Government of Western Australia
Department of Parks and Wildlife

Final report for assessment of genetic processes in *Lepidosperma* sp. Parker Range and *Lepidosperma* sp. Mt Caudan

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Executive Summary

This research was requested from Cazaly Iron Ore Pty Ltd to satisfy Condition 7-2(2) required under Ministerial Statement 892 for the approval of the Parker Range (Mount Caudan) Iron Ore Project. Condition 7-2(2) specifies that:

> *"The proponent shall undertake genetic analysis including:*
>
> *a) spatial analysis of population genetic structure;*
>
> *b) genetic analysis of the mating system; and*
>
> *c) genetic analysis of realized dispersal,*
>
> *to the satisfaction of the CEO to determine the relative genetic diversity of the populations of Lepidosperma sp. Parker Range and populations of Lepidosperma sp. Mt Caudan using the seed and plant material collected in accordance with condition 7-2-1"*

Lepidosperma sp. Parker Range and Lepidosperma sp. Mt Caudan have narrow distributions in the Parker Range area, with few populations and have a conservation status of Priority 1 (poorly known taxa), as determined by the Department of Parks and Wildlife. The development of the proposed mine would require the removal of one population from each species and thus, this research aims to determine the genetic and reproductive processes in these species prior to mine development, while assessing the impact of the removal of these populations. In doing so, recommendations can be made for ensuring the ongoing genetic persistence of *L.* sp. Parker Range and *L.* sp. Mt Caudan during and after the lifetime of the proposed mine.

The project addressed the requirement of the approval condition through research in two major areas, 1) the determination of genetic diversity and spatial genetic structure among populations, and 2) determination of the frequency and spatial extent of asexual reproduction (i.e. clonality) for each of *L.* sp. Parker Range and *L.* sp. Mt Caudan. To assess genetic diversity and structure, all known populations were broadly sampled and analysed using chloroplast and nuclear microsatellite markers. Various diversity parameters and measures of population connectivity were assessed, including an assessment of the contribution of each population to overall species' diversity. The clonality analyses required a narrower sampling design, focussing on two intensively sampled quadrats within two populations for each species and were assessed for clonality using nuclear microsatellite markers.

The main findings of this study include:

- Overall genetic diversity is low to moderate in both *L.* sp. Parker Range and *L.* sp. Mt Caudan.
- The geographically disjunct populations of *L.* sp. Parker Range exhibit high levels of differentiation, with strong geographic structuring particularly between the central populations and the more isolated, peripheral populations.
- *Lepidosperma* sp. Mt Caudan has a more restricted distribution, and populations are well connected by gene flow and show little geographic structuring.
- Peripheral populations of *L.* sp. Parker Range and *L.* sp. Mt Caudan (LPR 7, 8, 9 and LMC 3, 7) contribute the most to overall species diversity, while the impact populations of both species are similar to other populations and do not make a significant contribution to overall species diversity.

- Both species exhibited some level of clonality but this was more prevalent in *L.* sp. Parker Range than in *L.* sp. Mt Caudan.

Overall, the less diverse and more differentiated populations of *L.* sp. Parker Range are of greater conservation concern than the diverse and well-connected populations of *L.* sp. Mt Caudan. The removal of two populations for the mine operation should have little impact on short term genetic diversity and structure of these species. However, to maintain their genetic integrity in the long term, we recommend the safeguarding of plant and seed material, from both species, for rehabilitation purposes following the closure of the mine.

Project Outcome

This research program provides information toward ensuring the long term maintenance of genetic diversity in *Lepidosperma* sp. Parker Range and *Lepidosperma* sp. Mt Caudan.

Background

This research is in response to a request from Cazaly Iron Ore Pty Ltd for a program that addresses a specific condition, from the Environmental Protection Authority, in approving development and operation of an open cut iron ore mine at the Parker Range deposit. This condition requires the long term maintenance of genetic diversity of two conservation significant flora, *Lepidosperma* sp. Parker Range and *Lepidosperma* sp. Mt Caudan, and stipulates that this is to be achieved via an assessment of genetic diversity and genetic processes in each species. This assessment is now complete and this report provides a summary of all the data collected over the last 18 months, with final recommendations for the ongoing genetic persistence of *L.* sp. Parker Range and *L.* sp. Mt Caudan.

Research plan

The research involved genetic analysis of *Lepidosperma* sp. Parker Range and *Lepidosperma* sp. Mt Caudan, including:

i. spatial analysis of population genetic diversity and structure;
ii. determination of the reproductive system; and
iii. determination of dispersal parameters.

Determination of the mating system and pollen dispersal aspects of the reproductive system requires genetic assessment of seed. Seed set in *Lepidosperma* can be highly irregular and no seed was obtained from populations of either species during the course of the project. Therefore, a full study of the sexual mating system and pollen dispersal was not possible. It was suspected that there may be clonal reproduction in both species due to the pattern of growth and the absence of seed set. Hence, an assessment of clonality was carried out to determine this aspect of the reproductive system and satisfy objectives ii and iii.

Research Methods

Sample Collection

Initial population surveys for each of *Lepidosperma* sp. Parker Range (LPR) and *Lepidosperma* sp. Mt Caudan (LMC) were conducted by Jim Williams (Botanica Consulting), finding nine LPR and 12 LMC populations of various sizes (Figure 1). Following further morphological and genetic assessment, three populations thought to be *L.* sp. Parker Range were re-classified as *Lepidosperma* sp. A2 Inland Flat and omitted from further analysis. Additionally, genetic analysis of population LMC 5.5 showed strong inconsistencies with the rest of the species in both chloroplast and nuclear markers, indicating that it may be a different species, or perhaps a hybrid. Given that its genetic signature is not typical of *L.* sp. Mt Caudan and does not appear to contribute to gene flow amongst populations, this population was also omitted from further analysis to avoid overestimating genetic diversity and misinterpreting the dataset.

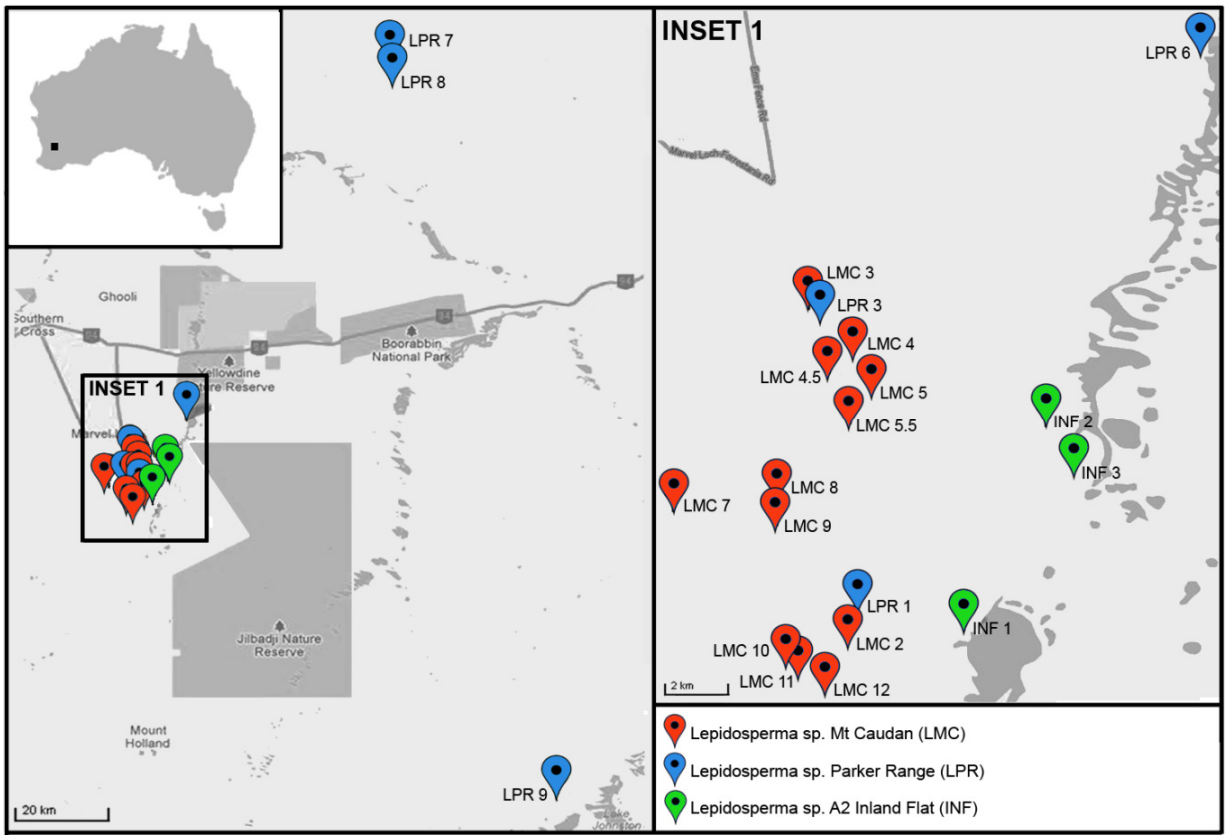


Figure 1: Location of all known populations sampled for each of *Lepidosperma* sp. Mt Caudan (LMC), and *Lepidosperma* sp. Parker Range (LPR). Also shown are three populations of the more widespread *Lepidosperma* sp. A2 Inland Flat (INF).

For analyses of spatial genetic structure and diversity (Objective i), 24 individuals were sampled from each population (408 total). These samples were collected across the full spatial extent of each population to estimate the genetic diversity within each population. For the nuclear microsatellite markers, all 24 samples were analysed for each population, while eight samples per population were randomly chosen for analysis with the chloroplast markers. Assessment of the occurrence and spatial extent of asexual reproduction with a study on clonality for each species required a more concentrated sampling design. From each population 75 samples were collected within two, 15m x 15m quadrats. In total, approximately 600 samples were collected from two populations per species; the impact populations (LPR 1 and LMC 2), as well as two other populations (LPR 3 and LMC 3) (Figure 1). For this study, eight nuclear microsatellite markers were used to genotype samples and assess clonality for each species.

Laboratory Analysis

Leaf material was freeze-dried before DNA was extracted using a modified CTAB method (Doyle & Doyle 1987), adding 1% PVP (polyvinylpyrrolodine) to the extraction buffer. Nuclear microsatellite markers were developed through partial genome sequencing on a 454 platform at the Australian Genome Research Facility using the methods of Gardner et al. (2011). From the resulting microsatellite libraries, 80 primers were trialled and 24 (12 for each species) were optimised and used for all three objectives in this project. Chloroplast microsatellite markers previously developed for other *Lepidosperma* species (Wallace et al. 2010), were tested and six of these were used with both species for Objective (i). Polymerase chain reactions (PCR) were performed in a 15 µl volume containing 50 mM KCl, 20 mM Tris–HCl (pH 8.4), 0.2 mM each dNTP, 2.5 – 3.5 mM $MgCl_2$, 0.032 mM forward primer, 0.16 mM reverse primer, 0.16 mM fluorescent M13 primer, 0.1 µl Taq DNA polymerase and 10 ng of template DNA. PCR cycling conditions entailed 4 min at 95 °C, followed by 15 cycles of 94 °C for 30 sec, 30 sec stepping down from 65 to 50 °C (-1 °C per cycle), and 72 °C for 45 seconds, followed by 30 cycles of 94 °C for 15 seconds, 50 °C for 15 seconds, and 72 °C for 45 seconds, with a final extension of 72 °C for 8 minutes. PCR products were visualised on a Biosystems 3730 Sequencer (Applied Biosystems) and genotypes were scored manually using Genemapper™ v.3.7 (Applied Biosystems) software.

Data Analysis

For the analysis of chloroplast microsatellite data, haplotypes were identified using GenAlEx v.6.5 (Peakall & Smouse 2006) and visualised using pie charts overlaid on a map to show the geographic distribution of haplotypes across populations. Genetic diversity and differentiation parameters were calculated using PERMUT v.1.0 (Pons & Petit 1996) and principle co-ordinate analysis (PCoA) was used to visualise the genetic differentiation within and among species using GenAlEx v.6.5 (Peakall & Smouse 2006).

The nuclear microsatellite data were assessed for genetic diversity and population differentiation using GenAlEx v.6.5 (Peakall & Smouse 2006). Exact tests for Hardy-Weinberg equilibrium and linkage disequilibrium were performed with Genepop v.4.2 (Raymond & Rousset 1995). GenAlEx

v.6.5 (Peakall & Smouse 2006) was also used to visualise population differentiation through PCoA and to test for isolation by distance using Mantel tests, while STRUCTURE v.2.3.4 (Pritchard et al. 2000) was used to identify any pattern of genetic structuring across the sampled populations for each species. Finally, analyses were performed in CONTRIB v.1.02 (Petit et al. 1998) to determine the relative contributions of populations LPR 1 and LMC 2 toward each species' overall genetic diversity to allow a quantitative assessment of the impact of their removal.

Clonality was assessed using GenClone v.2.0 (Arnaud-Haond & Belkhir 2006). This analysis includes the identification of clones, estimation of diversity parameters and spatial analyses of clonal structure.

Chloroplast microsatellite diversity and population structure

A total of 12 haplotypes were found across the 11 populations of *Lepidosperma* sp. Mt Caudan and six haplotypes across the six populations of *Lepidosperma* sp. Parker Range (Figure 2). All populations consisted of multiple haplotypes, with an average of 2.67 ± 0.02 and 3.64 ± 0.4 haplotypes per population for LPR and LMC, respectively. For both species, two to three common haplotypes were distributed widely across each species' range with the remaining haplotypes occurring in more localised areas. Average diversity, both within population diversity and total diversity, was significantly higher in *L.* sp. Mt Caudan than *L.* sp. Parker Range, while population differentiation was significantly higher among LPR populations than those of LMC (Table 1). Three *L.* sp. Parker Range populations (LPR 1, 7, 9) and three *L.* sp. Mt Caudan populations (LMC 4, 9, 11) each exhibited one private allele. There were no shared haplotypes between the two species.

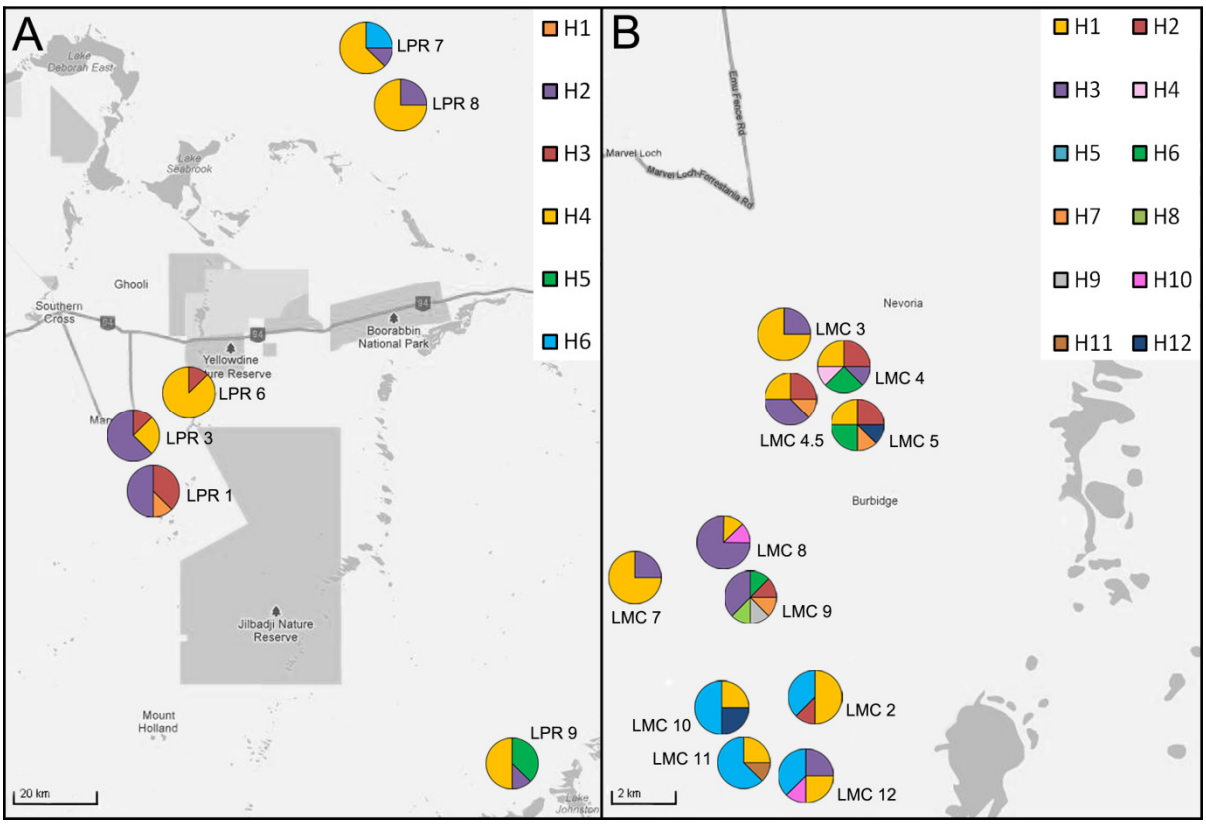


Figure 2: Geographic distribution of chloroplast haplotypes found in populations of A. *Lepidosperma* sp. Parker Range (LPR) and B. *Lepidosperma* sp. Mt Caudan (LMC). The size of the portions in each pie graph corresponds to the relative proportion of individuals with that haplotype in the population.

Within each species, there is limited evidence of geographical structuring. Overall differentiation among populations is low in both species because most populations share one or two common haplotypes across each species' range. And while there are some haplotypes that occur in specific geographic regions, such as Haplotype 3 in the central *L.* sp. Parker Range populations or Haplotype 5 in the southern *L.* sp. Mt Caudan populations (Figure 2), a comparison of the two differentiation parameters (Table 1) showed no significant difference between G_{ST} and R_{ST} for either species, indicating that haplotypes within a given population are not necessarily more closely related than haplotypes amongst different populations.

Finally, the principle co-ordinate analysis (Figure 3) shows the clear genetic differentiation amongst the *Lepidosperma* species collected. This figure includes the *L.* sp. A2 Inland Flat populations, as well as population LMC 5.5 to highlight their distinction from *L.* sp. Mt Caudan and *L.* sp. Parker Range. The populations of each species clustered together in three separate groups while population LMC 5.5 exhibited haplotypes with more affinity to *L.* sp. Parker Range despite having morphology more similar to *L.* sp. Mt Caudan.

Table 1: Genetic diversity (within population diversity, h_S/v_S and total species diversity, h_T/v_T) and differentiation (G_{ST}/R_{ST}) parameters for the chloroplast haplotypes of *Lepidosperma* sp. Parker Range (LPR) and *Lepidosperma* sp. Mt Caudan (LMC).

Species	N	N_a	h_S	h_T	G_{ST}	v_S	v_T	R_{ST}
A. LPR	48	6	0.542 (0.07)	0.697 (0.08)	0.223 (0.05)	0.444 (0.09)	0.602 (0.08)	0.262 (0.10)
B. LMC	88	12	0.695 (0.06)	0.820 (0.04)	0.153 (0.05)	0.689 (0.05)	0.781 (0.03)	0.118 (0.04)

N, total number of individuals; N_a, total number of haplotypes; diversity and differentiation parameters are averaged across populations for each species, with standard error in parentheses.

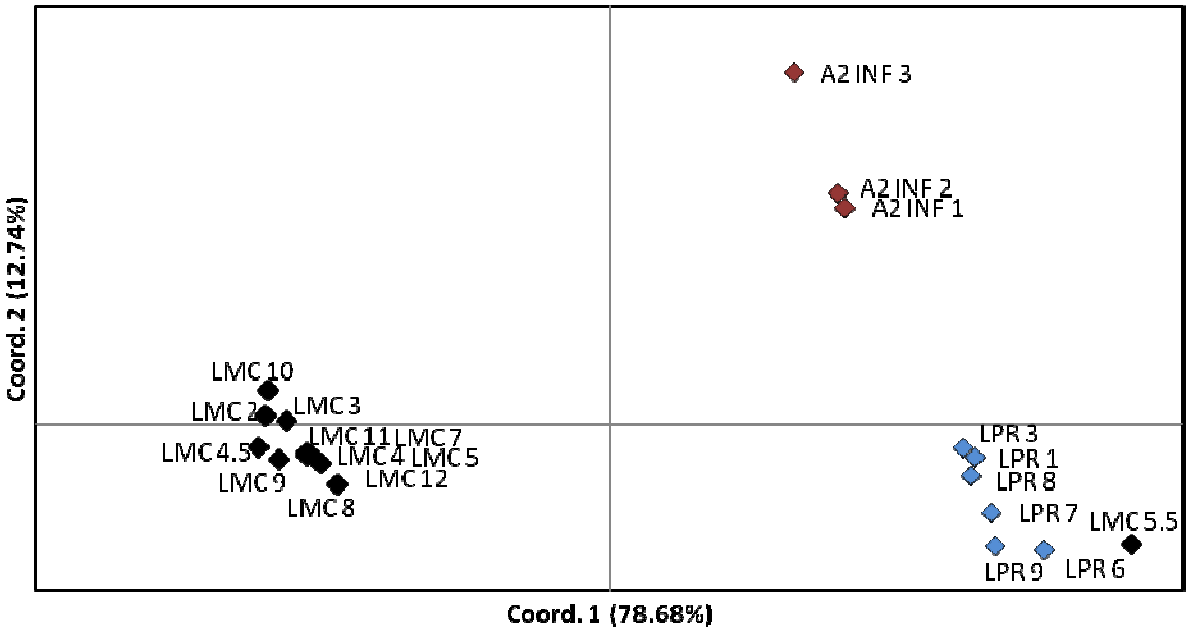


Figure 3: Principle co-ordinates analysis of genetic differentiation based on chloroplast microsatellites for all populations of three *Lepidosperma* species: *Lepidosperma* sp. Parker Range (LPR; blue), *Lepidosperma* sp. Mt Caudan (LMC; black) and *Lepidosperma* sp. A2 Inland Flat (A2 INF; red). The two co-ordinate axes account for 91.42% of the total genetic variation.

Nuclear microsatellite diversity and population structure

Both species of *Lepidosperma* exhibited moderate levels of nuclear genetic diversity (Table 2). Across 11 populations, allelic richness and expected heterozygosity averaged 3.97 + 0.03 and 0.498 + 0.01 respectively for *L.* sp. Mt Caudan, and similarly, 3.47 + 0.24 and 0.436 + 0.02 respectively, across the six *L.* sp. Parker Range populations.

While measures of diversity were similar between the two species, there are other important differences to note. Firstly, diversity in *L.* sp. Parker Range was less consistent across populations in comparison to *L.* sp. Mt Caudan. This is largely attributed to population LPR 1 which stands out with considerably low diversity relative to the other *L.* sp. Parker Range populations. Secondly, observed heterozygosity was significantly lower for *L.* sp. Parker Range than *L.* sp. Mt Caudan (Table 2). Thus, while both species experienced significant deviations from Hardy-Weinberg equilibrium and high inbreeding coefficients (F_{IS}) due to heterozygote deficiency, such deficits were much more pronounced in *L.* sp. Parker Range. This is most likely indicative of increased inbreeding or clonal reproduction reducing genetic variability within *L.* sp. Parker Range populations. Finally, both species exhibited private alleles and these alleles were more common in peripheral populations. Population LPR 9 in particular, which is the most geographically isolated of all populations, had the highest number of private alleles (eight), which contributed to the significantly greater number of private alleles for *L.* sp. Parker Range relative to *L.* sp. Mt Caudan.

Table 2: Genetic diversity statistics for populations of **A.** *Lepidosperma* sp. Parker Range (LPR) and **B.** *Lepidosperma* sp. Mt Caudan (LMC).

Species/ Population	Allelic Richness	Private Alleles	Observed Heterozygosity	Expected Heterozygosity	Inbreeding Coefficient
A. *L.* sp. Parker Range					
LPR 1	2.463 (0.34)	1	0.174 (0.06)	0.355 (0.07)	0.516 (0.14)*
LPR 3	3.356 (0.64)	1	0.304 (0.08)	0.423 (0.08)	0.413 (0.13)*
LPR 6	3.603 (0.75)	4	0.274 (0.06)	0.483 (0.07)	0.449 (0.09)*
LPR 7	3.482 (0.56)	2	0.347 (0.08)	0.401 (0.07)	0.180 (0.10)*
LPR 8	3.732 (0.57)	1	0.358 (0.09)	0.493 (0.07)	0.386 (0.13)*
LPR 9	4.218 (0.80)	8	0.304 (0.07)	0.462 (0.09)	0.337 (0.07)*
Mean (SE)	**3.475 (0.24)**	**2.83 (1.13)**	**0.293 (0.03)**	**0.436 (0.02)**	**0.380 (0.05)***
B. *L.* sp. Mt Caudan					
LMC 2	4.48 (0.57)	1	0.358 (0.06)	0.491 (0.05)	0.234 (0.11)*
LMC 3	3.95 (0.62)	2	0.442 (0.09)	0.540 (0.06)	0.200 (0.13)*
LMC 4	3.89 (0.62)	1	0.417 (0.07)	0.486 (0.05)	0.110 (0.13)*
LMC 4.5	3.55 (0.58)	0	0.438 (0.08)	0.476 (0.05)	0.088 (0.13)*
LMC 5	3.88 (0.76)	0	0.434 (0.09)	0.434 (0.06)	-0.004 (0.12)
LMC 7	4.14 (0.59)	3	0.534 (0.08)	0.547 (0.06)	-0.013 (0.10)
LMC 8	3.53 (0.52)	1	0.464 (0.07)	0.460 (0.05)	0.016 (0.09)
LMC 9	3.68 (0.48)	0	0.364 (0.08)	0.504 (0.05)	0.255 (0.14)*
LMC 10	4.26 (0.47)	2	0.367 (0.07)	0.520 (0.05)	0.281 (0.12)*
LMC 11	4.26 (0.78)	1	0.365 (0.06)	0.520 (0.05)	0.295 (0.10)*
LMC 12	4.05 (0.67)	1	0.379 (0.06)	0.499 (0.05)	0.242 (0.10)*
Mean (SE)	**3.97 (0.09)**	**1.50 (0.27)**	**0.415 (0.02)**	**0.498 (0.01)**	**0.155 (0.04)***

* indicates significant deviation from Hardy-Weinberg Equilibrium.
All parameters (except for the total number of private alleles) are averaged across loci for each population of 24 individuals with standard errors in parentheses.

Consistent with the chloroplast microsatellite data, the nuclear microsatellites showed that *L.* sp. Parker Range exhibits stronger population structure than *L.* sp. Mt Caudan. Pairwise F_{ST} values (Table 3) were an order of magnitude larger than those for *L.* sp. Mt Caudan, with a global F_{ST} value of 0.277, demonstrating considerable genetic differentiation among all *L.* sp. Parker Range populations. Variation in these pairwise comparisons also showed a clear geographic pattern, as shown by PCoA (Figure 4A) and IBD (Figure 5A), where genetic differentiation increased with increasing geographic distance between populations. Finally, STRUCTURE analysis identified two major genetic clusters (Figure 6A), one corresponding with the three central populations (LPR 1, 3 and 6) and the other cluster with the three more broadly distributed populations (LPR 7, 8 and 9), with slightly more admixture in population LPR 9. In contrast, there were very low levels of differentiation amongst *L.* sp. Mt Caudan populations (Table 4) with a global F_{ST} value of 0.051. Moreover, this differentiation had little geographic structuring. This is visualised in the principle coordinates analysis (PCoA; Figure

4B), which shows that geographically close populations did not necessarily cluster together and confirmed by mantel testing for isolation by distance (IBD; Figure 5B), in which there was no significant relationship between genetic and geographic distance among populations. Furthermore, STRUCTURE analysis did not detect any genetic structuring among *L.* sp. Mt Caudan populations (Figure 6B).

Table 3: Pairwise F_{ST} comparisons amongst *Lepidosperma* sp. Parker Range populations.

Population	LPR 1	LPR 3	LPR 6	LPR 7	LPR 8	LPR 9
LPR 1	-					
LPR 3	0.119	-				
LPR 6	0.228	0.067	-			
LPR 7	0.362	0.269	0.265	-		
LPR 8	0.305	0.240	0.231	0.108	-	
LPR 9	0.276	0.209	0.275	0.261	0.150	-

Table 4: Pairwise F_{ST} comparisons among *Lepidosperma* sp. Mt Caudan populations. Non-significant comparisons are bolded and asterisked.

Population	LMC 2	LMC 3	LMC 4	LMC 4.5	LMC 5	LMC 7	LMC 8	LMC 9	LMC 10	LMC 11	LMC 12
LMC 2	-										
LMC 3	0.064	-									
LMC 4	0.041	0.025	-								
LMC 4.5	0.038	0.060	0.023	-							
LMC 5	0.096	0.125	0.125	0.102	-						
LMC 7	0.072	0.066	0.064	0.069	0.069	-					
LMC 8	0.077	0.095	0.057	0.066	0.101	0.079	-				
LMC 9	0.063	0.070	0.083	0.082	0.091	0.074	0.078	-			
LMC 10	0.037	0.082	0.064	0.059	0.084	0.065	0.047	**0.01***	-		
LMC 11	0.029	0.059	0.053	0.034	0.085	0.052	0.064	0.061	0.042	-	
LMC 12	0.020	0.049	0.039	0.036	0.073	0.044	0.053	0.036	0.025	**0.00***	-

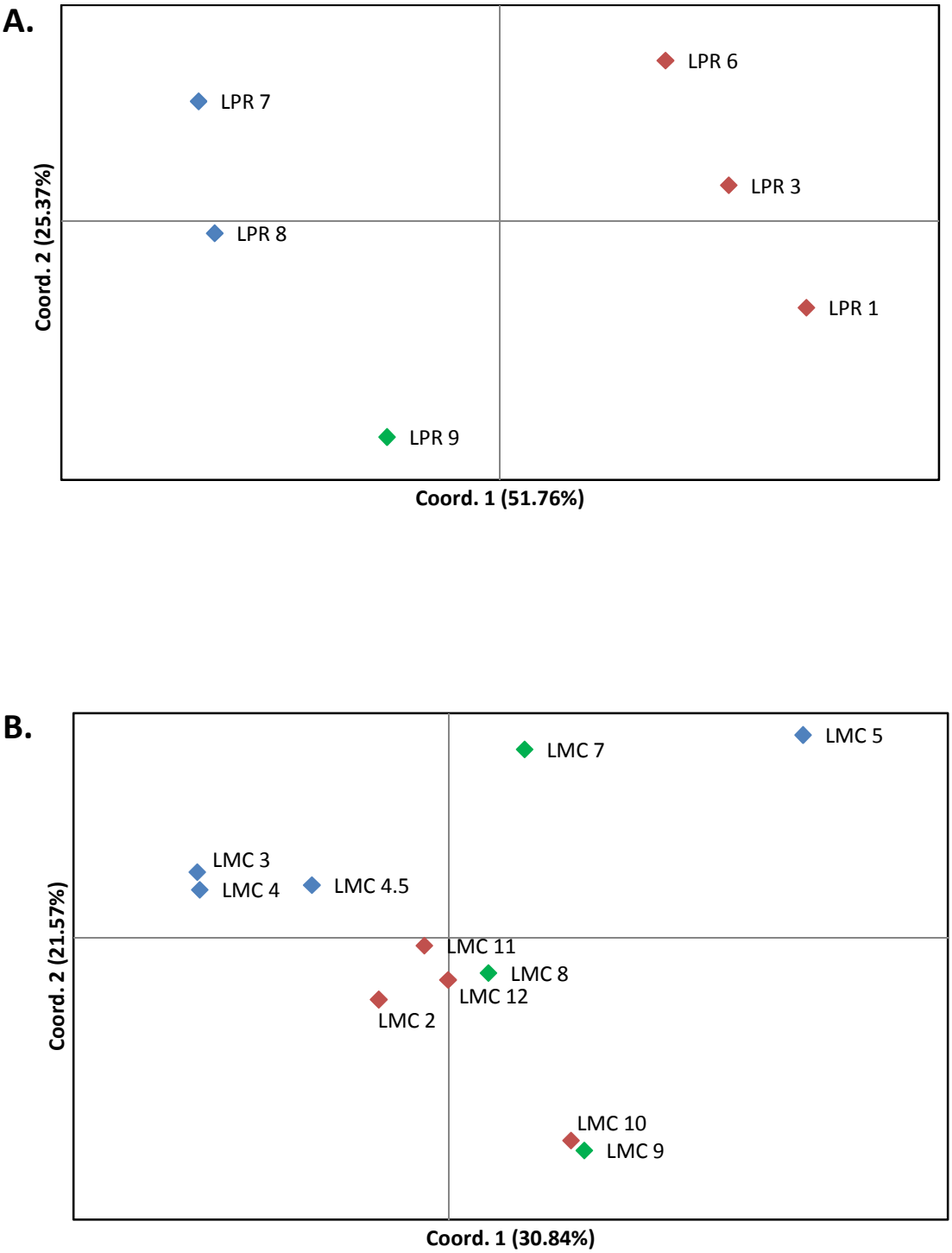


Figure 4: Principle co-ordinates analysis of genetic differentiation based on nuclear microsatellite markers among populations of **A.** *Lepidosperma* sp. Parker Range and **B.** *Lepidosperma* sp. Mt Caudan. The two co-ordinate axes account for 77.13% and 52.41% of the total genetic variation for each species, respectively. The coloured markers indicate geographic relationships; for each species, populations of the same colour are geographically closer to each other.

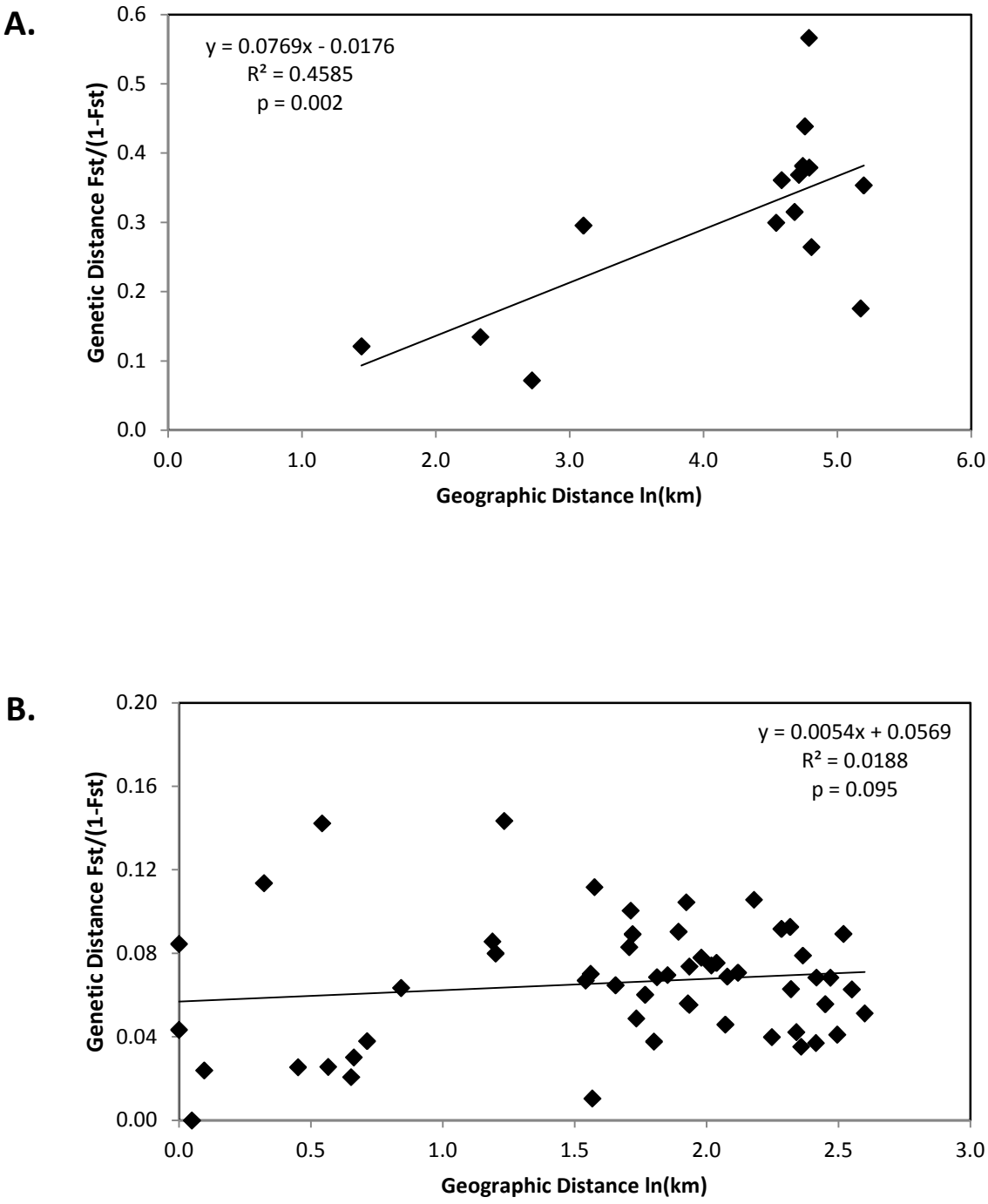


Figure 5: Mantel testing of isolation by distance for correlations between genetic and geographic distance using pairwise population comparisons for **A.** *Lepidosperma* sp. Parker Range (significant) and **B.** *Lepidosperma* sp. Mt Caudan (not significant).

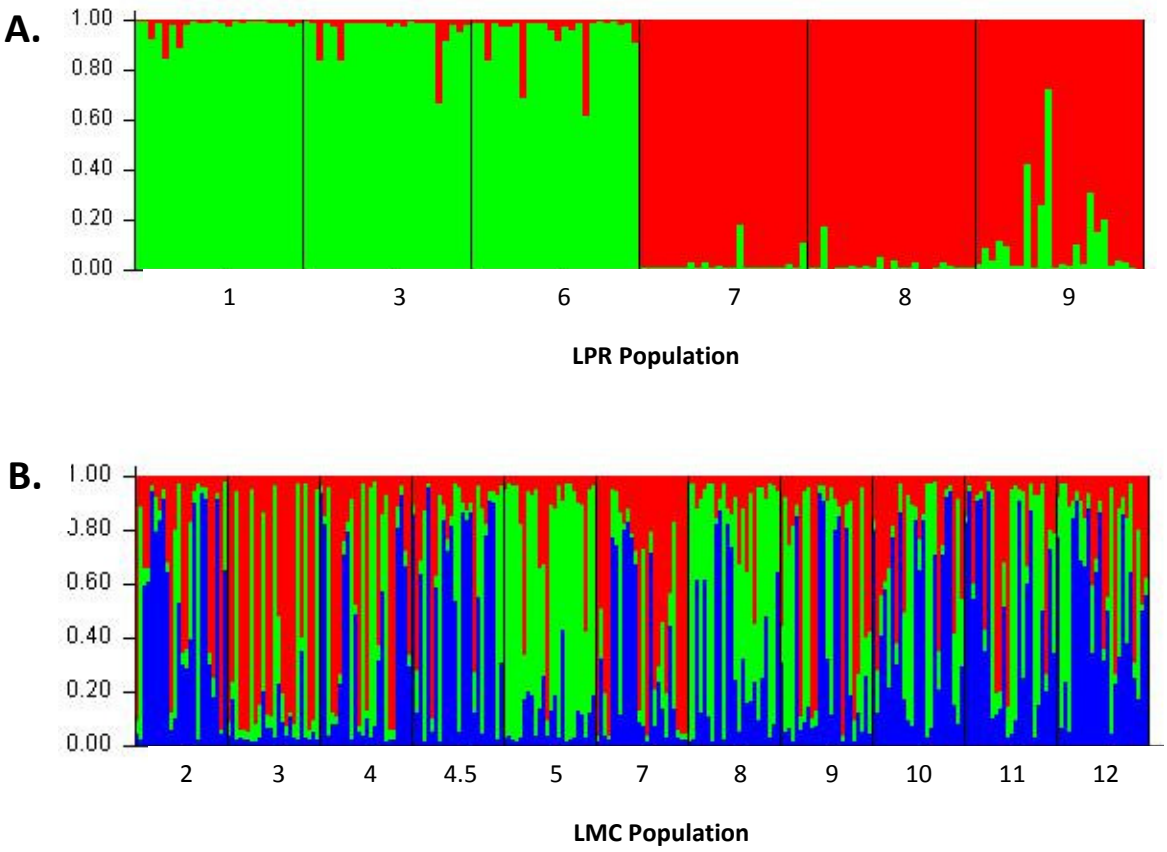


Figure 6: Structure analysis inferring genetic clusters across individuals of **A.** *Lepidosperma* sp. Parker Range and **B.** *Lepidosperma* sp. Mt Caudan. Each individual is represented by a vertical bar which is partitioned into the proportion of its affinity to each genetic cluster. Individuals are arranged in population order to show geographic structuring (or the lack thereof in the case of *Lepidosperma* sp. Mt Caudan).

Analysis of the contribution of each population to the genetic diversity and differentiation in the species showed a high variability among populations in both species. For *L.* sp. Parker Range, populations LPR 7 and LPR 8 contribute the most significantly to the diversity in terms of heterozygosity, while population LPR 9 is the most important contributor to the species' allelic richness. The contribution of population LPR 1, at the proposed mine site, to overall species diversity showed the same pattern for heterozygosity (CT) and allelic richness (CRT) (Figures 7A and 7B respectively). LPR 1 has the lowest diversity of all six *L.* sp. Parker Range populations, therefore having negative contributions to both heterozygosity and allelic richness. In turn and perhaps because of this, the population is quite differentiated from other populations, positively contributing to species diversity. However, the combined contribution of LPR 1 to species diversity was negative for both heterozygosity and allelic richness, indicating that species diversity would not be affected following the removal of LPR 1.

For *L.* sp. Mt Caudan, populations LMC 3 and LMC 7 contribute the most significantly to species diversity in terms of both heterozygosity and allelic richness. Population LMC 5 contributed little to

diversity but more to differentiation. Population LMC 2, the population to be impacted by mining, has a low, but positive contribution to total species diversity. In terms of heterozygosity (CT), LMC 2 was neither particularly diverse nor differentiated from other populations (Figure 8A). With the highest richness of all 11 populations, LMC 2 contributes positively to total allelic richness (CRT) for the species, but these alleles are not particularly unique and with a negative contribution for population differentiation, the population's overall contribution to total allelic richness is positive but low (Figure 8B).

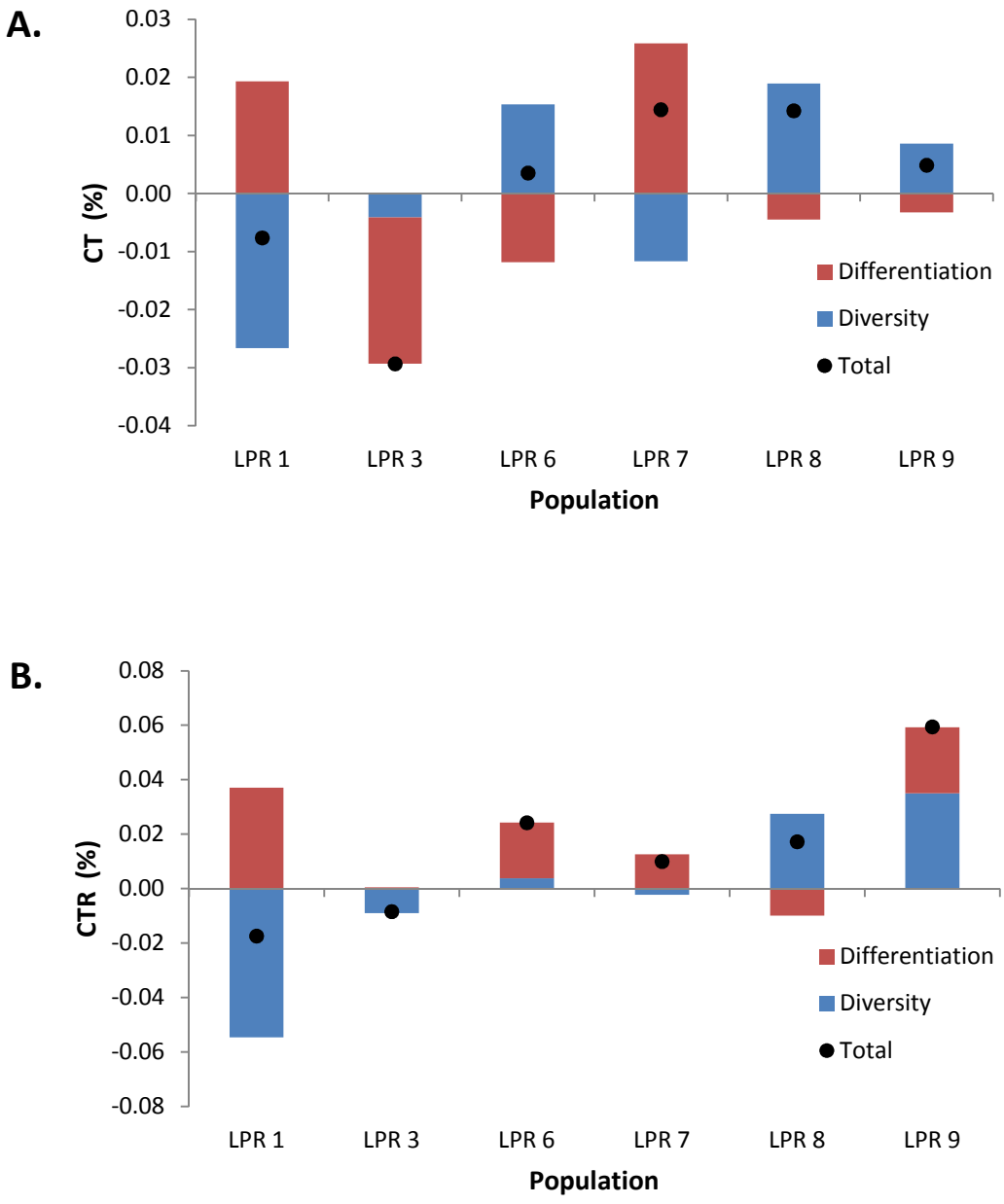
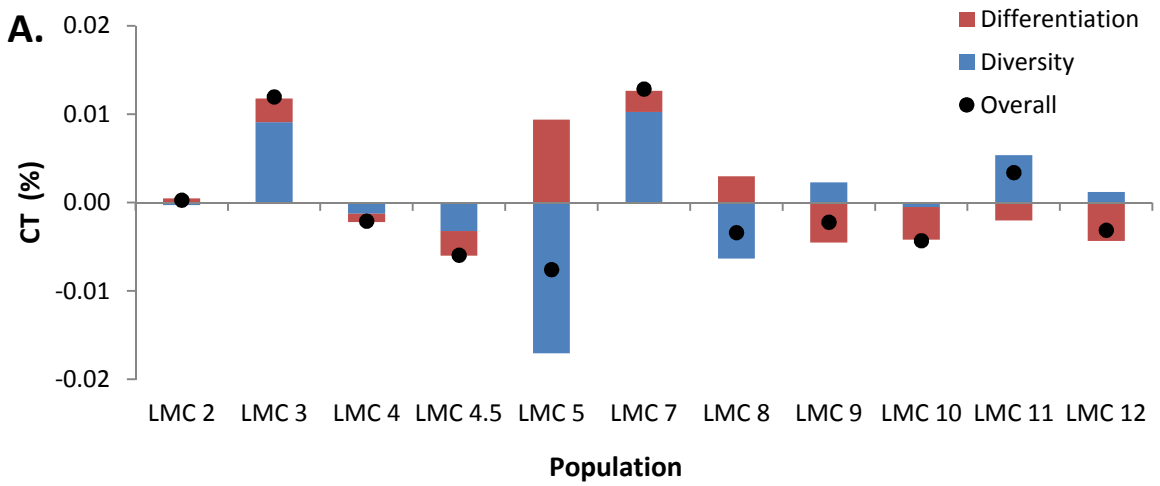


Figure 7: Contributions of *Lepidosperma* sp. Parker Range populations to **A.** total species diversity (CT) and **B.** allelic richness (CTR). The overall contribution of each population (black dot) is split into the contribution due to the population's diversity (blue) and it's differentiation from other populations (red).



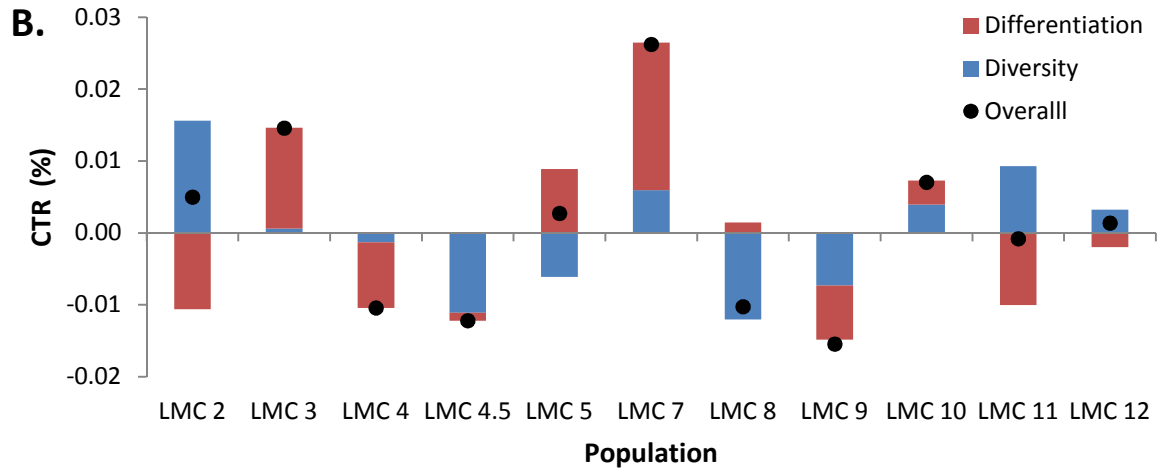


Figure 8: Contributions of *Lepidosperma* sp. Mt Caudan populations to **A.** total species diversity (CT) and **B.** allelic richness (CTR). The overall contribution of each population (black dot) is split into the contribution due to the population's diversity (blue) and it's differentiation from other populations (red).

Clonality analysis of reproductive system and dispersal

Analysis of clonality revealed some clonal reproduction in each species. While neither species exhibited high levels of clonality, *L.* sp. Parker Range was much more frequently clonal and these clones were spread over greater spatial scales than those of *L.* sp. Mt Caudan (Table 5). There was considerable variation among *L.* sp. Parker Range quadrats, with some being highly clonal (i.e. LPR1 Q1) and others less so (e.g. LPR1 Q2), giving an overall moderate level of clonal richness (mean = 0.437 ± 0.13). The number of ramets per multi-locus lineage (MLL) ranged from two to 18, although most MLL's were small with an overall average of 3.15 ± 1.07 ramets per lineage. Ramets within a given MLL were significantly aggregated, with little crossover among neighbouring MLL's (Figure 9). Clonal size, determined by the maximum distance between any two ramets within a given MLL, ranged from 2.8 to 5.8 m. In contrast, clonality was rare across all *L.* sp. Mt Caudan quadrats, where most of the ramets sampled had distinct multi-locus genotypes (Figure 10). As a result, clonal richness was high across all quadrats (mean = 0.889 ± 0.02) and the few MLL's detected consisted of just two to three ramets. These MLL's tended to consist of neighbouring ramets with significant aggregation scores and a clonal size averaging 1.3 ± 0.1 m. There was no evidence for an edge effect in any of the quadrats sampled in either species.

Table 5: Summary of diversity and spatial parameters for multi-locus lineages (MLLs) in populations of A. *Lepidosperma* sp. Parker Range and B. *Lepidosperma* sp. Mt Caudan.

Population /Quadrat	# Loci	# Ramets	# MLL	Richness	Mean #Ramets per MLL	Clonal Subrange (m)	Edge Effect	Agg. Index
A. *L.* sp. Parker Range								
LPR1 Q1	8	56	9	0.145	6.222 (1.58)	4.354	-0.313	0.810*
LPR1 Q2	8	75	51	0.766	1.471 (0.12)	2.814	-0.140	0.257*
LPR3 Q1	8	75	39	0.514	1.923 (0.26)	4.118	-0.047	0.423*
LPR3 Q2	8	75	25	0.324	3.000 (0.34)	5.796	-0.144	0.653*
Mean (SE)			**31.00 (9.06)**	**0.437 (0.13)**	**3.154 (1.07)**	**4.271 (0.6)**		
B. *L.* sp. Mt Caudan								
LMC2 Q1	8	75	64	0.851	1.172 (0.06)	1.342	0.028	0.183*
LMC2 Q2	8	75	69	0.919	1.087 (0.03)	1.249	0.004	0.105*
LMC3 Q1	8	75	69	0.919	1.087 (0.04)	1.583	-0.014	0.118*
LMC3 Q2	8	75	65	0.865	1.154 (0.06)	1.043	-0.032	0.169*
Mean (SE)			**66.75 (1.31)**	**0.889 (0.02)**	**1.125 (0.02)**	**1.304 (0.11)**		

*indicates a significant ($p < 0.05$) result

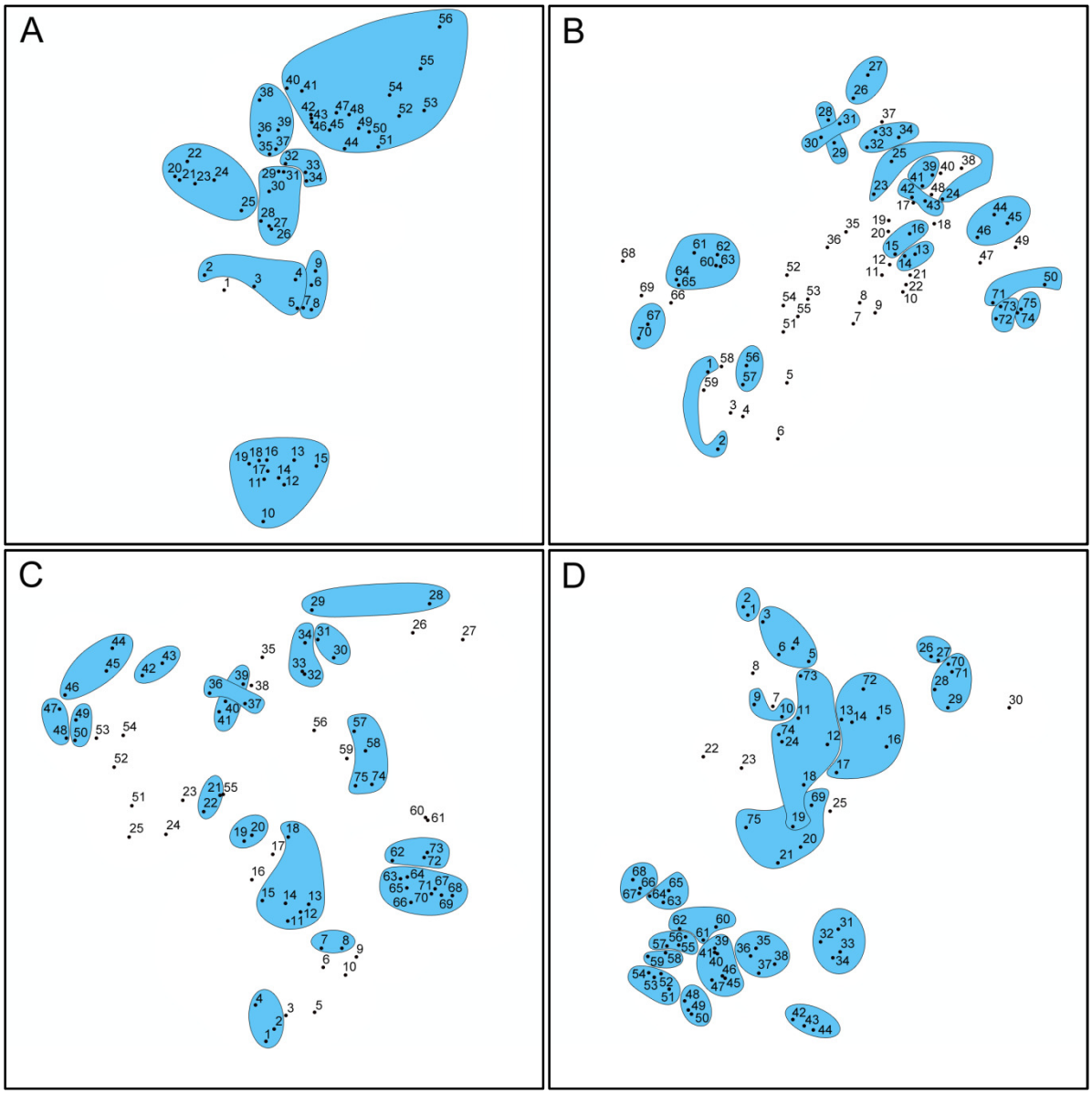


Figure 9: Size and distribution of clonal patches in populations of *Lepidosperma* sp. Parker Range. Each point represents a sample collected within 15 x 15m quadrats. Ramets within the same multi-locus lineage (MLL) are grouped by blue shading. **A.** LPR1 Q1, **B.** LPR1 Q2, **C.** LPR3 Q1, **D.** LPR3 Q2.

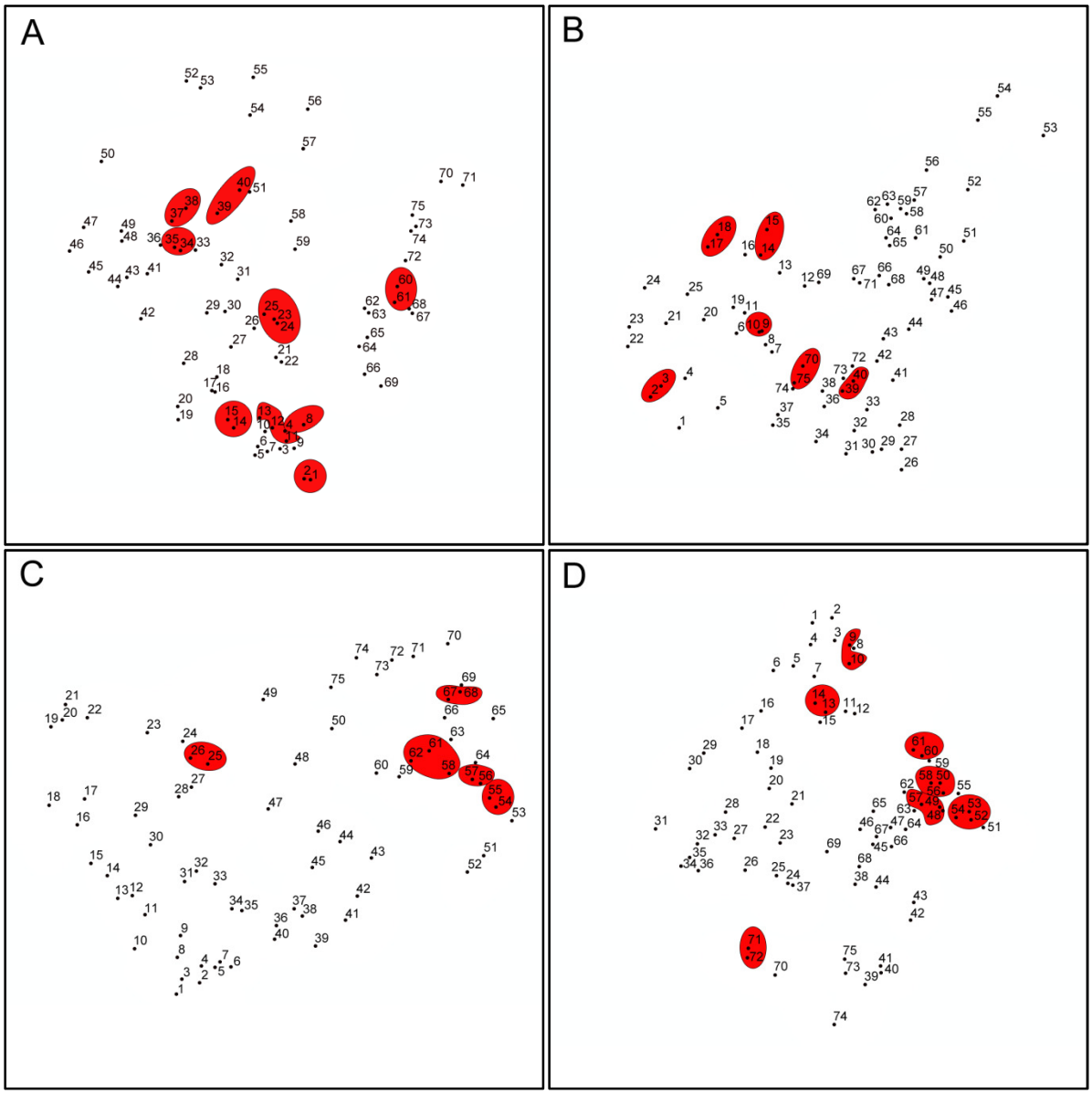


Figure 10: Size and distribution of clonal patches in populations of *Lepidosperma* sp. Mt Caudan. Each point represents a sample collected within 15 x 15m quadrats. Ramets within the same multi-locus lineage (MLL) are grouped by red shading. **A.** LMC2 Q1, **B.** LMC2 Q2, **C.** LMC3 Q1, **D.** LMC3 Q2.

Summary and Final Recommendations

This project assessed the genetic diversity and structure of *Lepidosperma* sp. Mt Caudan and *Lepidosperma* sp. Parker Range with particular regard to the impact of removing one population of each species for the development of an open cut iron-ore mine in the Parker Range. To date, there are no full studies of population genetic diversity or structure in Western Australian *Lepidosperma* species; however the levels of genetic diversity found in this study were considerably lower than those found in a brief study of *Lepidosperma gibsonii*, a congener with a similarly restricted distribution (Barrett et al. 2012). This suggests that the diversity in *L.* sp. Mt Caudan and *L.* sp. Parker Range is in the low to moderate range which is typical of species with small populations and restricted distributions. Moreover, the differing spatial arrangements of these populations are reflected in contrasting genetic signatures for each species.

All 11 populations of *L.* sp. Mt Caudan are clustered within a maximum range of 15 km of each other. Diversity across these populations is highly consistent with minimal differentiation among populations. As a result, there is little geographic structuring across their range, indicating that populations of *L.* sp. Mt Caudan are well connected by gene flow. Given that the species does not appear to favour asexual reproduction (from the low levels of clonality found) *L.* sp. Mt Caudan must primarily rely on sexual reproduction for population persistence, despite not producing viable seed in the last two flowering seasons. Such irregular sexual reproduction must occur sufficiently frequently and over an adequate spatial scale for maintaining gene flow across the restricted geographic range of *L.* sp. Mt Caudan. The CONTRIB analyses highlighted populations LMC 3 and LMC 7 as key populations contributing highly to the species' diversity, while population LMC 2 (i.e. the impact population) was shown to play a less important role.

In contrast to *L.* sp. Mt Caudan, populations of *L.* sp. Parker Range are fewer and much more geographically disjunct (maximum distances of 200 km apart). This is reflected in their genetic structuring, with much higher levels of differentiation among populations. This restriction of gene flow is particularly divisive across larger distances between the central populations (LPR 1, 3, and 6) and the peripheral populations (LPR 7, 8 and 9). The two populations studied exhibited a moderate level of clonality, which if extrapolated to all populations, suggests that effective population sizes for *L.* sp. Parker Range may be approximately one third of the number of plants, rendering population sizes smaller than originally thought. Consistent with small, isolated populations, genetic diversity was reduced and more inconsistent among populations, with particularly low diversity in the impact population LPR 1. Despite being more clonal than *L.* sp. Mt Caudan, clonal richness in *L.* sp. Parker Range was much higher than that typically found in fully clonal species (e.g. Gitzendanner et al. 2011) and therefore, clonality is also not likely to be the primary mode of reproduction in *L.* sp. Parker Range. Like *L.* sp. Mt Caudan therefore, the species must also experience irregular bouts of sexual reproduction when conditions are favourable. However, gene flow appears to be much more restricted among populations of *L.* sp. Parker Range, suggesting that the spatial extent of dispersal is unlikely to regularly surpass the geographic distance among populations. *Lepidosperma* sp. Parker Range, with fewer, small and geographically disjunct populations is therefore of greater conservation concern than *L.* sp. Mt Caudan with its larger, more connected populations. The

peripheral populations (in particular, LPR 7 and LPR 9) contribute the most to the species overall diversity, while the impact population LPR 1 makes no particular contribution.

Overall, the available data suggest that the removal of populations LPR 1 and LMC 2 in the development of an open cut iron-ore mine is unlikely to have an appreciable impact on the overall genetic diversity and structure for either species in the near future. Neither population contributes significantly to species diversity nor should their removal interrupt current processes of gene flow. Nevertheless, both species do have restricted distributions and few populations, with low to moderate levels of diversity and a tendency for clonality, which renders them vulnerable to disease and disturbance in the long term. Therefore, considerable effort should be made to restore the impact populations following the closure of the mine. In doing so, we offer several suggestions;

1. Plants, or cuttings, from the impacted populations could be translocated to a neighbouring population of similar genetic status prior to mining development and then returned back to their source during the land rehabilitation process following the mines closure. In this case, we would suggest translocating plants from LPR 1 to LPR 3 and LMC 2 to LMC 12 as these are the most geographically and genetically similar pairs of populations, maximising the chances of successful transplantation without incurring adverse effects. Regular monitoring of the translocated plants would be required to ensure their survival during the lifetime of the mine.

2. Alternatively, plants, or cuttings, from the impacted populations could be removed prior to the mining development and maintained in greenhouse facilities for the duration of the operation. This would eliminate any genetic or environmental impacts of combining populations but would require more resources and intensive care of the plants outside of their natural environment until they could be returned to their source populations following closure of the mine.

3. In addition to the safeguarding of plant material, we suggest that seed stocks be collected for rehabilitation purposes. These could be sourced from the impact sites before clearing for the mine development and/or from nearby, genetically similar populations (i.e. LPR 3 and LMC 12) during the lifetime of the mine. This is largely a protective mechanism in the event that the translocated plants have poor survivability, either in natural or greenhouse conditions. It would be especially important if plants are kept in greenhouse conditions, as they would cease to have the opportunity to exchange genes with neighbouring populations. Given the apparent irregular occurrence of a good reproductive season for either species, populations would need to be monitored annually to ensure that collections are made in a year of good seed production.

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