Assessment of genetic diversity captured in *Acacia saligna* populations from Tigray, Ethiopia

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Melissa A Millar and Margaret Byrne

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Summary

This report describes the assessment of levels of genetic diversity present in populations of *Acacia saligna* (Labill.) in Tigray, Ethiopia. This work was undertaken by the Department of Environment and Conservation (DEC) for World Vision Australia. Phyllode material was collected from 66 individuals from 4 different 'enclosures' and from the Tigray Research Station by Peter Cunningham and provided to DEC in late 2011. DeoxyriboNucleic Acid (DNA) was extracted and individuals genotyped at five polymorphic microsatellite loci. Individuals from Ethiopia were compared to a reference data set of genotypes of genetic entities known to be present in the native range of the *A. saligna* species complex in Western Australia (WA). This reference data set was used as a diagnostic tool to assign individuals from Ethiopia to the known native entities. Levels of genetic diversity captured in individuals from Ethiopia were also assessed and compared to that present across the species native range.

Individuals of *A. saligna* in enclosures in Ethiopia capture a range of native genetic diversity. At a broad level, the majority of individuals of *A. saligna* from Ethiopia were identified as the informal subsp. '*lindleyi*'. Some individuals were identified as the informal subsp. '*saligna*' and a number of individuals showed mixed co ancestry for two or more of the broad genetic entities. At a finer scale, individuals of *A. saligna* from Ethiopia were assigned predominantly to the Northern '*lindleyi*' entity and the informal subsp. '*lindleyi*' entity, although a few individuals were assigned to each of the other three lineages and a few individuals again showed a degree of mixed co ancestry. Principal coordinate analysis illustrated the degree of co ancestry among native genetic entities in the Ethiopian *A. saligna*. Genetic differentiation among enclosures in Ethiopia is low. The vast majority of diversity is partitioned within populations or enclosures, not among them. Diversity within enclosures is high with levels of heterozygosity typically exceeding that found in populations from across the native range. There is no evidence of inbreeding in 'populations' from Ethiopia.

Introduction

The aim of this work was to identify individuals of *A. saligna* present in Ethiopia in terms of ancestry with the genetic entities present across the native range of the species complex in WA and to assess the amount of genetic diversity captured in populations of *A. saligna* in Ethiopia.

Note on the taxonomy of *Acacia saligna*

There is no formal taxonomic description of the genetic entities present in the *A. saligna* species complex, therefore we refer to the entities as described in earlier studies, combining geographic descriptors with widely used yet informal 'subspecies' names (Millar *et al.* 2011). At a broad level, the *Acacia saligna* species complex is composed of three genetic entities; the informal subsp. '*stolonifera*'; the informal subsp. '*saligna*'; and the informal subsp. '*lindleyi*'. Further structure exists within the informal '*saligna*' and '*lindleyi*' subspecies so that, at a finer level, the species complex is comprised of the informal subsp. '*stolonifera*', the 'Western *saligna*' entity, the 'Eastern *saligna*' entity, the 'Northern *lindleyi*' entity and the remaining populations of the informal subsp. '*lindleyi*'.

Laboratory Work

On arrival samples were freeze dried overnight and stored on silica. Genomic DNA was extracted from 40 mg of lypholised phyllode material following Millar *et al*. (2008). Genotypes were obtained for all individuals using five diagnostic microsatellite loci as described by Millar and Byrne (2007). This set of loci was previously selected as being the most discriminating for differentiation of genetic structure in *A. saligna*.

Analysis

Assignment of Ethiopian individuals

A reference data set of genotypes for the five diagnostic microsatellite markers exists for 444 individuals sampled from 33 native populations of *A. saligna* (Millar *et al.* 2011). These populations cover the species native range in Western Australia. Previous analysis of this data set revealed the *A. saligna* species complex to be optimally resolved into three genetic entities at a broad scale, and five genetic entities at a finer scale. These entities show a high degree of genetic differentiation (average proportion of membership of populations to one of the five identified entities

Q > 0.82). This data set will be used to assign individuals of *A. saligna* sampled in Ethiopia to native genetic entities.

We followed the approach recommended by Pritchard, Wen *et. al.* (2000) for the STRUCTURE program and used individuals of known origin, i.e. the native populations from WA in the reference data set, as defined 'learning samples' to classify the additional individuals of unknown origin, i.e. the individuals sampled from Ethiopia.
A putative population of origin was given to each individual in order to organise the STRUCTURE output. STRUCTURE analysis was conducted using the USEPOPINFO and the 'update allele frequencies using only individuals with POPFLAG = 1' setting to enable clustering, with prior population information for individuals from the reference data set (POPFLAG parameter = 1), and assignment with no prior information for individuals from Ethiopia (POPFLAG = 0). For assignment of individuals without prior population information (Ethiopian individuals) an ancestory model with correlated allele frequencies but no admixture was allowed. The length of the burn-in period was set to 10,000, number of Markov chain repetitions was set to 100,000, and the number of clusters (*K*) was set to three for assignment at the broad scale, and to five for assignment at a finer scale. In this way, we used STRUCTURE to assign individuals from Ethiopia to clusters corresponding to the known genetic entities with a given proportion of membership (q_i). Individuals were considered as having a degree of common ancestry with each genetic entity for which the q_i value was greater than 0.10.

Genetic differentiation and partitioning of diversity

Multivariate principal coordinate analysis (PCA) was conducted using the full data set of 510 individuals with the reference data set partitioned either as three genetic entities, or for analysis at a finer scale as five genetic entities. Covariance genetic distance matrices were constructed by AMOVA (Excoffier *et al.* 1992) and ordinated in a multidimensional space by PCA using a standardised data set, then plotted using the Statistica software (StatSoft 2001). The hierarchical partitioning of genetic variation was also investigated for individuals from Ethiopia via AMOVA, conducted using a standardised data set and 999 permutations in the GenAlEx program (Peakall& Smouse 2006).

An overall mean measure of population genetic differentiation, Weir and Cockerham's θ (1984), was obtained for sample sites in Ethiopia, and a pairwise value was obtained for differentiation between sites in Ethiopia and five native genetic entities using the FSTAT program (Goudet 2001).

Genetic diversity

Genetic diversity parameters were estimated for each enclosure and the Tigray Research Station and compared to those for each of five known genetic entities from

within the native range. Genetic diversity parameters, including the mean percentage of polymorphic loci (*P*), mean number of alleles per locus (*A*), mean number of polymorphic alleles per locus (A_p), expected heterozygosity (H_e), observed heterozygosity (H_o) and Wrights inbreeding coefficient (F_{IS}), were calculated using GenAlEx.

Results

Assignment of Ethiopian individuals

At a broad level, STRUCTURE analysis assigned *A. saligna* individuals from Ethiopia largely to the informal subsp. '*lindleyi*' or to the informal subspecies '*saligna*' (Table 1). There were 52 or 78.78 % of individuals with the highest proportion of membership to the informal subsp. '*lindleyi*' and 13 or 19.69 % of individuals had the highest proportion of membership to the informal subsp. '*saligna*'. A single individual from the Tigray Research Centre had the highest proportion of membership to the informal subsp. '*stolonifera*'. The average proportion of membership (q_i) of each individual to each cluster is illustrated in Figure 1 and provided in Appendix A. While most individuals showed ancestry to one cluster, there were 18 individuals that showed a degree of co ancestry with $q_i > 0.10$ for more than one cluster.

Table 1 Average proportion of membership of 'populations' (*Q*) to each of three native genetic entities. Highest average proportion of membership values for each 'population' is in bold.

	Cluster		
Reference Population	1 '*stolonifera'*	2 '*saligna'*	3 '*lindleyi'*
'stolonifera'	**0.996**	0.003	0.001
'saligna'	0.010	**0.985**	0.005
'lindleyi'	0.002	0.006	**0.992**
Ethiopia	0.051	0.233	**0.716**

At a finer level, 50 or 75.75 % of *A. saligna* individuals from Ethiopia were assigned to the Northern '*lindleyi*' entity and 5 or 7.57 % of individuals, to the informal subsp. '*lindleyi*' (Figure 2). A degree of mixed ancestry with the two entities was evident for many of these individuals. A single individual from the Tigray Research Centre was assigned solely to the informal subsp. '*stolonifera*' and another single individual from enclosure three was assigned solely to the Western '*saligna*' entity. There were 21 individuals that showed a degree of mixed ancestry with a majority of proportion of membership with the Northern '*lindleyi*' entity or the informal subsp. '*lindleyi*', and also a proportion of membership ($q_i > 0.10$) with one or more of the other entities. Average proportions of membership values for individuals are provided in Appendix B and average proportion of membership for 'populations' are provided in Table 2.

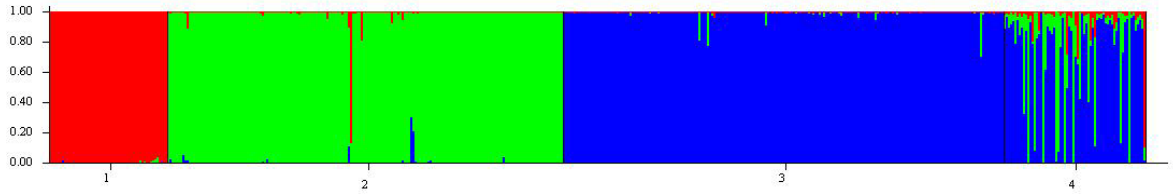

Figure 1 Bar plot representing the identity of sampled individuals of *Acacia saligna* via assignment using Bayesian modeling. Each individual is represented as a vertical line partitioned into coloured segments whose length is proportional to the individual coefficients of membership in the three clusters (q_i). Individuals from the reference data set of known native genetic entities in WA are represented by clusters 1 to 3 (cluster 1 (red) - subsp. '*stolonifera*', cluster 2 (green) - subsp. '*saligna*', cluster 3 (blue) - subsp. '*lindleyi*'). Individuals of *Acacia saligna* from Ethiopia are represented by cluster 4.

Table 2 Average proportion of membership of 'populations' (*Q*) to each of five native genetic entities. Highest average proportion of membership values for each 'population' is in bold.

	Cluster				
Reference Population	1 *'stolonifera'*	2 Western '*saligna'*	3 Eastern '*saligna'*	4 *'lindleyi'*	5 Northern '*lindleyi'*
'stolonifera'	**0.996**	0.002	0.001	0.001	0.001
Western *'saligna'*	0.010	**0.968**	0.015	0.004	0.004
Eastern '*saligna'*	0.000	0.009	**0.989**	0.001	0.001
'lindleyi'	0.001	0.003	0.002	**0.987**	0.007
Northern *'lindleyi'*	0.001	0.006	0.001	0.009	**0.982**
Ethiopia	0.058	0.055	0.093	0.260	**0.534**

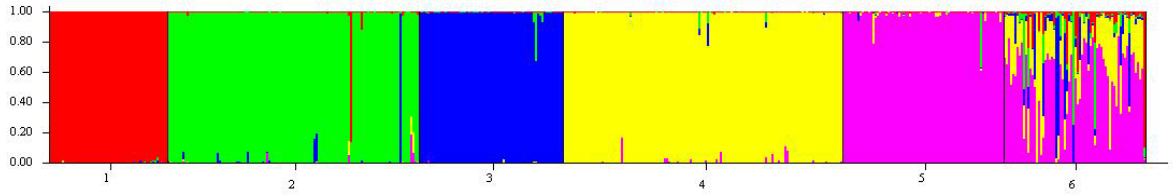

Figure 2 Bar plot representing the identity of sampled individuals of *Acacia saligna* via assignment using Bayesian modelling. Each individual is represented as a vertical line partitioned into coloured segments whose length is proportional to the individual coefficients of membership in the five clusters. Individuals from the reference data set of known native genetic entities in WA are represented by clusters 1 to 5 (cluster 1 (red) - subsp. '*stolonifera*', cluster 2 (green) - Western '*saligna*', cluster 3 (blue) - Eastern '*saligna*', cluster 4 (yellow) - subsp. '*lindleyi*', cluster 5 (pink) - Northern '*lindleyi*'). Individuals of *Acacia saligna* from Ethiopia are represented by cluster 6.

Genetic differentiation and partitioning of diversity

Principal coordinate analysis illustrates the degree of mixed ancestry present in *Acacia saligna* from Ethiopia in terms of the native genetic entities (Figure 3 and Figure 4). The first three axes explain 71.73% of the genetic variation present. For analysis at the broad scale, samples from Ethiopia appear to be ordinated largely in the centre of the three native entities (Figure 3).

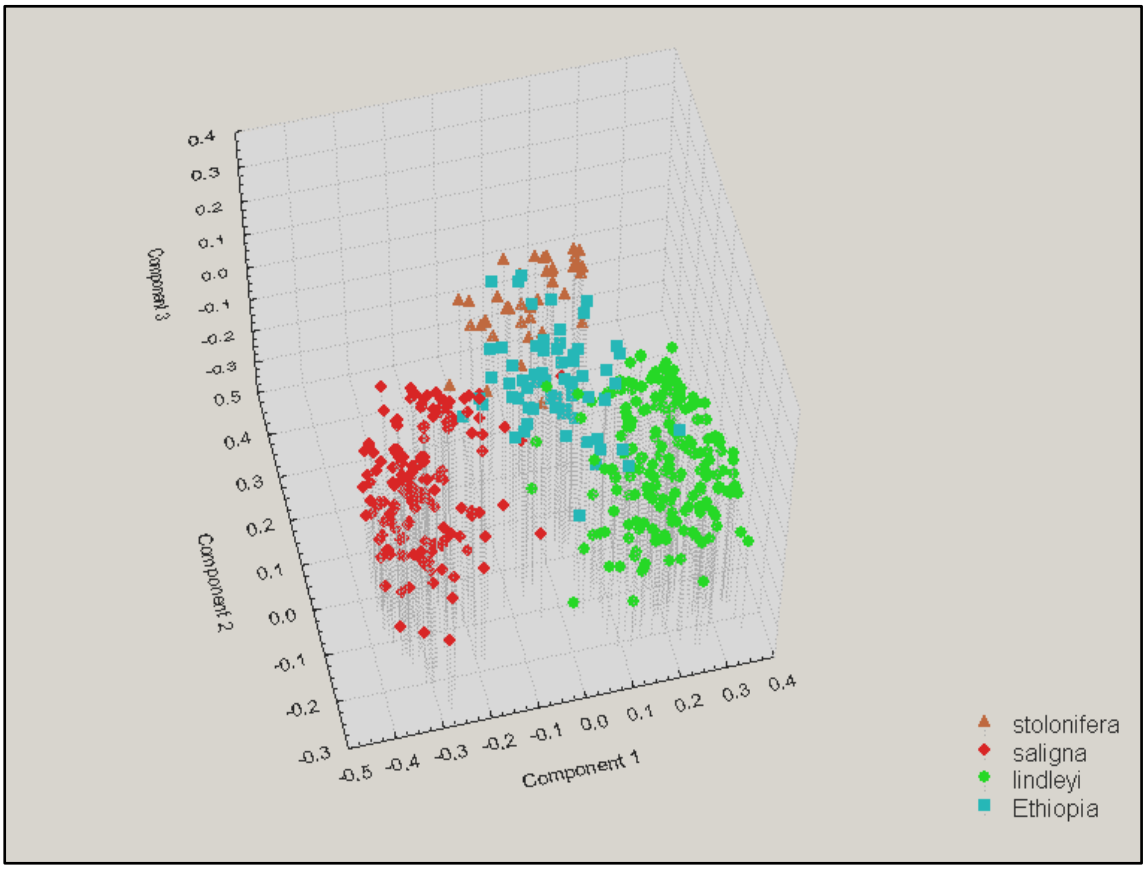

Figure 3 Principal coordinates analysis of *Acacia saligna* 'populations' from Ethiopia and populations of three genetic entities across the native range in WA.

At the finer scale, samples from Ethiopia are again ordinated largely in the centre of the three native entities, although their association with the Northern '*lindleyi*' entity is clearer (Figure 4).

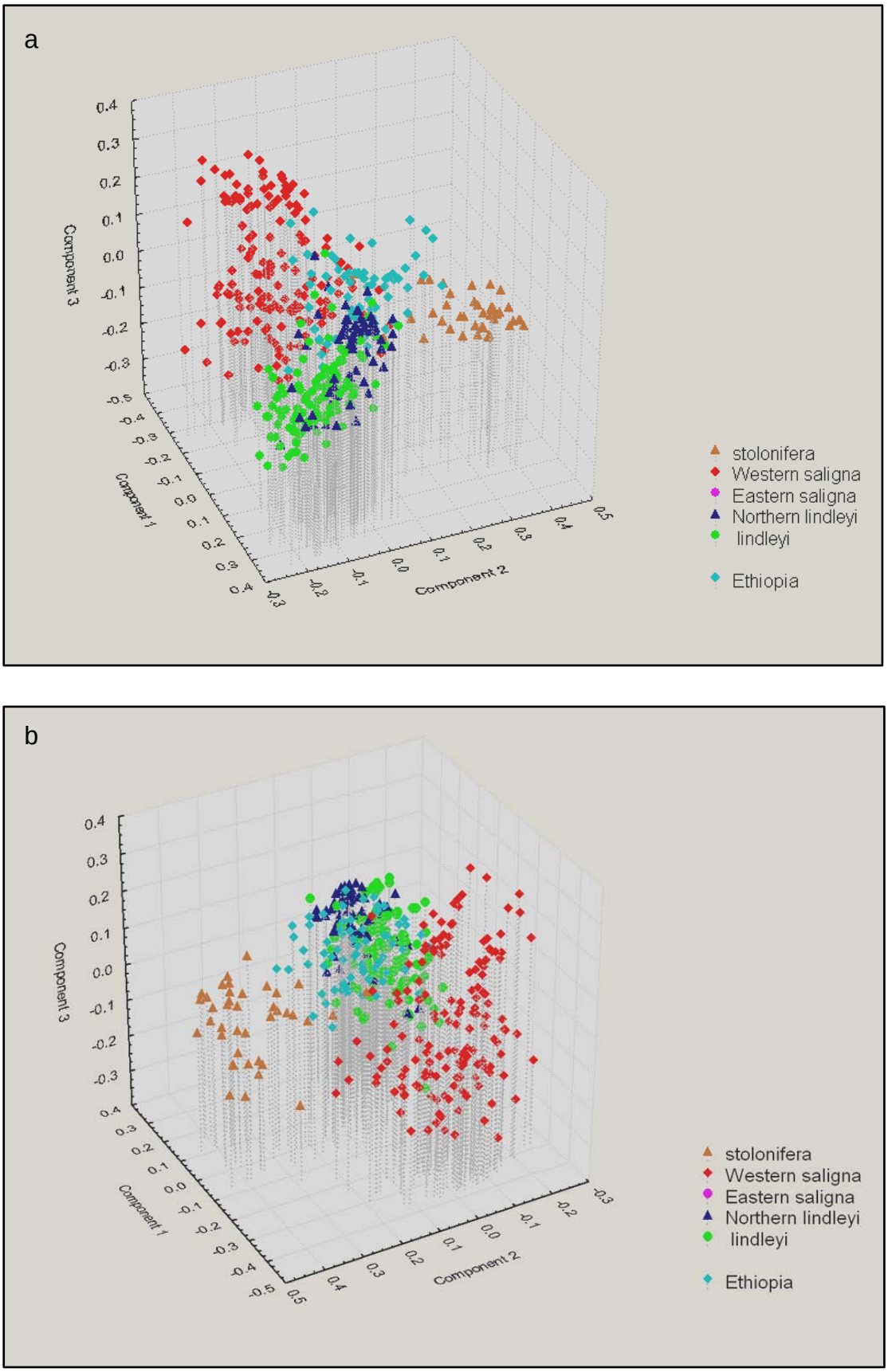

Figure 4 Principal coordinates analysis of *Acacia saligna* 'populations' from Ethiopia and populations of five genetic entities across the native range in WA. Panels (a) and (b) show differing views of the same analysis.

Genetic differentiation and partitioning of diversity

AMOVA analysis illustrated the high degree of genetic diversity captured within individuals of *A. saligna* within Ethiopian 'populations' or enclosures. Only 8% of diversity occurs among populations or enclosures and 92 % of variation occurs within enclosures.

Overall genetic differentiation among enclosures of *A. saligna* in Ethiopia was low, θ = 0.056 with a standard error of 0.012. Individuals within Tigray and enclosure one (E1) showed the most genetic similarity (lowest θ value) and individuals within enclosures two and three were the most divergent (greatest θ value, Table 3). However standard errors were high for all values and divergence among populations overall is low.

Table 3 Pairwise values of genetic differentiation (θ) among populations of *Acacia saligna* from Ethiopia.

	E1	E2	E3	E4	Tigray
E1	0.0000				
E2	0.0506	0.0000			
E3	0.0492	0.1036	0.0000		
E4	0.0188	0.0644	0.0683	0.0000	
Tigray	0.0181	0.0954	0.0816	0.0310	0.0000

When compared to known genetic entities in the species native range, populations of *A. saligna* from Ethiopia are least divergent from the informal subsp. '*lindleyi'* in particular the Northern '*lindleyi'* entity, in support of the STRUCTURE analysis, and most divergent from the Western and Eastern '*saligna'* entities (Table 4).

Table 4 Pairwise values of genetic differentiation (θ) among populations of *Acacia saligna* from Ethiopia and five genetic entities from the species native range in WA.

	'stolonifera'	Western '*saligna'*	Eastern '*saligna'*	*'lindleyi'*	Northern '*lindleyi'*	Ethiopia
'stolonifera'	0.0000					
Western *'saligna'*	0.3984	0.0000				
Eastern '*saligna'*	0.4700	0.1825	0.0000			
'lindleyi'	0.3079	0.3340	0.3688	0.0000		
Northern *'lindleyi'*	0.2804	0.3611	0.3708	0.0731	0.0000	
Ethiopia	0.2445	0.2602	0.2805	0.1713	0.1692	0.0000

All values are significant at p = 0.05 adjusted for multiple comparisons

Genetic diversity

A high level of genetic diversity is captured in *A. saligna* within enclosures in Ethiopia. All loci were polymorphic at all sites and the effective number of polymorphic alleles per locus was not significantly different to that of populations across the native range (Table 5). Enclosure 4 holds the greatest level of allelic diversity, although standard errors are high for all populations suggesting there is little variation in diversity among enclosures. The Ethiopian *A. saligna* showed high levels of expected and observed heterozygosity, with mean values significantly greater than those observed for 33 populations from across the species native range. The inbreeding coefficient was not significantly different from zero for Ethiopian *A. saligna* suggesting there is no evidence of inbreeding among individuals within enclosures.

Table 5 Population genetic parameters for *Acacia saligna* from Ethiopia and for known genetic entities in Western Australia. Site refers to the sample site in Ethiopia. Natural entity refers to known genetic entities in the native range in Western Australia as described by Millar, Byrne et al. (2008). *P*, mean proportion of polymorphic loci; *A*, mean number of alleles per locus; A_e, mean number of effective alleles; H_e, expected heterozygosity; H_o, observed heterozygosity; F_{IS}, Wrights inbreeding coefficient. Standard errors are provided in parentheses.

Site	*P*	*A*	A_e	H_e	H_o	F_{IS}
Enclosure 1	100	5.40 (0.510)	3.95 (0.406)	0.737 (0.025)	0.769 (0.045)	-0.129 (0.048)
Enclosure 2	100	5.80 (1.068)	3.65 (0.711)	0.670 (0.077)	0.704 (0.063)	-0.036 (0.097)
Enclosure 3	100	5.40 (1.030)	3.03 (0.522)	0.621 (0.076)	0.652 (0.079)	-0.133 (0.108)
Enclosure 4	100	7.20 (0.860)	4.20 (0.495)	0.746 (0.034)	0.766 (0.084)	-0.081 (0.144)
Tigray	100	5.40 (0.812)	3.56 (0.520)	0.702 (0.037)	0.743 (0.075)	0.102 (0.091)
Mean	100	5.84 (0.386)	3.68 (0.228)	0.695 (0.024)	0.691 (0.037)	-0.002 (0.047)
Natural entity						
Western '*saligna*'	100	7.80 (1.463)	2.27 (0.471)	0.480 (0.103)	0.373 (0.097)	0.200 (0.095)
subsp. *stolonifera*'	100	5.80 (1.393)	2.47 (0.786)	0.457 (0.119)	0.379 (0.379)	0.128 (0.092)
Eastern '*saligna*'	100	6.40 (1.568)	2.27 (0.4770	0.466 (0.123)	0.312 (0.112)	0.286 (0.141)
subsp. '*lindleyi*'	100	14.00 (3.962)	4.13 (1.193)	0.672 (0.081)	0.589 (0.081)	0.124 (0.042)
Northern '*lindleyi*'	100	16.40 (3.723)	7.36 (2.086)	0.729 (0.148)	0.651 (0.136)	0.112 (0.032)
Mean	100	10.80 (1.405)	3.70 (0.623)	0.561 (0.053)	0.461 (0.051)	0.170 (0.039)

Conclusion

Individuals of *A. saligna* within enclosures and located at the Tigray Research Centre in Ethiopia encompass a range of native genetic entities and a reasonably high degree of genetic diversity. The majority of individuals of *A. saligna* from Ethiopia have an origin in the informal subsp. '*lindleyi*', specifically the Northern '*lindleyi*' entity. However 31.81 % of individuals appear to have mixed ancestry, with membership to more than one genetic entity. This suggests material for initial

establishment was also of high diversity and comprised of a number of native genetic entities. A degree of interbreeding among native genetic entities appears to have occurred in Ethiopian *A. saligna*. The individuals with divergent genotypes or genetic origins are distributed among enclosures resulting in low levels of genetic differentiation among enclosures. A reasonable amount of allelic diversity is captured within enclosures, compared to that present in populations located across the species native range. Levels of diversity within enclosures indicate that seed collections made from within single enclosures may be suitable for establishment of further populations, although mixing seed collections from a greater number of enclosures will result in the maximum levels of diversity being captured. Levels of heterozygosity are very high in *A. saligna* from Ethiopia and there is no evidence of inbreeding within populations, suggesting selection against inbreeding may be taking place.

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Appendix A

Proportion of assignment of *Acacia saligna* from Ethiopia to each of three native genetic entities.

		Proportion of Assignment to Cluster		
Individual	% Missing Data	1 '*stolonifera'*	2 '*saligna'*	3 'lindleyi'
E1T01	0	0.020	0.092	0.888
E1T02	0	0.009	0.023	0.968
E1T02(2)	0	0.015	0.087	0.897
E1T03	0	0.007	0.074	0.919
E1T04	0	0.018	0.042	0.939
E1T05	0	0.001	0.207	0.792
E1T06	0	0.030	0.027	0.943
E1T07	0	0.009	0.143	0.848
E1T08	-20	0.029	0.075	0.896
E1T09	0	0.010	0.665	0.325
E1T010	0	0.017	0.111	0.872
E1T011	0	0.045	0.955	0.001
E2T01	0	0.024	0.043	0.933
E2T02	0	0.199	0.014	0.786
E2T03	0	0.001	0.918	0.081
E2T04	0	0.003	0.170	0.826
E2T05	-40	0.122	0.024	0.855
E2T06	-40	0.001	0.000	0.999
E2T07	-20	0.004	0.996	0.000
E2T08	0	0.021	0.363	0.616
E2T09	0	0.009	0.084	0.907
E2T010	0	0.011	0.111	0.878
E2T011	-20	0.015	0.129	0.856
E2T012	0	0.035	0.065	0.900
E3T01	0	0.000	0.990	0.010
E3T02	0	0.001	0.921	0.078
E3T03	-20	0.026	0.208	0.765
E3T04	0	0.008	0.031	0.961
E3T05	0	0.002	0.994	0.004
E3T06	0	0.283	0.222	0.495
E3T07	-20	0.025	0.074	0.901
E3T08	-20	0.038	0.084	0.878
E3T09	-20	0.000	0.998	0.002
E3T010	-20	0.087	0.206	0.707
E3T011	0	0.311	0.037	0.652
E3T012	0	0.001	0.574	0.425

E4T01	0	0.008	0.000	0.992
E4T02	-20	0.022	0.124	0.853
E4T03	0	0.018	0.056	0.926
E4T04	0	0.009	0.585	0.406
E4T05	-20	0.190	0.037	0.773
E4T06	0	0.039	0.063	0.895
E4T07	-20	0.171	0.715	0.114
E4T08	0	0.009	0.044	0.947
E4T09	0	0.021	0.019	0.957
E4T010	0	0.014	0.036	0.950
E4T011	0	0.005	0.014	0.980
E4T012	0	0.037	0.040	0.923
E4T013	-20	0.024	0.056	0.920
E4T014	0	0.041	0.016	0.942
E4T015	0	0.020	0.060	0.920
E4T016	-40	0.109	0.107	0.784
E4T017	0	0.067	0.058	0.874
E4T018	0	0.005	0.031	0.964
E4T019	0	0.001	0.867	0.132
E4T020	0	0.011	0.256	0.733
TARI01	-20	0.025	0.040	0.933
TARI02	0	0.022	0.026	0.952
TARI03	0	0.000	1.000	0.000
TARI04	0	0.017	0.021	0.963
TARI05	0	0.005	0.019	0.976
TARI06	0	0.054	0.072	0.869
TARI07	0	0.033	0.046	0.922
TARI08	-20	0.016	0.039	0.945
TARI09	0	0.046	0.065	0.890
TARI010	-20	0.897	0.082	0.020

Appendix B

Proportion of assignment of *Acacia saligna* from Ethiopia to each of five native genetic entities.

		Proportion of Assignment to Cluster				
Individual	% Missing Data	*'stolonifera'*	Western *'saligna'*	Eastern *'saligna'*	*'lindleyi'*	Northern *'lindleyi'*
E1T01	0	0.009	0.015	0.007	0.272	0.698
E1T02	0	0.008	0.005	0.008	0.327	0.651
E1T02(2)	0	0.006	0.007	0.012	0.153	0.822
E1T03	0	0.013	0.000	0.011	0.458	0.517
E1T04	0	0.005	0.004	0.014	0.389	0.588
E1T05	0	0.000	0.001	0.207	0.217	0.574

E1T06	0	0.016	0.003	0.009	0.207	0.765
E1T07	0	0.007	0.059	0.008	0.216	0.71
E1T08	-20	0.013	0.03	0.009	0.148	0.8
E1T09	0	0.012	0.499	0.107	0.223	0.159
E1T010	0	0.013	0.015	0.034	0.221	0.717
E1T011	0	0.242	0.257	0.14	0.292	0.069
E2T01	0	0.012	0.009	0.008	0.343	0.628
E2T02	0	0.237	0.005	0.004	0.174	0.58
E2T03	0	0.006	0.002	0.440	0.050	0.501
E2T04	0	0.002	0.002	0.003	0.988	0.005
E2T05	-40	0.127	0.004	0.000	0.458	0.411
E2T06	-40	0.001	0.000	0.000	0.944	0.055
E2T07	-20	0.144	0.193	0.354	0.138	0.171
E2T08	0	0.024	0.011	0.089	0.193	0.683
E2T09	0	0.008	0.007	0.022	0.239	0.725
E2T010	0	0.009	0.008	0.042	0.258	0.683
E2T011	-20	0.006	0.006	0.047	0.234	0.708
E2T012	0	0.091	0.022	0.007	0.194	0.686
E3T01	0	0.000	0.040	0.926	0.032	0.002
E3T02	0	0.001	0.022	0.794	0.158	0.025
E3T03	-20	0.013	0.148	0.026	0.160	0.653
E3T04	0	0.006	0.004	0.018	0.420	0.552
E3T05	0	0.049	0.092	0.568	0.203	0.087
E3T06	0	0.346	0.033	0.145	0.148	0.328
E3T07	-20	0.009	0.007	0.044	0.398	0.542
E3T08	-20	0.022	0.040	0.008	0.209	0.721
E3T09	-20	0.000	0.748	0.252	0.000	0.000
E3T010	-20	0.160	0.015	0.054	0.212	0.558
E3T011	0	0.399	0.004	0.006	0.199	0.393
E3T012	0	0.001	0.033	0.287	0.257	0.422
E4T01	0	0.010	0.000	0.000	0.275	0.715
E4T02	-20	0.009	0.057	0.011	0.100	0.823
E4T03	0	0.005	0.002	0.023	0.171	0.799
E4T04	0	0.008	0.040	0.298	0.046	0.609
E4T05	-20	0.141	0.033	0.001	0.053	0.772
E4T06	0	0.069	0.003	0.015	0.084	0.828
E4T07	-20	0.201	0.576	0.039	0.067	0.118
E4T08	0	0.008	0.011	0.014	0.206	0.761
E4T09	0	0.033	0.004	0.004	0.173	0.786
E4T010	0	0.007	0.008	0.013	0.206	0.766
E4T011	0	0.004	0.004	0.002	0.304	0.687
E4T012	0	0.023	0.011	0.008	0.32	0.638
E4T013	-20	0.016	0.010	0.013	0.385	0.575
E4T014	0	0.047	0.011	0.000	0.85	0.091
E4T015	0	0.009	0.014	0.006	0.429	0.543
E4T016	-40	0.080	0.057	0.029	0.32	0.513

E4T017	0	0.070	0.067	0.001	0.488	0.374
E4T018	0	0.005	0.004	0.012	0.137	0.843
E4T019	0	0.007	0.088	0.256	0.321	0.328
E4T020	0	0.009	0.010	0.125	0.132	0.725
TARI01	-20	0.006	0.002	0.010	0.283	0.699
TARI02	0	0.017	0.014	0.005	0.186	0.777
TARI03	0	0.045	0.096	0.481	0.042	0.336
TARI04	0	0.012	0.005	0.007	0.256	0.720
TARI05	0	0.004	0.003	0.008	0.252	0.733
TARI06	0	0.032	0.017	0.031	0.320	0.600
TARI07	0	0.022	0.012	0.014	0.283	0.669
TARI08	-20	0.014	0.021	0.009	0.399	0.556
TARI09	0	0.030	0.012	0.015	0.315	0.628
TARI010	-20	0.894	0.064	0.008	0.012	0.022