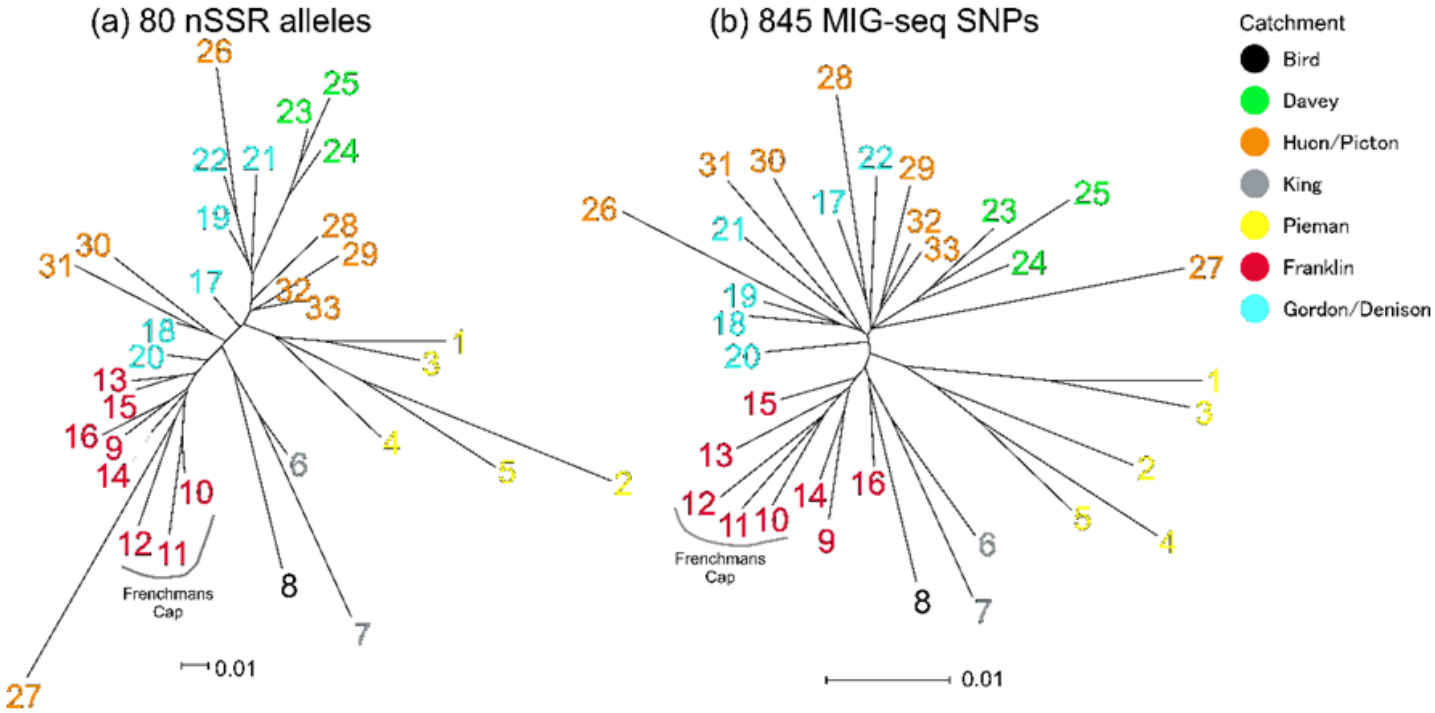

Figure 4

Neighbour joining tree depicting genetic relationships of the 33 sampled *Lagarostrobos franklinii* populations based on Nei's *Da* distance for the nuclear SSR dataset and Nei's distance for the MIG-seq dataset. Populations are coloured according to the catchment within which they occur with the high elevation non-riverine populations from Frenchmans Cap indicated.

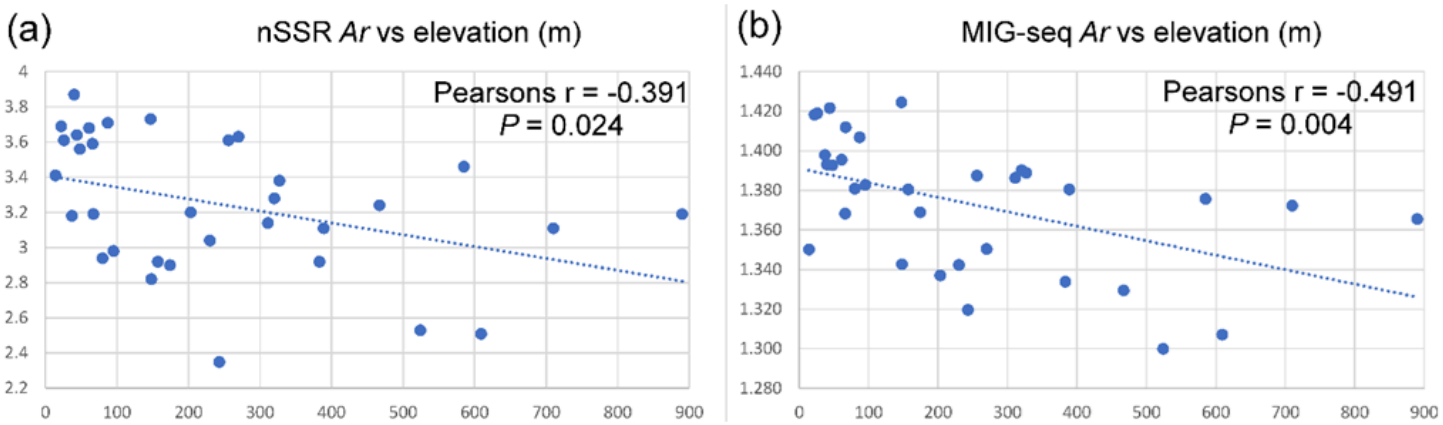

Figure 5

Graphs displaying correlation between population elevation (m asl) and *Ar* for the (a) nSSR and (b) MIG-seq datasets.

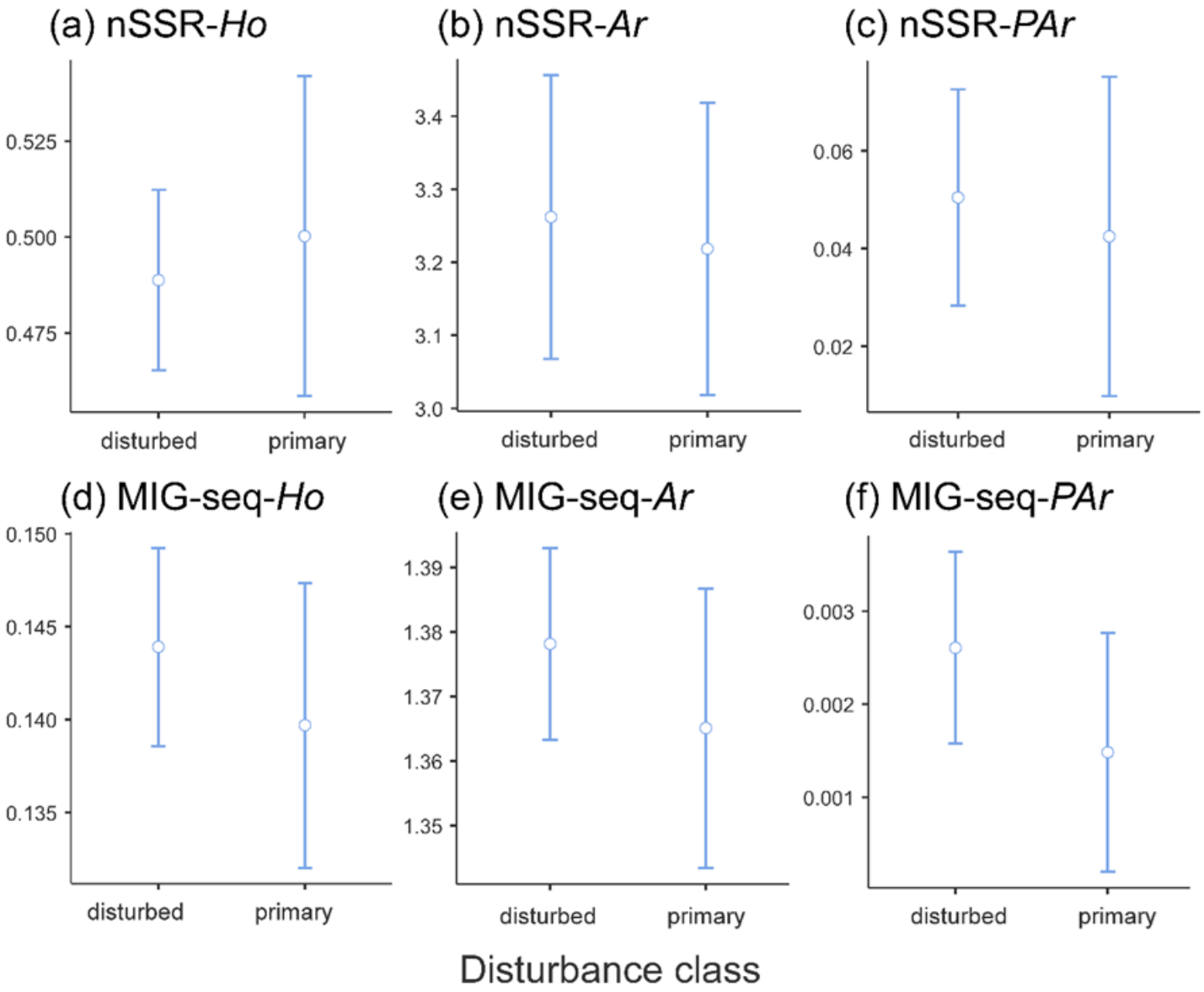

Figure 6

Comparison of genetic diversity means with 95% confidence intervals (blue bars) of observed heterozygosity (*Ho*), rarefied allelic richness (*Ar*) and rarefied private allelic richness (*PAr*) between disturbed and primary stands based on nuclear SSR (a, b and c) and MIG-seq (d, e and f) markers. All differences were insignificant at the < $P = 0.05$ level.

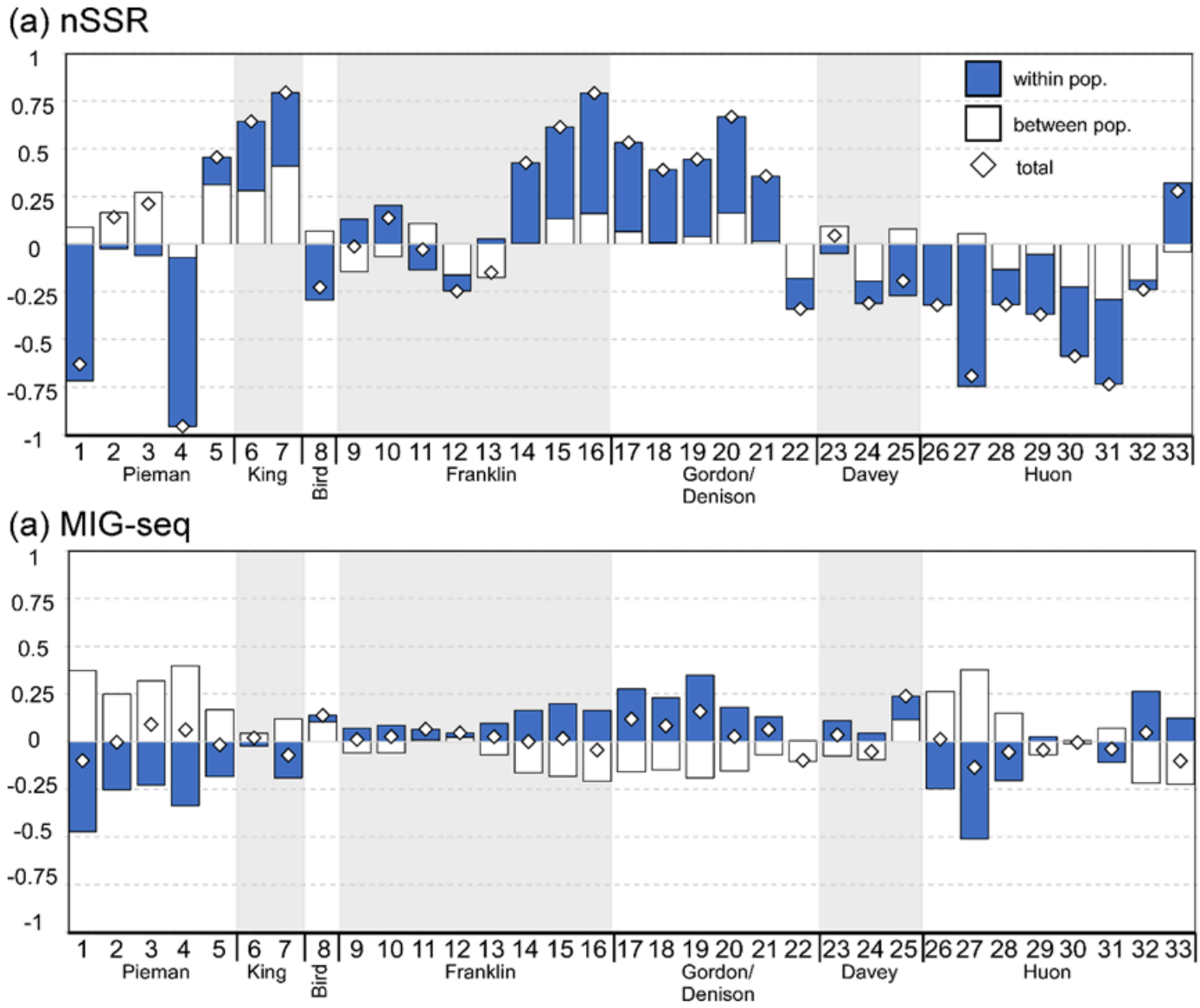

Figure 7

Percentage of loss (+) or gain (-) of allelic diversity after removal of each population represented in terms of total allelic diversity (white diamond), average allelic diversity within populations (blue columns) and average allelic diversity between populations (white columns). The shaded areas delimit the seven catchments. Population numbers follow the order in Table 1.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- nuclearSSRgenepopfile.txt
- SupplementaryMaterials.docx
- MIGseqgenepopfile.txt