Genetic diversity in *Eucalyptus leucophloia* across the Pilbara: Provenance zone implications

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Executive Summary

Rehabilitation of disturbed sites through revegetation requires knowledge of the genetic adaptation of a species to the targeted sites in order to manage these sites on an ecologically sustainable basis. It has been generally agreed that local seed sources should be used in rehabilitation and traditional approaches to seed collection for revegetation typically advocate a narrow definition of the 'local provenance' for a species. This approach is being adopted by the Environmental Protection Authority through the environmental impact assessment and approvals process for mining activity in the rangelands of Western Australia, in particular the Pilbara. The EPA and other regulatory agencies have sort to prescribe or conditioned new approvals with the use of 'locally' sourced seed based on anecdotal 'local' seed collection zones of 15km. This restriction relates simply to geographic distance and does not take into consideration other factors relevant to the supply of seed for revegetation.

Identification of appropriate seed source distances requires information on the level and extent of local adaptation and underlying genetic diversity within species. General guidelines on seed collection zones can be developed for implementation in rehabilitation programs in order to maintain the genetic integrity of Pilbara ecological communities on a sustainable basis.

The aim of this exemplar study which is a collaboration between Rio Tinto and the Science Division of the Department of Environment and Conservation is to define the provenance seed collection zones for two common species of the Pilbara, *Eucalyptus leucophloia* (Snappy Gum) and *Acacia ancistrocarpa* (Fitzroy Wattle). This preliminary report details information on genetic analysis conducted on the first of these species *E. leucophloia.* Collections of *E. leucophloia* were made from 20 populations across the Pilbara bioregion and genetic analysis was conducted using microsatellite markers.

Genetic diversity in *E. leucophloia* was high and was typical of that found in other eucalypt species with wide spread distributions. Across the species the level of population differentiation was low and the majority of the diversity was maintained within populations with only 6% of variation partitioned between populations. Genetic variation in *E. leucophloia* showed little structure across the Pilbara with no clustering of populations based on any geographical proximity or in association with obvious topographical, physiogeographical or geological features such as the Hamersley or Chichester Ranges. Populations towards the edges of the species distribution within the Pilbara showed greater levels of differentiation from populations within the species main range.

The high levels of genetic diversity and low levels of differentiation within *E. leucophloia* implies that seed resources for land rehabilitation and mine-site revegetation programs can be selected from a wide range within the Pilbara distribution of the species, thus maintaining genetic diversity of restoration areas. We therefore recommend that seed collections be focussed within the same Pilbara biogeographical subregions of the species distribution as the disturbance site to ensure limited mixing of seed between these sub-regions. Within sub-regions we recommend a composite provenancing approach to maintain local adaptive genetic variation.

Background

Rehabilitation of sites through revegetation requires knowledge of the genetic adaptation of the species to the disturbance site in order to manage such sites on an ecologically sustainable basis. Appropriate strategies for collecting seed that provide the raw genetic material for restoration activities is an important issue for land rehabilitation and mine site revegetation programs.

It has been generally agreed that local seed sources should be used in rehabilitation so as to protect the genetic integrity of sites and maintain any adaptive variation that has developed through evolutionary history. Traditional approaches regarding seed collection follow a restrictive precautionary principle that advocates a narrow definition of seed of ‘local provenance’ for revegetation. This approach is being adopted by the Environmental Protection Authority and other regulatory agencies through the environmental impact assessment and approvals process for mining and native vegetation clearing applications in the Pilbara. In many instances conditions are being prescribed which advocate the use of ‘locally’ sourced seed, with local variously defined based on anecdotal ‘local’ seed collection zones of 15km commonly used in the revegetation of State Forest areas. This prescription relates simply to geographic distance and does not take into consideration other factors relevant to seed supply. Such prescriptions have also caused concern for the resources industry as they are significantly more restrictive than those recommended in the early 1990s for the Pilbara (Coates & van Leeuwen 1997; van Leeuwen 1994).

There is no clearly articulated definition of ‘local’ in the scientific literature on use of seed sources for restoration or revegetation programs. The concept of ‘local provenance’ arises from the assumption that seed from local plants are best adapted to local conditions. However, patterns of morphological and physiological variation may or may not be reflective of genetic adaptation to local environmental conditions. Furthermore, site conditions for rehabilitation may be dramatically altered, particularly during mining operations, so that locally adapted material may be less appropriate than maximising genetic diversity and adaptive potential by combining seed collections from multiple sites within an area (Millar *et al*. 2009, Broadhurst *et al*. 2008).

Previous recommendations (Coates & van Leeuwen 1997; Fletcher *et al.* 1994; van Leeuwen 1994, 1995) delimiting ‘local provenance’ in the Pilbara followed the following spatial hierarchy:

- Seed to be sourced from the biogeographical subregion in which the rehabilitation site is located.
- Seed to be sourced from the physiogeographic unit (after Beard, J.S. 1975) in which the rehabilitation site is located.
- Seed to be sourced from the appropriate 1:250,000 map on which the rehabilitation site is located (or an area equivalent to a 1:250,000 map sheet (typically 18,400km^2)) while honouring above recommendations.
- Endeavour to restrict seed collecting to the catchment in which the rehabilitation site occurs while adhering to the previous recommendations.
- For the rehabilitation of habitat specific sites (e.g. hill tops, clay playas) restrict seed collection to the next nearest similar sites.
- When the rehabilitation program involves rare, endangered, uncommon, habitat specific, range-end and/or disjunctly distributed species restrict seed collection to the next nearest population.

Identification of appropriate seed source distances requires information on the level and extent of local adaptation and underlying genetic diversity within a species. A reasonable surrogate for adaptive variation can be obtained through analysis of molecular diversity. By determining this information for a range of species with different life histories and breeding systems, general guidelines on seed collection zones can be developed for implementation in rehabilitation programs in order to maintain the genetic integrity of Pilbara ecological communities on a sustainable basis. Such guidelines will deliver enduring value to the resources industry through articulating the requirements for seed collection programs. They will provide regulatory authorities with a means of reducing uncertainty and risk through increasing the likelihood of establishing self sustaining plant communities through rehabilitation programs.

Objectives

The aim of this study is to define the provenance seed collection zones for two common species in the Pilbara, *Eucalyptus leucophloia* (Snappy Gum) and *Acacia ancistrocarpa* (Fitzroy Wattle). This preliminary report details information on genetic analysis conducted on the first of these species *E. leucophloia.*

Methods

Distributional information on *E. leucophloia* was determined from herbarium vouchers lodged at the Western Australian Herbarium (PERTH) through Florabase. Figure 1 depicts the overall distribution of the species across the Pilbara where it is found to occur predominantly across the central and eastern Hamersley Ranges, throughout the Chichester Ranges and extending with a non-continues distribution into the eastern and north eastern Pilbara to the edge of the Great Sandy and Little Sandy Deserts. Within the Pilbara the species has been recorded from the Hamersley, Chichester and Fortescue subregions and is absent from the Roebourne subregion. Populations for sampling were selected to cover the range of the distribution across the Pilbara region.

Collections of leaf material were made from 24 trees from 20 populations as detailed in Table 1 and Figure 1. DNA was extracted from all plants using a scaled-down version of the CTAB method of Doyle and Doyle (1987) with the addition of 0.1% sodium sulfite (Byrne *et al*. 2001) and 1% PVP (polyvinylpyrrolodine) to the extraction buffer. Microsatellite primers previously developed for eucalypts were tested and the most informative 12 loci were used to genotype all samples of *E. leucophloia.* Primers used were EMBRA 2, 3, 5, 8, 9, 10, 11, 18 EN 6, EMCRC 2, 6, 12 (Brondani *et al.* 1998, Byrne *et al. 1*996, Steane *et al.* 2001). Amplification reactions were carried out using 1.25 – 1.75 mM $MgCl_2$ in a PCR of 96°C for 2 min; 30 cycles of 94°C for 30 s, 56°C for 30 s, 72°C for 30 s, followed by 72°C for 5 min. Amplification products were separated on an Applied Biosystems 3730 capillary sequencer.

Genotypes were scored from the raw data using GENEMAPPER version 4.0. Genetic diversity parameters *N*a (average number of alleles per locus), *P* (percentage polymorphic loci), *H*o (observed heterozygosity) and *H*e (gene diversity, expected heterozygosity) were determined for each population using GenAlEx 6.1. (Peakall and Smouse 2006). The partitioning of genetic variation within and among populations was estimated by analysis of molecular variation (AMOVA) in GenAlEx 6.1.

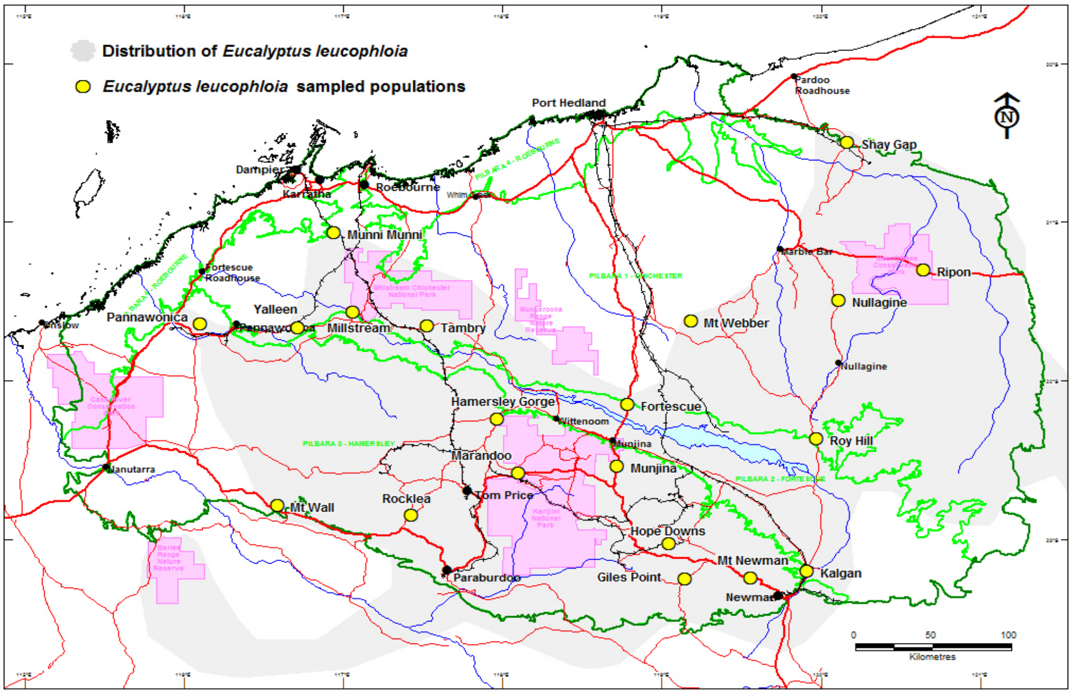

Figure 1 Location of sampling sites of *Eucalyptus leucophloia* in the Pilbara.

Differentiation among populations (F_{ST}) and Nei's unbiased genetic distance (D) were calculated using GenAlEx 6.1. The relationships among populations were identified through ordination in multidimensional space using a principal co-ordinate analysis, and through a UPGMA analysis based on genetic distance using the program GDA (Lewis and Zaykin 2002). A Bayesian analysis using the program STRUCTURE (Pritchard 2000) was undertaken to identify any pattern of structure across the sampled populations.

Results

The microsatellite loci showed high allelic diversity with 272 alleles over the 12 loci. All loci were polymorphic in all populations except for EMBRA 18 in the Ripon population. The high number of alleles had generally similar frequency distributions with very few common alleles. Populations had high levels of observed heterozygosity ranging from 0.484 to 0.646 and expected heterozygosity ranging from 0.747 to 0.819 (Table 2).

Estimates of genetic differentiation between populations (F_{ST}) were low and ranged from 0.022 to 0.067 with an average across all populations of 0.036. Analysis of variation using AMOVA showed that 94% of the variation was maintained within populations with only 6% between populations.

Analysis of genetic relationships between individuals by a principal co-ordinates analysis showed no geographical association between populations. Individuals did not cluster into discrete groups based on population or region and occupied wide areas of the ordination space.

Table 1 Details of *Eucalyptus leucophloia* populations sampled in the Pilbara for genetic analysis.

Location	Population Code	Voucher No.	Latitude	Longitude	Pilbara subregion
Mt Newman	MNE	SvL5292	23 14 30.1	119 33 20.5	Hamersley
Giles Point	GPT	SvL5294	23 15 01.6	119 08 37.8	Hamersley
Hope Downs	HDO	SvL5296	23 01 41.9	119 02 46.8	Hamersley
Munjina	MUN	SvL5298	22 32 12.7	118 43 07.8	Hamersley
Fortescue	WKN	SvL5299	22 08 53.0	118 46 55.4	Fortescue
Hamersley Gorge	HAM	SvL5301	22 14 36.5	117 57 47.5	Hamersley
Marandoo	MDO	SvL5303	22 34 42.5	118 05 47.0	Hamersley
Rocklea	ROC	SvL5305	22 50 48.7	117 25 24.1	Hamersley
Mt Wall	MWA	SvL5306	22 47 14.7	116 34 58.3	Hamersley
Pannawonica	PAN	SvL5312	21 38 37.2	116 05 49.4	Hamersley
Yalleen	YAL	SvL5314	21 40 06.1	116 42 40.3	Chichester
Millstream	MIL	SvL5315	21 33 57.4	117 03 31.9	Chichester
Tambry	TAM	SvL5318	21 03 56.6	117 31 25.0	Chichester
Munni Munni	MON	SvL5320	21 03 56.6	116 56 28.1	Chichester
Mt Webber	MWE	SvL5323	21 37 20.1	119 10 51.6	Chichester
Shay Gap	SGP	SvL5333	20 29 40.6	120 09 53.8	Chichester
Ripon	RIP	SvL5335	21 17 56.8	120 38 40.4	Chichester
Nullagine	NUL	SvL5337	21 29 43.1	120 06 39.5	Chichester
Roy Hill	RHL	SvL5338	22 21 52.9	119 58 07.8	Chichester
Kalgan	KAL	SvL5339	23 12 08.9	119 54 29.2	Fortescue

Table 2 Genetic diversity parameters for populations of *Eucalyptus leucophloia* in the Pilbara. (*Na*, average number of alleles per locus; *P*, polymorphic loci; *Ho*, observed heterozygosity; *He* expected heterozygosity. Standard error in parentheses).

Pop	*Na*		*P*	*Ho*		*He*	
GPT	12.2	(1.8)	100	0.614	(0.067)	0.813	(0.041)
HDO	11.8	(1.6)	100	0.616	(0.064)	0.819	(0.035)
MNE	11.3	(1.7)	100	0.598	(0.070)	0.805	(0.033)
MUN	11.7	(1.7)	100	0.609	(0.065)	0.773	(0.066)
HAM	12.3	(1.7)	100	0.600	(0.069)	0.805	(0.059)
MDO	10.3	(1.4)	100	0.565	(0.071)	0.775	(0.048)
MIL	12.2	(1.6)	100	0.529	(0.066)	0.807	(0.043)
MWA	11.0	(1.3)	100	0.588	(0.084)	0.784	(0.044)
PAN	9.8	(1.2)	100	0.583	(0.064)	0.778	(0.042)
ROC	11.3	(1.5)	100	0.509	(0.083)	0.778	(0.066)
WKN	11.8	(1.6)	100	0.646	(0.056)	0.822	(0.032)
YAL	11.8	(1.6)	100	0.484	(0.072)	0.794	(0.057)
KAL	9.2	(1.2)	100	0.628	(0.079)	0.764	(0.045)
MON	10.8	(1.5)	100	0.606	(0.077)	0.747	(0.068)
MWE	10.3	(1.3)	100	0.581	(0.092)	0.780	(0.044)
NUL	12.1	)1.6)	100	0.552	(0.060)	0.769	(0.060)
RHL	11.3	(1.6)	100	0.556	(0.072)	0.758	(0.066)
RIP	9.5	(1.2)	92	0.580	(0.074)	0.747	(0.072)
SGP	10.6	(1.5)	100	0.580	(0.065)	0.782	(0.054)
TAM	10.1	(1.3)	100	0.583	(0.073)	0.784	(0.045)
Total	11.1	(0.3)	99	0.580	(0.016)	0.784	(0.011)

Analysis of population relationships using a principal co-ordinates analysis also showed no geographical association between populations (Figure 2). The Pannawonica population was the most genetically differentiated of the populations, and the Kalgan, Mt Webber, Ripon and Roy Hill populations were also more differentiated than other populations.

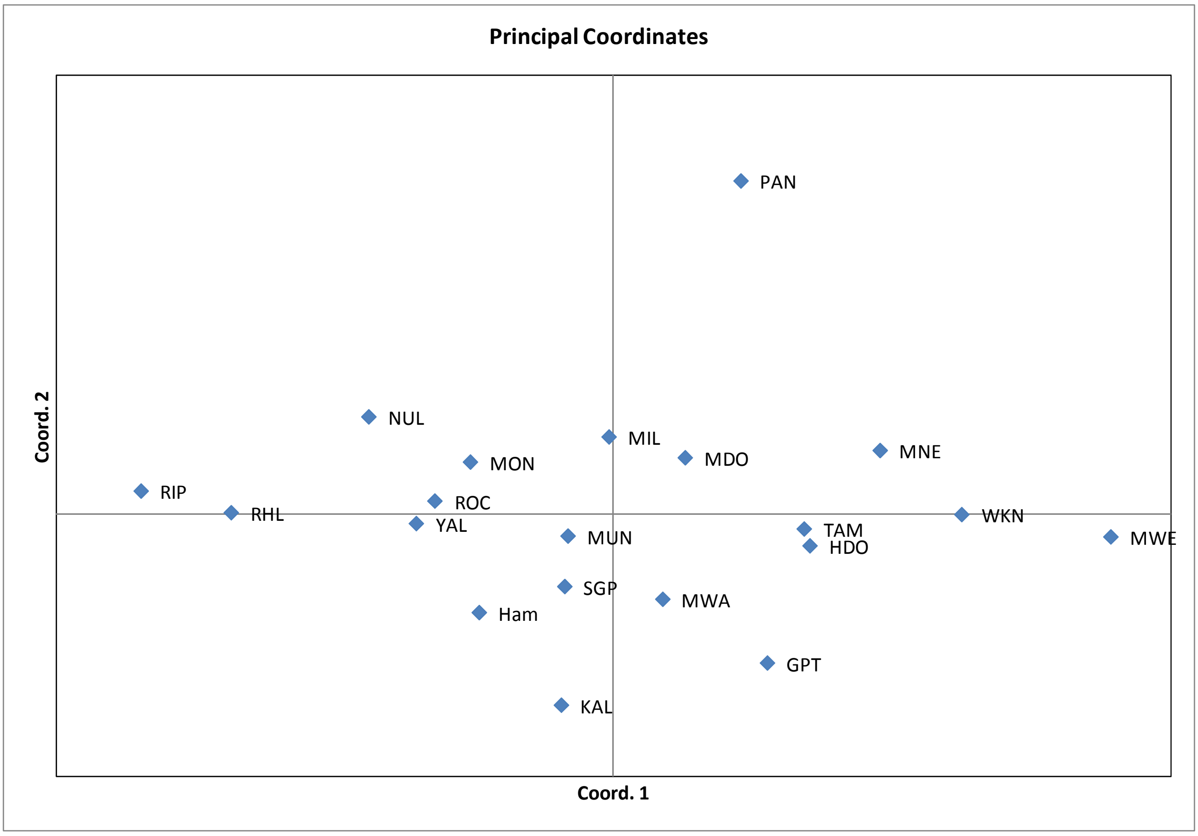

Figure 2 Principal Co-ordinates Analysis of the relationships between populations of *Eucalyptus leucophloia* in the Pilbara. Circled populations are more on the periphery of the geographical distribution of this species and tend to be genetically more discrete.

A dendrogram of population relationships showed some minor geographical structure among populations (Figure 3). The Pannawonica population was the most differentiated, and the Mt Webber and Kalgan populations were also more differentiated than other populations.

The Bayesian analysis did not identify any level of structure among the populations. Assignment of individuals to the lowest possible level of structure of two genetic groups showed no association of populations to groups and all individuals were assigned to both groups (Figure 4).

Discussion

Genetic diversity in *E. leucophloia* was high and was typical of that found in other eucalypt species with wide spread distributions (Byrne 2008). Populations showed similar levels of diversity with a high number of alleles in all populations. Across the species the level of population differentiation was low and the majority of the diversity (94%) was maintained within populations with only 6% of variation partitioned between populations.

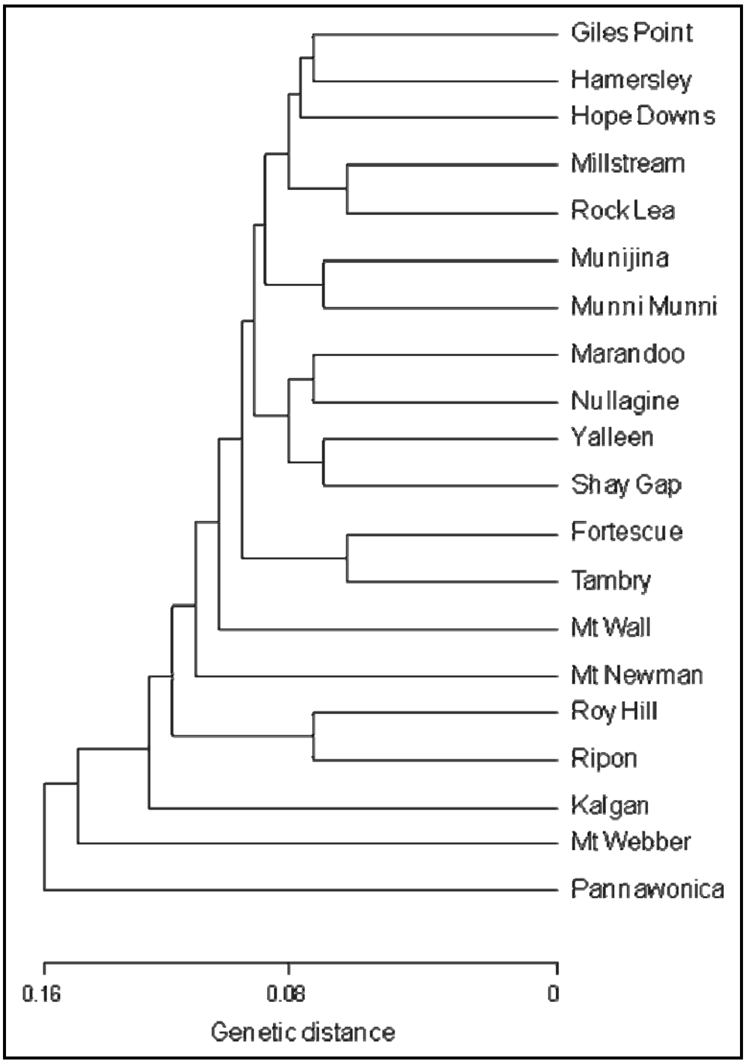

Figure 3 Genetic relationships among populations of *Eucalyptus leucophloia* based on a UPGMA analysis of genetic distance.

Eucalyptus leucophloia showed little genetic structure across the Pilbara region with no clear clustering of populations based on geographical proximity or in association with obvious topographical and geological features, such as the Hamersley and Chichester Ranges. Some populations on the edge of the species distributional range tended to show greater levels of differentiation from other populations, in particular the Pannawonica population in the west Pilbara, the Mt Webber population in the central Pilbara, and the Kalgan, Ripon and Roy Hill populations in the eastern Pilbara.

There are a number of possible explanations for the overall lack of association between genetic differentiation and geographical location of populations. The maintenance of significant levels of connectivity between populations through gene flow either by pollen movement or seed dispersal seems likely. In particular, seed dispersal perhaps through major flooding events associated with cyclonic activity may result in occasional widespread movement over large distances. Similarly seed dispersal associated with localised wind phenomena such as willy willies or dust devils may also transport seed of large distances. The lack of any clear genetic structure associated with the four Pilbara biogeographical subregions also indicates that there has been little historical isolation involving *E. leucophloia* populations between these areas.

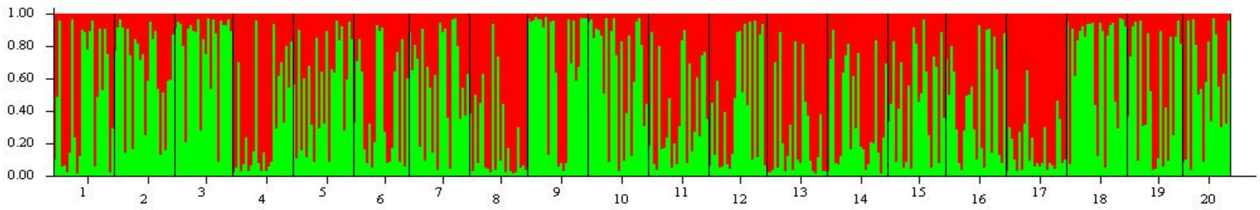

Figure 4 Assignment analysis for individuals of *E. leucophloia* for the lowest possible level of structure of two genetic groups. Plots show assignment of individuals to two putative genetic groups shown by different colours. Each individual is represented as a vertical bar broken into coloured segments representing components of different genetic groups.

The high levels of genetic diversity and low levels of differentiation within *E. leucophloia* implies that seed for land rehabilitation and mine-site revegetation programs can be selected from a wide distributional range across the Pilbara, thus maintaining genetic diversity in the areas being restored. However, with some differentiation of populations on the edge of the species range, we recommend that a precautionary approach be adopted with seed collection being restricted to the biogeographical subregion (see Coates and van Leeuwen 1996) in which it will be eventually used thereby limiting the opportunity for mixing of seed between subregions (Figure 5).

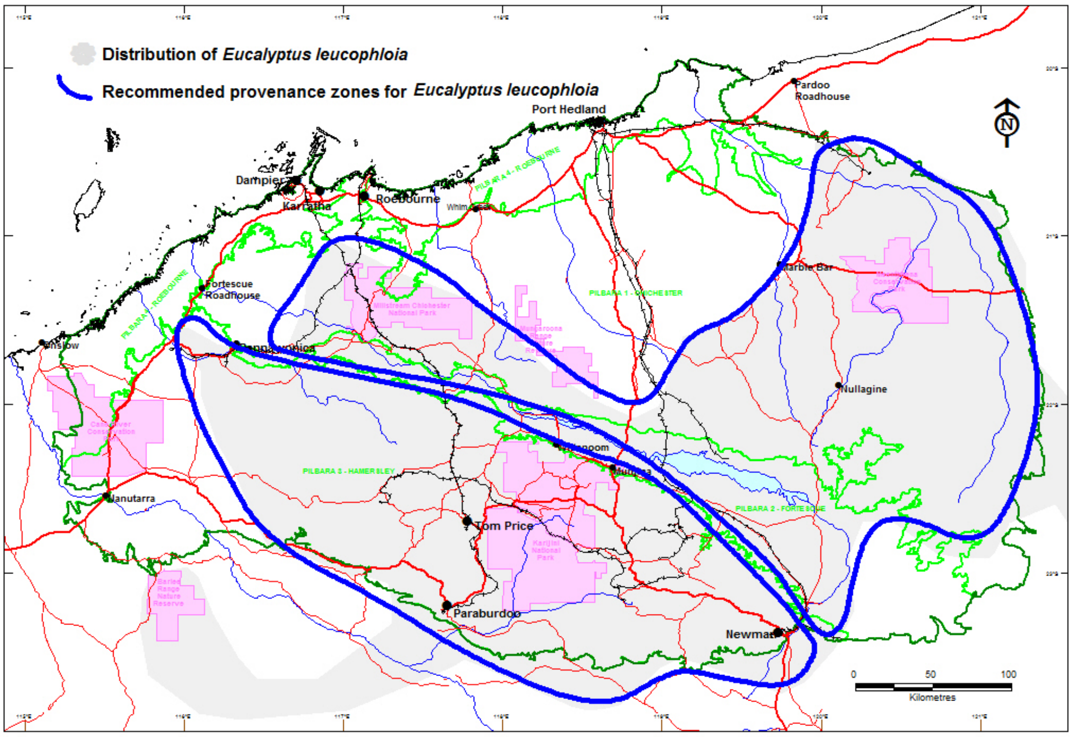

Figure 5 Recommended provenance zones for *Eucalyptus leucophloia* in the Pilbara.

Furthermore, we recommend maximising genetic variation of seed collections within a subregion. This “composite provenancing” approach involves the proportionate mixing of seed collections from several populations sourced within a single subregion. Such an approach facilitates the opportunity for new gene combinations that may potentially be able to respond to environmental heterogeneity, while maintaining the majority of any locally adaptive genetic variation (Broadhurst *et al.* 2008).

Current patterns of genetic diversity may also be influenced by historical population processes. A greater understanding of the factors influencing the distribution of genetic diversity in *E. leucophloia* may be obtained from an analysis of the variation in the chloroplast genome which would give an insight into the historical patterns of diversity in this species across the Pilbara.

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