
***Acacia saligna* as a Sustainable Agroforestry Crop for southern Australia: A Genetic Assessment**



Melissa Ann Millar, BSc. Hons

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Statement of Originality

This work contains no material that has been accepted for the award of any other degree or diploma in any other University or other tertiary institution. This thesis does contain material that has been either published or submitted for publication in peer-reviewed scientific journals.

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Abstract

Acacia saligna is a native species complex with a widespread natural distribution throughout the south west of Western Australia. It is being developed as an agroforestry crop to produce low value, bulk biomass products in the low rainfall agricultural areas of southern Australia. This thesis develops knowledge to assist the domestication and breeding program of *A. saligna* as an agroforestry cultivar. It also furthers development of a risk management plan for utilisation of the *Acacia saligna* species complex.

Highly informative microsatellite markers for *A. saligna* were developed for use in mating system studies, paternity analysis and in the development of a diagnostic tool for the identification of individuals and populations at the subspecific level. Microsatellites developed in other *Acacia* species were also screened for utility in *A. saligna*. A high level of outcrossing (mean multilocus outcrossing rate of 0.98) and little true selfing was found for a planted stand of *A. saligna* subspecies *saligna*. Paternity analysis indicated heterogeneity in pollen clouds experienced by maternal trees and an essentially random pattern of mating within the stand. Inter-subspecific pollen immigration into the stand from trees of subspecies *lindleyi* was detected for 14% of progeny analysed and occurred over distances greater than 1500 m. Extensive intra-subspecific pollen-mediated gene flow is maintained between remnant natural populations of *A. saligna* subspecies *lindleyi*, and a high level of inter-subspecific pollen immigration from trees in the planted stand of *A. saligna* subspecies *saligna* was detected in remnant populations of subspecies *lindleyi* (32% of analysed progeny) occurring over distances greater than 1500 m.

Polymorphic microsatellite markers used to investigate genetic structuring within *A. saligna* revealed a high level of genetic divergence between subspecific entities congruent with a taxonomic reclassification of the species complex. Selected microsatellite markers also proved suitable for use as a rapid diagnostic tool that can be used to characterise populations into one of the proposed subspecies of *A. saligna* with high probability.

These results indicate that high levels of outcrossing and essentially random patterns of mating that maintain genetic diversity in seed crops should be achievable with the suitable management of seed production stands of *A. saligna*. Appropriate management techniques that limit genetic contamination into seed production stands will need to be employed to achieve this goal. Management techniques will also be required to minimise the risk of genetic contamination from stands planted for agroforestry purposes into remnant natural populations. Isolation distances greater than 1500 m between genetically divergent agroforestry crops and natural populations are suggested in both cases and key areas of further research are suggested.

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Table of Contents

STATEMENT OF ORIGINALITY	II
ABSTRACT.....	III
ACKNOWLEDGEMENTS	V
TABLE OF CONTENTS	VII
THESIS BY PUBLICATION	X
LIST OF FIGURES	XII
LIST OF TABLES.....	XIV
ACCOUNT OF RESEARCH PROGRESS	XV
CHAPTER ONE	1
GENERAL INTRODUCTION AND RESEARCH OBJECTIVES	1
INTRODUCTION	1
RESEARCH AIM.....	3
RESEARCH OBJECTIVES	3
CHAPTER TWO	5
LITERATURE REVIEW	5
VEGETATION CLEARANCE AND SALINISATION	5
<i>Vegetation Clearance</i>	5
<i>Salinisation</i>	6
<i>Extent of Salinisation</i>	7
<i>Impacts of Salinisation</i>	8
LAND USE CHANGE	8
<i>Addressing Salinity</i>	8
AGROFORESTRY.....	9
<i>Perennial Crops</i>	10
<i>Native Species</i>	11
<i>Species Evaluation</i>	11
ACACIA SALIGNA.....	11
<i>Species Description</i>	11
<i>Occurrence</i>	12
<i>Utilisation</i>	13
<i>Intersubspecific Variation and Distribution</i>	15
SPECIES DEVELOPMENT FOR SUSTAINABLE AGROFORESTRY	18
KNOWLEDGE GAPS	19
<i>Reproductive system</i>	19
<i>Patterns of pollen-mediated gene flow</i>	21
<i>Likelihood of genetic contamination</i>	22
<i>Impacts of pollen dispersal</i>	23
<i>Structuring of genetic diversity</i>	26
<i>Subspecific population identification</i>	27
<i>Methodology for Genetic Analysis</i>	28
ADDRESSING THE KNOWLEDGE GAPS IN <i>A. SALIGNA</i> : SPECIFIC OBJECTIVES.....	32
CHAPTER THREE	33
HIGH OUTCROSSING AND RANDOM POLLEN DISPERSAL IN A PLANTED STAND OF ACACIA SALIGNA SUBSP. SALIGNA REVEALED BY PATERNITY ANALYSIS USING MICROSATELLITES.	33
STATEMENT OF CONTRIBUTION	33
ABSTRACT	35
INTRODUCTION	36
MATERIALS AND METHODS	37

<i>Study species</i>	37
<i>Study site and sample collection</i>	38
<i>Microsatellite marker development</i>	39
<i>DNA extraction and genotyping</i>	41
<i>Data analysis</i>	41
RESULTS	44
<i>Microsatellite marker development and utility</i>	44
<i>Mating system</i>	45
<i>Paternity Assignment</i>	46
<i>Spatial Analysis</i>	46
<i>Genetic Contamination</i>	49
DISCUSSION	51
ACKNOWLEDGMENTS	53
CHAPTER FOUR	54
LONG DISTANCE INTER-SUBSPECIFIC GENE FLOW INTO REMNANT ACACIA SALIGNA (MIMOSACEAE) FROM A PLANTED STAND	54
STATEMENT OF CONTRIBUTION	54
ABSTRACT	56
INTRODUCTION	57
METHODS	60
<i>Study species</i>	60
<i>Study site and sample collection</i>	60
<i>DNA extraction and genotyping</i>	61
<i>Data analysis</i>	62
RESULTS	63
<i>Microsatellite marker utility</i>	63
<i>Population differentiation</i>	65
<i>Paternity assignment</i>	65
<i>Pollen-mediated gene flow</i>	68
DISCUSSION	69
<i>Pollen-mediated gene flow among remnant patches</i>	69
<i>Inter-subspecific hybridisation</i>	70
<i>Pollinator behaviour</i>	72
<i>Recommendations for management practices</i>	72
ACKNOWLEDGEMENTS	73
CHAPTER FIVE	74
CHARACTERISATION OF POLYMORPHIC MICROSATELLITE DNA MARKERS FOR ACACIA SALIGNA (LABILL.) H.L.WENDL. (MIMOSACEAE)	74
STATEMENT OF CONTRIBUTION	74
ABSTRACT	76
ACKNOWLEDGEMENTS	79
CHAPTER SIX	81
A RAPID PCR BASED DIAGNOSTIC TEST FOR THE IDENTIFICATION OF SUBSPECIES OF ACACIA SALIGNA	81
STATEMENT OF CONTRIBUTION	81
ABSTRACT	83
INTRODUCTION	84
MATERIALS AND METHODS	86
<i>Study species</i>	86
<i>Sample populations</i>	87
<i>DNA extraction and microsatellite genotyping</i>	90
<i>Determination of species structure</i>	91
<i>Assignment of Test Individuals</i>	92
<i>Population Genetic Parameters</i>	92
RESULTS	93
<i>Determination of species structure</i>	93

<i>Test Population Assignment</i>	97
<i>Population Genetic Parameters</i>	98
DISCUSSION	100
CONCLUSIONS	103
ACKNOWLEDGEMENTS	104
CHAPTER SEVEN	105
GENERAL DISCUSSION	105
PRINCIPAL SIGNIFICANCE OF THE FINDINGS	106
DOMESTICATION AND BREEDING	107
ENVIRONMENTALLY SUSTAINABLE MANAGEMENT	108
GENETIC RELATIONSHIPS AND SUBSPECIES IDENTIFICATION	109
RECOMMENDATIONS FOR FUTURE RESEARCH	112
<i>Assessment of management techniques for planted stands</i>	112
<i>Assessment of floral phenology</i>	113
<i>Assessment of hybridisation and introgression</i>	113
<i>Assessment of invasive populations</i>	114
CONCLUSION	115
BIBLIOGRAPHY	116

Thesis by Publication

This doctoral thesis is submitted as a portfolio of publications either published or submitted for publication by peer-reviewed journals according to the 'PhD Rules and Specifications for Thesis' of the University of Adelaide. The journals in which these papers were published are closely related to the research field of this work. The citation information is listed in the order of their appearance in this thesis. The thesis is based on the following papers,

Millar MA, Byrne M, Nuberg I, Sedgley M (2008) High outcrossing and random pollen dispersal in a planted stand of *Acacia saligna* subsp. *saligna* revealed by paternity analysis using microsatellites. *Tree Genetics and Genomes*. 4: 367-377.

Millar MA, Byrne M, Nuberg I, Sedgley M (2008) Long distance inter-subspecific gene flow detected by paternity analysis in remnant *Acacia saligna* (Mimosaceae). *Heredity*. In prep.

Millar MA, Byrne M (2007) Characterisation of polymorphic microsatellite DNA markers for *Acacia saligna* (Labill.) H.L.Wendl. (Mimosaceae). *Molecular Ecology Notes*. 7: 1372-1374.

Millar MA, Byrne M, Nuberg I, Sedgley M (2008) A rapid PCR based diagnostic test for the identification of subspecies of *Acacia saligna*, *Tree Genetics and Genomes*. doi 10.1007/s11295-008-0138-0.

The following additional publications are of relevance to the present work and were published over the course of the doctoral candidature:

Millar MA, Byrne M, (2004) Genetic systems and issues in the development of woody perennials for revegetation. In: Ridley, A, Feikema, P, Bennet, S, Rogers, MJ, Wilkinson, R, Hirth, J (Eds) *Proceedings of the Conference Salinity Solutions: Working with Science and Society*, Bendigo Victoria CRC for Plant-Based Management of Dryland Salinity, 2-5 August, 2004: 1-5.

Millar MA, Byrne M, Nuberg I, Sedgley M (2005) Risk assessment for *Acacia saligna* agroforestry. Advances in plant conservation biology symposium; Implications for flora management and restoration, Perth, Western Australia, 25 - 27 October, 2005.

Millar MA, Byrne M, (2007) Pollen contamination in *Acacia saligna*: assessing the risks for sustainable agroforestry. In: Sixth International Conference on Ecosystems and Sustainable Development Tiezzi E, Marques JC, Brebbia CA, Jorgensen SE (Eds), Portugal, Coimbra, 3-6 September, 2007, 101-110.

Sampson JF, Millar MA, Byrne M (2007) Pollen dispersal between planted populations and remnant native populations in a fragmented agricultural landscape. In: Rokich D, Wardell-Johnson G, Yates C., Stevens J, Dixon K, McLellan R, Moss, G (Eds), The International Mediterranean Ecosystems Conference Perth, Western Australia, Kings Park and Botanic Garden, 2-5 September, 2007, 221-222.

Byrne M, Stone L, Millar M.A. (2007) Environmental risk in agroforestry, In: Agroforestry for Natural Resource Management, Nuberg I, George B, Reid R (Eds), CSIRO Publishing, in press.

List of Figures

Figure 2.1 The extent of native vegetation clearance (in brown) in Australia as at 2001 (NLWRA 2001).	6
Figure 2.2 Areas of land forecast to be of high hazard or risk of dryland salinity (in green) in 2050 (NLWRA 2001).	8
Figure 2.3 The distribution of <i>Acacia saligna</i> in Western Australia. Image used with the permission of the W.A. Herbarium, Department of Environment and Conservation.	13
Figure 2.4 The naturalised distribution of <i>Acacia saligna</i> in Australia. Image used with the permission of Australia's Virtual Herbarium.	14
Figure 2.5 The four main subspecies of <i>Acacia saligna</i> (McDonald and Maslin, in prep; Photography MA Millar). Mature specimens of subspecies (A) <i>lindleyi</i> (B) <i>saligna</i> (C) <i>stolonifera</i> and (D) <i>pruinescens</i> .	16
Figure 2.6 The Western Australian distribution of four proposed subspecies of <i>Acacia saligna</i> (McDonald and Maslin, in prep). Image used with the permission of the W.A. Herbarium, Department of Environment and Conservation.	17
Figure 3.1 Map of the study site near Toodyay, Western Australia showing the planted stand comprised of 107 <i>A. saligna</i> subsp. <i>saligna</i> trees (open circles) and individual locations of sampled maternal trees (closed circles). Broken grey lines are roadways and the blue line is the Avon River.	39
Figure 3.2 Male mating success of the 64 trees in the planted stand of <i>Acacia saligna</i> subsp. <i>saligna</i> that sired progeny assayed from nine maternal trees.	47
Figure 3.3 Distribution of pollen dispersal distance classes in progeny assayed from nine maternal trees of a stand of <i>Acacia saligna</i> subsp. <i>saligna</i> . The line is the exponential curve fitted to the mating frequencies averaged over maternal trees.	48
Figure 3.4 Comparison of the potential (light bars) and observed (dark bars) pollinating male parent distribution as a function of distance to the female parent. The potential distribution comprised all 107 trees within the planted stand assuming random pollen dispersal.	49
Figure 4.1 Map of the study site near Toodyay, Western Australia showing locations of sampled trees of <i>Acacia saligna</i> . Green circles represent sampled trees of subsp. <i>lindleyi</i> ; open black circles represent sampled trees of subsp. <i>saligna</i> in the planted stand; broken grey lines represent roadways and the blue lines represent the Avon River.	62
Figure 4.2 Distribution of outcrossed pollination events for maternal trees of <i>Acacia saligna</i> subsp. <i>lindleyi</i> . The fitted geometric pollen-mediated gene flow curve is shown.	69

Figure 6.1 Geographic location of sampled populations of <i>Acacia saligna</i> . Population names refer to the populations in Table 6.1. ■ subspecies <i>saligna</i> , ▲ subspecies <i>stolonifera</i> , ◆ subspecies <i>pruinescens</i> , ● subspecies <i>lindleyi</i>	89
Figure 6.2 Bar plots representing the identity of individuals of <i>Acacia saligna</i> based on assignment using Bayesian modelling. Each individual is represented as a vertical line partitioned into K coloured segments whose length is proportional to the individual coefficients of membership in the K clusters that represent subspecies of <i>A. saligna</i> . a: reference populations using an admixture model with $\alpha = 0.032$ for $K = 4$, b: reference populations using prior population information and a model without admixture for $K = 4$, c: reference and test populations combined using a model without admixture for $K = 4$. Cluster 1 (red): subsp. <i>saligna</i> , cluster 2 (green): subsp. <i>stolonifera</i> , cluster 3 (blue): subsp. <i>pruinescens</i> , cluster 4 (yellow): subsp. <i>lindleyi</i>	94
Figure 6.3 Identification of optimal number of clusters in Bayesian assignment analysis of reference populations of <i>Acacia saligna</i> . a: Increase of $\ln P(D)$ against the number of K clusters obtained for $K = 1-8$ and b: $D. \Delta K$ computed after Evanno et al. (2005) against the number of clusters for $K = 1-8$ for populations in the reference data set.	95
Figure 6.4 Principal component analysis of genotypic data using five microsatellite loci for individuals in the reference populations of <i>Acacia saligna</i>	97

List of Tables

Table 3.1	Details of five microsatellite loci used to genotype individuals of <i>Acacia saligna</i>	41
Table 3.2	Utility of five microsatellite loci for <i>Acacia saligna</i> subsp. <i>saligna</i> at the study site.	44
Table 3.3	Details of outcrossing rate, pollen dispersal and gene flow estimates obtained via paternity analysis for nine maternal trees of <i>Acacia saligna</i> subsp. <i>saligna</i>	45
Table 3.4	Pollen allele frequencies (only two most common alleles are shown) and significance for G^2 tests of homogeneity between pollen clouds for nine maternal trees of <i>A. saligna</i> subsp. <i>saligna</i>	50
Table 4.1	Details of polymorphism and utility of five microsatellite loci for trees of <i>Acacia saligna</i> subsp. <i>lindleyi</i> and trees of <i>Acacia saligna</i> subsp. <i>saligna</i>	64
Table 4.2	Allele frequencies for sampled trees of <i>Acacia saligna</i> subsp. <i>lindleyi</i> and sampled trees of <i>Acacia saligna</i> subsp. <i>saligna</i> for five microsatellite loci. Private alleles are indicated in bold.....	66
Table 4.3	Pollen sources in remnant patches of <i>Acacia saligna</i> subsp. <i>lindleyi</i> . Gene flow events and distances are average values for maternal trees in each patch of <i>A. saligna</i> subsp. <i>lindleyi</i> , and for all maternal trees of subsp. <i>lindleyi</i> from all patches.	67
Table 5.1	Primer sequence, GenBank Accession No., Repeat units and magnesium chloride concentration for amplification of 10 microsatellite loci in <i>Acacia saligna</i>	78
Table 5.2	Diversity characteristics of ten microsatellite loci for 30 individuals (15 each) of <i>Acacia saligna</i> subsp. <i>saligna</i> (ms) and subsp. <i>lindleyi</i> (ms).....	80
Table 6.1	Details of sampled populations of <i>Acacia saligna</i> used as reference and test populations to demonstrate the use of the diagnostic genetic identification.....	90
Table 6.2	Values of average proportion of membership (Q) of pre-defined reference populations in four inferred clusters of <i>Acacia saligna</i>	96
Table 6.3	Values of average proportion of membership (Q) of test populations in four inferred clusters computed using STRUCTURE 2.1, under a model not allowing for admixture with five microsatellite loci.	98
Table 6.4	Population genetic parameters of five microsatellite loci used to genotype individuals of <i>Acacia saligna</i> reference populations.	99
Table 6.5.	Gene diversity and inbreeding coefficients for reference samples of subspecies of <i>Acacia saligna</i> . For comparison, overall mean values for RFLP markers are provided from George et al. (2006).	100

Account of Research Progress

This research has investigated a number of key areas to assist the domestication and breeding program and further the development of a risk management plan for utilisation of the *Acacia saligna* species complex as an agroforestry cultivar. The research is presented as a series of research papers written for submission to peer reviewed scientific journals. The progress of the research is described in the order of the chapters and papers presented in this thesis.

Chapter One

Chapter One is a general introduction to the thesis that introduces the context, study species and impetus for this research. The overall research aim is stated and the overall research objectives described.

Chapter Two

This chapter provides a background of the relevant literature underpinning this research. The need for the development of native woody perennial species as agroforestry crops is discussed in the context of increasing salinisation of the landscape and waterways of the agricultural areas of southern Australia. Relevant current knowledge regarding *Acacia saligna*, the priority species selected for further development as an agroforestry cultivar, is presented and areas where further knowledge of the species complex is required are identified and discussed. Suitable methodological approaches for investigating some of the key areas of research are introduced and the specific research objectives stated.

Chapter Three

This chapter presents a study of the mating system and patterns of intra-subspecific pollen-mediated gene flow within a planted stand of *A. saligna* subspecies *saligna*. The production of an initial unenriched microsatellite library and the development of two polymorphic microsatellite markers for *Acacia saligna*, and the screening of known microsatellite markers developed in other *Acacia* species is presented. Patterns of genetic contamination within the planted stand from inter-subspecific pollen immigration from natural remnant populations of subspecies *lindleyi* are assessed. The study is conducted using maximum likelihood paternity analysis of genotypic data at the five microsatellite loci.

Chapter Four

This chapter presents a study that investigates patterns of pollen-mediated gene flow. It investigates both intra-subspecific pollen-mediated gene flow among natural remnants of *A. saligna* subspecies *lindleyi* and patterns of inter-subspecific gene flow into natural remnants of subspecies *lindleyi* via pollen dispersal from a planted stand of subspecies *saligna*. The study is conducted using maximum likelihood paternity analysis of genotypic data obtained for the five microsatellite markers described in Chapter Three.

Chapter Five

This chapter details the production of a second microsatellite library using enrichment techniques, and the development of ten highly informative polymorphic microsatellite markers. A method incorporating several enrichment procedures was used to construct the microsatellite library because of the poor efficiency of primer development in the unenriched microsatellite library described in Chapter Three. The utility of the ten primers is described for two populations of *A. saligna*, one of subspecies *saligna* and one of subspecies *lindleyi*. These markers were screened for suitability of developing a diagnostic tool for the accurate identification of subspecies of *A. saligna*.

Chapter Six

Presents the development of a rapid and accurate diagnostic tool via Bayesian assignment analysis using genotypic data obtained for five of the diagnostic microsatellite markers described in Chapter Five. Its utility in characterising a number of cryptic populations of *A. saligna* at the subspecies level is presented.

Chapter Seven

This chapter is a general discussion of the how this work achieved the overall research objectives outlined, the main findings of the research and the principal significance of each of the main outcomes. A number of recommendations are made for the domestication and breeding program and for the sustainable management of *A. saligna* as an agroforestry cultivar. Key areas requiring further research for the development of *A. saligna* are identified and suitable approaches for further research are suggested.