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## Reproduction of threatened, long lived semi arid Acacia within highly fragmented stands in far western NSW

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# **Reproduction of threatened, long lived semi arid *Acacia* within highly fragmented stands in far western NSW.**

A thesis submitted in fulfilment of the requirements for the award of the  
degree

**DOCTOR OF PHILOSOPHY**

from the

**UNIVERSITY OF WOLLONGONG**

by

**Cairo N. Forrest B. Sc. (Hons)**

Department of Biological Sciences

2016



## **Certification**

I, Cairo N. Forrest, declare that this thesis, submitted in fulfilment of the requirements for the award of Doctor of Philosophy, in the School of Biological Sciences, University of Wollongong, is wholly my own work unless otherwise referenced or acknowledged. The document has not been submitted for qualifications at any other academic institution.

Cairo Forrest

22 March 2016



## **Abstract**

The contraction and aging of stands of overstory tree species in rangelands is well documented worldwide and largely reflects anthropogenic pressures such as agricultural land clearing and increased grazing leading to increased mortality and reduced recruitment. Without recruitment, stands that largely comprise old and senescent plants may soon go locally extinct. Complicating assessments of plant population health however, is the diversity of dynamics in populations of plant species; what constitutes reproductive failure and population contraction in one species can simply represent a natural cycle in another. In far western New South Wales (NSW), several *Acacia* species subject to intense grazing by domestic and feral herbivores display prolonged recruitment failure. Surveys over the past two decades have also failed to detect fruit sets suggesting they are trending to extinction. Hypotheses explaining the failure of these fragmented populations to reproduce sexually have included restrictions to mating systems, insufficient genetic diversity, prolonged drought period, and the widely supported claim that extant plants are senescent. In stark contrast, some shorter lived co-occurring *Acacia* species such as *A. ligulata* and *A. victoriae* are thriving and reproducing regularly under the same conditions. It is not understood why this difference exists. Reproductive effort has not yet been monitored outside a prolonged period of drought, demographic surveys to date have only been qualitative, and little is known about their mating systems. Without this information it is impossible to know which of the competing hypotheses explain their decline, or to recommend conservation strategies for the future. Here I use a multidisciplinary and comparative approach combining surveys, genetic analysis and manual pollination and growth experiments to gain this information.

Initially, I conducted the first formal surveys of the condition of populations of the threatened *Acacia* species (*A. melvillei*, *A. loderi*, and *A. carneorum*), one potentially threatened species (*A. homalophylla*) and the thriving co-occurring *A. ligulata*, across

approximately 430,000 km<sup>2</sup> of the semi arid region of western NSW. Specifically I estimated the age of plants within stands as well as their health via five demographic, three environmental and four plant health measures within 47, 26, 30, 10 and 20 separate stands of each species respectively. I took advantage of a rare *La Niña* rain event across the region to assess and quantify the reproductive (sexual) capacity of these stands under conditions where reproduction could not be limited by lack of water. I attempt to explain variation in seed set between stands as measured as 1) the presence of any mature fruit on plants and 2) the percentage of plant's canopies covered in mature fruit. I used microsatellite markers to look for differences in levels of genetic diversity as allelic and genotypic richness among stands of *A. loderi* and *A. carneorum* that did and did not set fruit in response to the *La Niña* rain event. The viability, fitness and capacity for these species to contribute to a long lived soil stored seed bank was assessed using 875 seed set after the *La Niña* rain event from 92 stands by performing controlled germination and growth experiments and sowing seed in the field. Region-wide surveys of seedling recruitment in 133 stands across 336,000 km<sup>2</sup> were also conducted, as well as long term growth and survival surveys of these seedlings in the field, to 1) assess the vigour of the seed currently being produced, 2) quantify the natural recruitment response within stands across the region, 3) assess the health and long term survival prospects of seedlings and 4) determine what local environmental conditions appear to be key drivers of recruitment success and failure. Finally I combined pollinator observations, pollen tube analysis, manual pollination experiments, and paternity analysis techniques, to attempt to characterize the state of current mating systems operating in several highly fragmented *A. carneorum* and *A. ligulata* stands within Kinchega National Park in western NSW, during this same period of high water availability.

I found that 100, 69, and 100% of *A. melvillei*, *A. loderi* and *A. carneorum* stands surveyed across the region respectively were populated predominantly by large mature plants

only with the majority of *A. loderi* and *A. carneorum* plants within these stands displaying reduced canopy cover indicative of senescence. In contrast, all *A. homalophylla* and *A. ligulata* stands were comprised of plants of a range of sizes with *A. homalophylla* stands displaying evidence of substantial recent recruitment through suckering and *A. ligulata* stands displaying a more even spread among morphological classes and a high proportion of putative recruits.

For the first time, I documented landscape wide sexual reproductive success in the form of generally high levels of fruiting / seed set in 83, 80, and 81% of *A. melvillei*, *A. homalophylla* and *A. loderi* stands surveyed respectively, in at least one of the two consecutive years following the *La Niña* rain. While every *A. ligulata* stand surveyed produced seed, only 13% of all *A. carneorum* stands surveyed in the region set seed and only in relatively small amounts. I also found that the seemingly oldest plants of all five *Acacia* species within these stands were equivalently fecund as plants of seemingly younger age. In contrast however, only four of the 30 *A. carneorum* stands were found to set any fruit, with fecundity levels being extremely low in comparison to the other four species.

Great intra-specific variance in demographic characteristics of stands of all five species was uncovered including stand size, density and proximity to other stands. However, all were likely to have populations greatly reduced by clearing combined with subsequent recruitment failure. 64% to 100% of *A. melvillei*, *A. homalophylla*, *A. loderi* and *A. carneorum* stands surveyed across the region now consist of fewer than 200 plants while 47, 89, 73, and 90% of these same stands are now isolated from the closest neighbouring stand by at least 4 km. Nevertheless, I found great similarities between stands of all four threatened species in the apparent condition of plants within them, the level of flowering effort and the amount of pollen deposited on stigmas by their pollinators. Furthermore, I found that differences in the five structural, three environmental, four plant health and two climatic variables, often associated with reproductive success or failure in others species, were not good indicators in

these species. For all four threatened species, the most fragmented stands with senescent plants were found to be comparably fecund as those in the largest, densest stands populated by apparently vigorous mature plants. The lack of genetic diversity found in all five of the 26 *A. loderi* stands surveyed that failed to set fruit (all 5 were monoclonal), despite most stands containing much genetic diversity however, suggests a genetic component to maternal sterility in these species.

I found high levels of seed viability in unparasitized seed ranging from 68% ( $SE\pm0.1$ ) to 77% ( $SE\pm0.0$ ) which was comparable with *A. ligulata* 46% ( $SE\pm0.1$ ). A substantial proportion of the seedlings grown from the *A. melvillei*, *A. homalophylla*, *A. loderi* and *A. carneorum* seed survived to two years of age under coastal conditions, ranging from 50% ( $SE\pm5.0$ ) to 54% ( $SE\pm5.1$ ), which was also comparable with *A. ligulata* (44 % ( $SE\pm1.8$ )). Highly variable recruitment was found within stands and even among plants within stands of *A. melvillei*, *A. homalophylla*, *A. loderi* and *A. ligulata*, ranging from as high as 369 seedlings per plant to zero seedlings. In contrast, no sexual recruitment was found in any of the *A. carneorum* stands surveyed irrespective of whether they were observed to set fruit or not. Understory vegetation was found to be important in protecting seedlings from grazing, with *A. melvillei*, *A. loderi* and *A. ligulata* seedlings located outside the canopy of nurse plants being grazed more often than seedlings located under a 'nurse plant'.

A diverse pollinator assemblage of 17 and 23 native insects was found successfully depositing viable pollen onto the majority of *A. carneorum* (37.3% ( $SE\pm0.3$ )) and *A. ligulata* flowers (55.1% ( $SE\pm0.1$ )) in all stands surveyed, irrespective of their reproductive histories. In contrast however, I found that while many of the same native insects visited both *A. carneorum* and *A. ligulata*, the pollination system of *A. ligulata* was now dominated by European honeybees (*Apis mellifera*), which tended to move pollen within plants and between local plants more so than the native pollinators. Paternity analysis of fruit collected from seed

produced in a fruiting *A. carneorum* stand revealed approximately one third of fruit in both stands to be selfed and the other two thirds to be outcrossed pollen from the only neighbouring stand 1 km away. This indicates that while these plants are self compatible, a mate choice mechanism preferentially selects for outcrossed pollen, given the largely localized movements the insect pollinators were found to be making. Naturally produced *A. ligulata* seed grew on average 18% and 26% slower than seed produced through manual outcrossing in the first and second years the experiment was run respectively, irrespective of the source of the outcrossed pollen applied.

My findings provide the first rigorous support for the claim that stands of these threatened species are at risk due to senescence and prolonged recruitment failure. Moreover, my findings suggest that while cyclical large scale rain events allow plants to initiate sexual reproduction in *A. melvillei*, *A. homalophylla* and *A. loderi*, persistent reproductive failure within all *A. carneorum* stands and a few *A. melvillei* and *A. loderi* stands, implies that for some, despite appearing equally healthy and receiving ample viable pollen, reproduction is connected to some other cryptic deficiency, or is limited by their natural reproductive strategy. For *A. carneorum* at least, this hypothesis is further supported by my own and previous carbon dating results which found that the clonal structure of stands likely pre-dates the worst effects of anthropogenic disturbance. For species that clearly rely on sexual reproduction to maintain populations and genetic diversity, degeneration of ground conditions suitable for sexual recruitment is likely to explain why current recruitment rates in many stands at least, are grossly inadequate. This combined with the unnaturally intense grazing regime in the region, means that long term mortality rates of these new recruits is likely to be unacceptably high.

Whilst *A. ligulata* seems to be recruiting far better than the threatened species, the finding that honeybees dominate pollination of many populations is cause for concern given

that they are almost certainly increasing the level of inbreeding, promoting inbreeding depression and reducing the adaptive capacity of these populations.

Taken together, my results suggest that conservation strategies to date, which have focused on excluding grazers in the most critically endangered stands, are likely to fall short of their goal. Active efforts will be needed in many stands to restock and recover dwindling numbers, and should also take into account the loss of genetic diversity that is expected even if some sexual recruitment is achieved. With climate change expected to make conditions in arid areas considerably harsher in the future, it has been strongly argued that local species already surviving on a physiological knife edge should be buffered from these predicted effects by maintaining, or even increasing, natural levels of genetic diversity to facilitate future adaptation.

## Acknowledgements

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This research and my scholarship was supported by an ARC linkage grant between the University of Wollongong and the NSW Department of Environment and Heritage. I also received support from competitive awards I attained from the Nature Conservancy of \$7000, administered via the Ecological Society of Australia (ESA) for expenses relating to this research and conference fees, and the Australian Institute of Nuclear Science and Engineering (AINSE) of \$30,000 towards use of the Carbon dating and ITRAX facilities at the Australian Nuclear Science and Technology Organisation (ANSTO). I would like to thank Quan Hua for assistance with this carbon dating work and Patricia Gadd for assistance with ITRAX scanning work undertaken at ANSTO.

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## **Background to thesis**

This thesis was part of a larger group effort supported by an ARC linkage grant which represents a collaboration between the University of Wollongong and the primary partner; the NSW Office of Environment and Heritage (formally the NSW Department of Environment and Climate Change) as well as The Lower Murray Darling and Murray Catchment Management Authorities, Sunraysia Nurseries, Darling Shire & Mildura City Councils, with in kind assistance from NSW National Parks and Wildlife Service and the Australian Inland Botanic Gardens, aimed at better managing and conserving these species in the region from local extinction. This work flows on from over two decades of study of these species facilitated by the Office of Environment and Heritage.

The overarching aim of the wider project as stipulated by the grant was to “evaluate the potential of genetic rescue to conserve critically endangered arid zone plants that have been judged unable to be saved by conventional management of remnant populations because fruit and seed production has diminished to extremely low levels and indeed zero fruit production in most populations”.

Before such genetic rescue attempts however, it was conceded that several critical questions required answering, namely:

- (i) Is there sufficient flowering to allow genetic rescue?
- (ii) Do populations lack critical genetic diversity either for neutral DNA markers or histocompatibility loci (determined by experimental pollinations)?
- (iii) Are populations currently failing to produce recruits?
- (iv) Do outcross pollinations from any source increase seed set?

So as to answer these questions and determine the suitability of a range of threatened and critically endangered plants, for both short and long term conservation efforts, the specific aims of this project were to:

- (1) Assess the current reproductive effort and output and levels and patterns of genetic variation present within such arid zone remnant populations.

(2) Measure the diversity of pollinators and frequency of pollinator visits within both small and large populations (where present).

(3) Infer plant mating systems from genetic comparison of mothers and offspring and experimentally determining levels of self-compatibility.

(4) Compare the fitness of offspring generated by experimental pollinations.

(5) Augment populations by facilitating the recruitment of the seedlings that performed best in glasshouse trials.

(6) Devise long-term management guidelines to take into account genetic diversity, effective population size, pollinator requirements and habitat requirements (Figure 1).

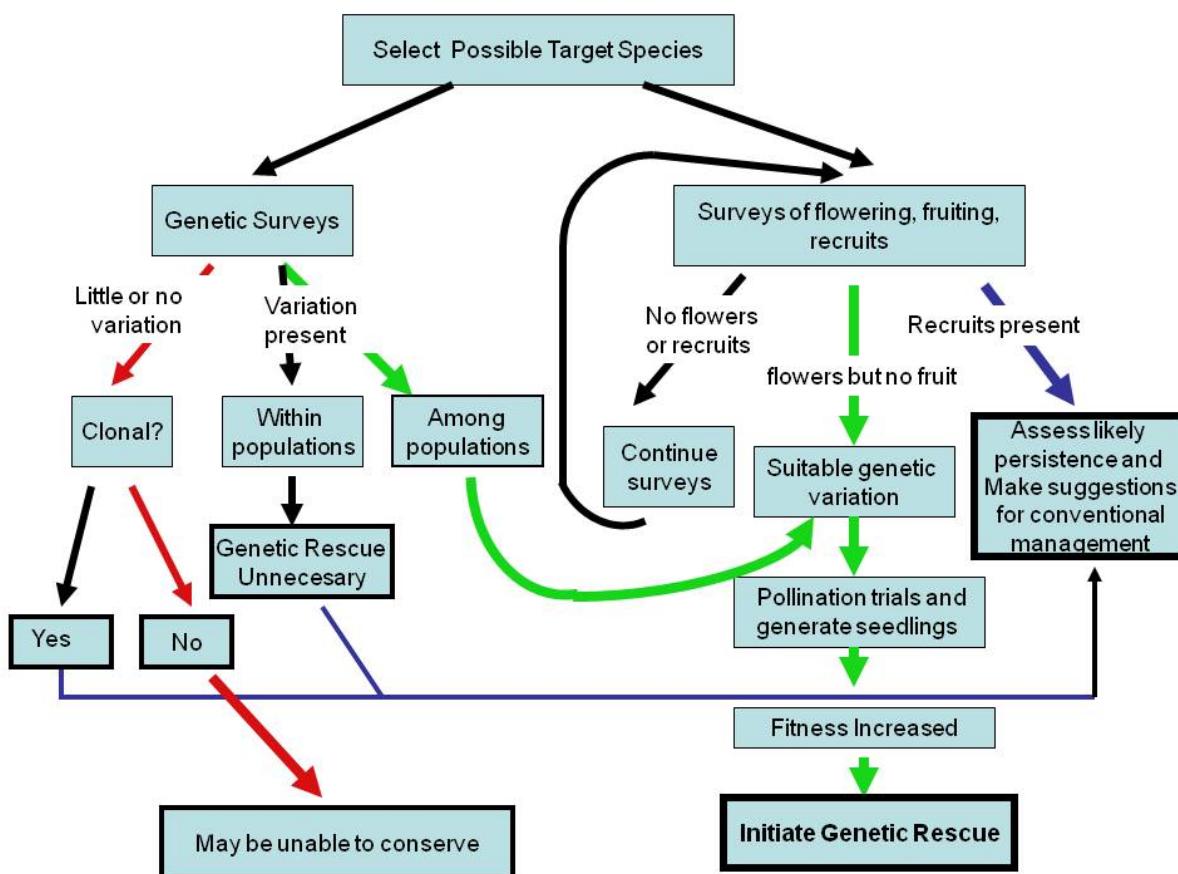


Figure 1. Decision tree indicating how our genetic and demographic survey results would be integrated with modelling and experimental pollinations to either initiate genetic rescue or recommend alternative conservation outcomes.

My PhD research was to a large degree guided and constrained by the aims of the grant and the expectation that key questions would be answered. The La Niña rain event of 2010 / 2011 was both a blessing and a curse as it provided a unique research opportunity, while destroying a year's worth of experiments which I was required to complete the following flowering season to meet the terms of the grant.

## Table of Contents

Certification .....	ii
Abstract .....	iii
Acknowledgements .....	ix
Background to Thesis .....	xi
Table of Contents .....	xiv
List of Tables .....	xxi
List of Figures .....	xxii
<b>Chapter 1: General Introduction .....</b>	<b>1</b>
1.1 Threats to plant populations world wide .....	1
1.1.1 Assessing threats to plant species and populations .....	2
1.2 Plant reproductive strategies and their importance to population health and persistence.....	6
1.2.1 Reproductive effort (fecundity and seed size).....	7
1.2.2 Modes of reproduction .....	7
1.2.3 Timing of reproduction /germination .....	9
1.3 Plant mating systems and their importance to plant population health and persistence.....	10
1.3.1 Inbreeding and outbreeding depression- a consequence of mating system disruption .....	12
1.3.2 Uncovering the mating system of plants .....	13
1.4 Reproduction and recruitment failure in plants .....	16
1.5 The threat of fragmentation .....	19
1.5.1 The effects of fragmentation .....	21
1.5.2 Assessing vulnerability to fragmentation .....	25
1.6 Threatened Acacia of semi arid NSW-A model system to investigate the effects of severe fragmentation on the functioning and persistence of long lived arid plants. ....	29
1.6.1 Competing hypothesis for the prolonged lack of sexual reproduction .....	31
1.6.2 Conservation considerations .....	33
1.6.3 La Niña rain event provides a golden opportunity .....	34
1.6.4 Thesis Questions.....	35
1.7 Study Species .....	36
1.7.1 <i>Acacia melvillei</i> and <i>Acacia homalophylla</i> .....	37

1.7.2 <i>Acacia loderi</i> .....	38
1.7.3 <i>Acacia carneorum</i> .....	38
1.7.4 <i>Acacia ligulata</i> .....	39
1.8 Thesis Structure.....	40
<b>Chapter 2: Assessing the demographics of threatened and thriving semi-arid <i>Acacia</i> in far west NSW.</b> .....	<b>43</b>
2.1 Abstract .....	43
2.2 Introduction .....	44
2.3 Methods .....	45
2.3.1 Study species and study area .....	45
2.3.2 Selection of stands .....	48
2.3.3 Sampling of plants within stands .....	49
2.3.4 Defining and assigning morphological “classes” of plants for each species ....	49
2.3.5 Stand morphology .....	51
2.4 Results .....	51
2.4.1 Defining and assigning morphological “classes” .....	52
2.4.2 Stand morphology .....	55
2.5 Discussion .....	63
2.5.1 Characterizing and comparing the demographic structure of stands .....	63
2.5.2 Predicting the fate of existing stands .....	65
2.6 Appendix .....	67
<b>Chapter 3: The importance of a rare, region-wide rain event for the reproduction of threatened semi arid <i>Acacia</i>.</b> .....	<b>69</b>
3.1 Abstract .....	69
3.2 Introduction .....	71
3.3 Methods .....	72
3.3.1 Study species, stands, plants and study area .....	72
3.3.2 Assessment of flowering effort among populations in response to the 2010 La Niña rainfall .....	73
3.3.3 Assessment of the sexual morphology of flowers and pollen tube growth ...	73
3.3.4 Assessment of sexual reproductive success in response to La Niña rain .....	74
3.3.5 Effect of plant age on reproductive capacity and reproductive effort (fruiting intensity) after rain event .....	78
3.3.6 Statistical analysis of data .....	78

3.4 Results .....	79
3.4.1 Assessment of flowering effort .....	79
3.4.2 Assessment of the sexual morphology of flowers and pollen tube growth ....	80
3.4.3 Assessment of sexual reproductive response to La Niña rain .....	83
3.4.4 Effect of plant age on reproductive capacity and reproductive effort (fruiting intensity) after rain event .....	90
3.5 Discussion .....	94
3.6 Appendix .....	101
<b>Chapter 4: Looking for drivers of reproductive success and failure in several threatened and one thriving semi arid <i>Acacia</i> species in far west NSW.</b> .....	105
4.1 Abstract .....	105
4.2 Introduction .....	106
4.3 Methods .....	108
4.3.1 Study species and study area .....	108
4.3.2 Selection of plants within stands .....	108
4.3.3. Assessing the condition (health) of stands across the region .....	108
4.3.4 Assessing the role of stand condition and climate on sexual reproductive success .....	112
4.3.5 Comparison of genetic diversity between stands with and without fruit .....	112
4.4 Results .....	113
4.4.1 Assessing the condition (health) of stands across the region .....	113
4.4.2 Assessing the role of stand condition and climate on sexual reproductive success .....	118
4.4.3 Comparison of the genetic make up of stands of <i>A. carneorum</i> and <i>A. loderi</i> with and without fruit set .....	119
4.5 Discussion .....	123
4.6 Appendix .....	129
<b>Chapter 5: Recruitment dynamics of long lived overstory <i>Acacia</i> in a degraded and heavily grazed arid landscape: effects of a rare La Niña rain event.</b> .....	139
5.1 Abstract .....	139
5.2 Introduction .....	141
5.3 Methods .....	144
5.3.1 Study species and study area .....	144
5.3.2 Assessing the viability and fitness of seed produced after the 2010-2011 La	

Niña rainfall event .....	145
5.3.3 Measuring seed health under lab conditions .....	145
5.3.4 Assessing the capacity for seed to recruit and contribute to a long lived soil stored seed bank .....	146
5.3.5 Quantifying the scale of recruitment following the 2010–2011 La Niña rainfall event .....	148
5.3.6 Assessment of the potential driving factors of initial recruitment success and failure within stands.....	151
5.3.7 Assessing the performance and survival of recruits .....	151
5.3.8 Driving factors for long term survival of recruits .....	152
5.3.9 Statistical analysis of data .....	153
5.4 Results .....	154
5.4.1 Assessment of the viability and fitness of seed produced after the 2010–2011 La Niña rainfall event .....	154
5.4.2. Assessing the capacity for seed to recruit and contribute to a long lived soil stored seed bank .....	158
5.4.3 Quantifying the scale of recruitment following the 2010–2011 La Niña rainfall event .....	160
5.4.4 Assessment of the potential driving factors of initial recruitment success and failure within stands .....	168
5.4.5 Assessing the long term performance and survival of recruits .....	171
5.4.6 Driving factors for long term survival of recruits .....	177
5.5 Discussion .....	184
5.5.1 Assessment of the quality of seed produced post a La Niña rain fall event ...	185
5.5.2 Survey of recruitment in the field following a La Niña rain fall event .....	187
5.5.3 Assessment of the driving factors for initial recruitment success and failure in stands .....	189
5.5.4 Assessing the long term survival of recruits .....	191
5.5.5 Driving factors for long term survival of recruits .....	192
5.5.6 Future persistence of semi arid Acacia in the region .....	194
5.6 Appendix .....	196
<b>Chapter 6: Is the threatened <i>A. carneorum</i> pollen or pollinator limited?</b> .....	209
6.1 Abstract .....	207
6.2 Introduction .....	208

6.3 Methods .....	209
6.3.1 Choice of stands and plants .....	209
6.3.2 Sampling method .....	209
6.3.3 Characterising the assemblage of flower visitors .....	210
6.3.4 Quantifying the abundance of potential pollinators in stands .....	210
6.3.5 Assessing the effectiveness of potential pollinators to carry and deposit pollen .....	210
6.3.6 Assessing foraging behaviour of potential pollinators .....	212
6.3.7 Statistical analysis of data .....	212
6.4 Results .....	213
6.4.1 Characterising the assemblage of flower visitors .....	213
6.4.2 Quantifying the abundance of potential pollinators in stands .....	215
6.4.3 Assessing the effectiveness of potential pollinators to transport and deposit pollen .....	218
6.4.4 Assessing foraging behaviour of potential pollinators .....	225
6.5 Discussion .....	232
6.5.1 Comparing and contrasting fruit setting and non fruit setting <i>A. carneorum</i> stands in and around Kinchega National Park .....	233
6.5.2 Pollination of thriving <i>A. ligulata</i> stands in Kinchega National Park .....	234
6.6 Appendix .....	237
<b>Chapter 7: Effect of pollen quality on the reproductive output and fitness of offspring of a threatened semi arid <i>Acacia</i> species (<i>A. carneorum</i>) and a thriving and co-occurring <i>Acacia</i> species (<i>A. ligulata</i>) in far western NSW .....</b>	<b>243</b>
7.1 Abstract .....	243
7.2 Introduction .....	245
7.3 Methods .....	246
7.3.1 Pollination treatments .....	246
7.3.2 Design of pollination experiments .....	246
7.3.3 Statistical analysis of data .....	257
7.4 Results .....	258
7.4.1 Assessing the effect of pollen source on reproductive success .....	258
7.5 Discussion .....	265
<b>Chapter 8: Genetic estimation of mating systems within rare fruiting populations of <i>A. carneorum</i> .....</b>	<b>269</b>

8.1 Abstract .....	269
8.2 Introduction .....	270
8.3 Methods .....	271
8.3.1 Selection of plants and stands .....	271
8.3.2 Experimental design / sample collection .....	271
8.3.3 Genetic analysis of leaf and seed .....	272
8.3.4 Assigning paternity to seed .....	272
8.3.5 Viability of rare <i>A. carneorum</i> seed .....	273
8.4 Results .....	273
8.4.1 Genetic analysis of leaf .....	273
8.4.2 Genetic analysis of seed .....	274
8.4.3 Assigning paternity to seed .....	275
8.4.4 Viability of <i>A. carneorum</i> seed .....	276
8.5 Discussion .....	276
8.6 Appendix .....	282
<b>Chapter 9: Carbon dating of recently deceased <i>A. carneorum</i> trees in Kinchega National Park.</b> .....	287
9.1 Abstract .....	287
9.2 Introduction .....	288
9.3 Methods .....	289
9.3.1 Sample collection .....	289
9.3.2 Estimating the age of plants (bomb-pulse $^{14}\text{C}$ dating) .....	291
9.3.3 Analysis of bomb pulse $^{14}\text{C}$ dating data .....	291
9.4 Results .....	291
9.5 Discussion .....	291
9.6 Appendix .....	294
<b>Chapter 10: General Discussion</b> .....	297
10.1 Overview .....	297
10.2 Are stands old and senescing across their whole range in western NSW? .....	297
10.3 Are stands highly fragmented and in poor condition, or is there important heterogeneity between them? .....	300
10.4 Are stands still capable of sexual reproduction and recruitment after a large scale rain event? .....	301
10.5 Is reproductive success and failure determined by the age or condition of	

stands? .....	304
10.6 Are mating systems of these species currently suboptimal or particularly susceptible to any increased levels of fragmentation? .....	309
10.7 What conservation strategies should managers adopt to conserve these stands? .....	312
10.8 Final conclusions about the effects of fragmentation on the persistence of long lived semi-arid <i>Acacia</i> existing within severely fragmented landscapes of far western NSW .....	317
References .....	321
Appendix to General Introduction / Discussion.....	395
Papers published from this project .....	428

## List of Tables

Table 2.1. Characteristics of the study species <i>A. melvillei</i> , <i>A. homalophylla</i> , <i>A. loderi</i> , <i>A. carneorum</i> and <i>A. ligulata</i> .....	47
Table 2.2. Characteristics of morphological classes assigned to <i>A. melvillei</i> , <i>A.</i> <i>homalophylla</i> , <i>A. loderi</i> , <i>A. carneorum</i> and <i>A. ligulata</i> plants in 133 stands across western NSW .....	54
Table 3.1: Assessment of reproductive effort, reproductive success and fecundity of <i>A. melvillei</i> , <i>A. homalophylla</i> , <i>A. loderi</i> , <i>A. carneorum</i> and <i>A. ligulata</i> in western NSW in response to a large scale La Niña rain event .....	76
Table 3.2: Statistical results for two way ANOVAs of flowering effort over two consecutive years .....	79
Table 3.3: Statistical results for two way ANOVAs of the percentage pollen tube initiation, pollen tube growth to ovules and the proportion of flowers with pollen tubes that reach ovules, over two consecutive years .....	83
Table 4.1. Measures of <i>Acacia</i> stand condition and plant condition .....	110
Table 4.2: Comparison of genetic diversity in fruit setting and non fruit setting <i>A.</i> <i>loderi</i> and <i>A. carneorum</i> stands .....	122
Table 6.1. Identification of potential pollinators of <i>A. carneorum</i> and <i>A. ligulata</i> plants within Kinchega National Park, NSW .....	214
Table 7.1. Details of experimental pollination of <i>A. carneorum</i> and <i>A. ligulata</i> plants in Kinchega National Park .....	249
Table 7.2. Treatment types used for manual pollination experiments of <i>A. ligulata</i> and <i>A. carneorum</i> plants on Kinchega National Park in far western NSW .....	254
Table 7.3. Measurements of reproductive effort and offspring fitness in stands of <i>A.</i> <i>ligulata</i> and <i>A. carneorum</i> . .....	257
Table 8.1. Observed and expected ratios of heterozygosity of 73 <i>A. carneorum</i> seed suspected to have been a product of selfing / local inbreeding, at four loci that were found to be heterozygous for the maternal genotype .....	275
Table 9.1. <i>A. carneorum</i> samples for carbon dating .....	290

## List of Figures

Figure 1.1. Arial picture of Australia's fragmented landscape.....	21
Figure 1.2. Senescing <i>A. melvillei</i> plants at Mungo National Park in far western NSW...	31
Figure 1.3. <i>A. carneorum</i> stands fenced to exclude grazers (namely rabbits and goats) at Kinchega National Park in western NSW.....	34
Figure 1.4. Australian Rainfall Deciles 1 December 2009 to 31 May 2011.....	35
Figure 2.1. Distribution of 47 <i>A. melvillei</i> , 10 <i>A. homalophylla</i> , 26 <i>A. loderi</i> , 30 <i>A. carneorum</i> and 20 <i>A. ligulata</i> stands surveyed across NSW.....	48
Figure 2.2. Example of a typical senescing <i>A. melvillei</i> plant within extant stands in Mungo National Park, western NSW that died during the period of study.....	50
Figure 2.3 a-e. Grouping of <i>Acacia</i> plants from each of five species into two to five morphological classes using nearest neighbour model cluster analysis (Primer 6)...	53
Figure 2.4. Typical shape of trees in each of the morphological classes that plants were assigned to.....	55
Figure 2.5 a-f. Defining five stand 'Types' .....	56
Figure 2.6. Structure of typical overstory <i>Acacia</i> stands across western NSW.....	57
Figure 2.7 a-e. Estimates of the age of <i>A. melvillei</i> , <i>A. homalophylla</i> , <i>A. loderi</i> , <i>A. carneorum</i> and <i>A. ligulata</i> plants spread throughout the semi-arid region of far western NSW.....	61
Figure 2.8 a-e. Estimation of the age structure of <i>A. melvillei</i> , <i>A. homalophylla</i> , <i>A. loderi</i> , <i>A. carneorum</i> and <i>A. ligulata</i> stands in the semi-arid region of far western NSW.....	62
Figure 3.1. Flowering effort of five semi arid <i>Acacia</i> species across western NSW.....	80
Figure 3.2. Presence of pollen tubes in stigmas of <i>A. melvillei</i> , <i>A. homalophylla</i> , <i>A. loderi</i> , <i>A. carneorum</i> and <i>A. ligulata</i> flowers.....	82
Figure 3.3. Fruit set on overstory <i>Acacia</i> in western NSW after a region wide La Niña driven rain event in 2011.....	84
Figure 3.4. Sexual reproductive success or failure of surveyed <i>Acacia</i> stands in western NSW.....	84
Figure 3.5. Reproductive capacity of <i>Acacia</i> stands.....	86
Figure 3.6. Variance in fecundity (fruiting intensity) across multiple <i>Acacia</i> stands.....	88
Figure 3.7. Average number of seed per <i>Acacia</i> fruit.....	89
Figure 3.8. Average (+SE) number of seed per <i>Acacia</i> plant.....	90

Figure 3.9. Effect of morphological class on the capacity of <i>Acacia</i> plants to set fruit.....	92
Figure 3.10. Effect of morphological structure on the fecundity of <i>Acacia</i> plants.....	93
Figure 4.1. Structure and condition of <i>A. melvillei</i> , <i>A. loderi</i> , <i>A. carneorum</i> and <i>A. homalophylla</i> stands.....	116
Figure 4.2. Condition of <i>A. melvillei</i> , <i>A. loderi</i> , <i>A. carneorum</i> and <i>A. homalophylla</i> plants	117
Figure 5.1. Design of seed sowing and seed burial experiments.....	148
Figure 5.2. Subset of five stands each of <i>A. melvillei</i> , <i>A. loderi</i> and <i>A. ligulata</i> used to determine the contribution of seedlings from the canopy seed and the soil stored seed bank after a La Niña rain event and for monitoring their long term persistence and condition.....	150
Figure 5.3. Viability and performance of seed tested under lab and coastal conditions...	157
Figure 5.4. Recruitment rates of manually sown <i>A. melvillei</i> and <i>A. loderi</i> seed in Kinchega National Park during the time of natural recruitment.....	158
Figure 5.5. Assessments of the dormancy characteristics of <i>A. melvillei</i> and <i>A. loderi</i> seeds after burial at three sites (s1, s2, s3) within Kinchega National Park.....	159
Figure 5.6. Seedling recruitment around overstory <i>Acacia</i> in western NSW after a region wide La Niña driven rain event in 2011.....	161
Figure 5.7. Sexual recruitment success or failure of surveyed <i>Acacia</i> stands in western NSW.....	162
Figure 5.8. Surveys of sexual recruitment from 20 to 47 stands of <i>A. melvillei</i> , <i>A. homalophylla</i> , <i>A. loderi</i> and <i>A. ligulata</i> spread across western NSW utilizing timed searches of 10 mins each of ten mature plants within each stand.....	162
Figure 5.9. Surveys of sexual recruitment under 30 <i>A. melvillei</i> , <i>A. loderi</i> and <i>A. ligulata</i> plants spread across five stands for each species to determine actual numbers of recruits.....	164
Figure 5.10. Estimates of the average ratio of seed produced to seedlings recruited on ten plants in each of 5 <i>A. melvillei</i> , <i>A. loderi</i> and <i>A. ligulata</i> stands respectively.....	167
Figure 5.11. Comparison of local structural and climatic conditions between <i>Acacia</i> stands that recruited on average 0, 1-5, 6-20, 21-100 and <100 seedlings per plant...	169
Figure 5.12. Assessments of the long term performance and survival of naturally occurring sexual recruits under 30 <i>A. melvillei</i> , <i>A. loderi</i> and <i>A. ligulata</i> plants spread across five separate stands.....	173
Figure 5.13. Average height of seedling recruits located under 30 <i>A. melvillei</i> , <i>A. loderi</i> and <i>A. ligulata</i> plants spread across five separate stands.....	176

Figure 5.14. Assessments of the long term performance and survival of naturally occurring sexual recruits under thirty <i>A. melvillei</i> , <i>A. loderi</i> and <i>A. ligulata</i> plants spread across five separate stands with respect to their proximity to the canopy of parental plants.....	178
Figure 5.15. Quantifying the growth rates of naturally occurring sexual recruits under thirty <i>A. melvillei</i> , <i>A. loderi</i> and <i>A. ligulata</i> plants spread across five separate stands with respect to their proximity to the canopy of parental plants.....	179
Figure 5.16. Assessments of the long term performance and survival of naturally occurring sexual recruits under thirty <i>A. melvillei</i> , <i>A. loderi</i> and <i>A. ligulata</i> plants spread across five separate stands positioned in relation to understory vegetation..	181
Figure 5.17. Assessments of the height of naturally occurring sexual recruits under thirty <i>A. melvillei</i> , <i>A. loderi</i> and <i>A. ligulata</i> plants spread across five separate stands positioned in relation to understory vegetation.....	183
Figure 6.1. Examples of flower visitors of <i>A. carneorum</i> and / or <i>A. ligulata</i> flowers.....	214
Figure 6.2. Abundance of potential pollinators visiting flowers on <i>A. carneorum</i> and <i>A. ligulata</i> in and around Kinchega National Park in western NSW.....	217
Figure 6.3. Effectiveness of potential <i>A. carneorum</i> and <i>A. ligulata</i> pollinators at transporting pollen in and around Kinchega National Park in western NSW.....	220
Figure 6.4. Effectiveness of the <i>A. carneorum</i> and <i>A. ligulata</i> pollinator assemblages at transporting pollen in and around Kinchega National Park in western NSW.....	222
Figure 6.5. Examples of <i>A. carneorum</i> flowers with and without pollen tubes stained with Bromothymol blue and viewed with florescence microscopy with and without pollen tubes growing.....	224
Figure 6.6: Proportion of <i>A. carneorum</i> and <i>A. ligulata</i> flowers with pollen tubes initiating.....	224
Figure 6.7. Average time (mins) insects spent foraging between flowers within branchlets of <i>A. carneorum</i> and <i>A. ligulata</i> before moving away in and around Kinchega National Park in western NSW.....	226
Figure 6.8. Average proportion of confirmed movements potential pollinators make between branchlets of the same on <i>A. carneorum</i> and <i>A. ligulata</i> plant after foraging, in and around Kinchega National Park, in western NSW.....	228
Figure 6.9. Overall foraging style of the <i>A. carneorum</i> and <i>A. ligulata</i> pollinator assemblages in and around Kinchega National Park in western NSW.....	231
Figure 7.1. Location of <i>A. carneorum</i> and <i>A. ligulata</i> stands used as recipient and donor	

stands for manual pollination experiments conducted over two consecutive years in and around Kinchega National Park in far western NSW.....	247
Figure 7.2. Manual pollination of an <i>A. ligulata</i> plant at Kinchega National Park in western NSW.....	256
Figure 7.3. Effect of several pollen addition treatments and open pollination on pollen tube growth in multiple stands of <i>A. carneorum</i> and <i>A. ligulata</i> within Kinchega National Park.....	258
Figure 7.4. Effect of several pollen addition treatments and open pollination on pollen tube growth to the ovules of flowers, in multiple stands of <i>A. carneorum</i> and <i>A. ligulata</i> within Kinchega National Park.....	260
Figure 7.5. The effect of pollen quality on fruit set on <i>A. carneorum</i> and <i>A. ligulata</i> plants within two recipient stands in Kinchega National Park over two consecutive years.....	261
Figure 7.6. The effect of pollen source on the number of seed produced per fruit on <i>A. ligulata</i> plants within two recipient stands in Kinchega National Park over two consecutive years.....	262
Figure 7.7. The effect of pollen source on the average weight of fruit and seed produced on <i>A. ligulata</i> plants within two recipient stands in Kinchega National Park over two consecutive years.....	262
Figure 7.8. The effect of pollen source on the viability of seed produced on <i>A. ligulata</i> plants within two recipient stands in Kinchega National Park over two consecutive years.....	263
.	
Figure 7.9. The effect of pollen source on the growth rates of seedlings produced by <i>A. ligulata</i> plants within two recipient stands in Kinchega National Park over two consecutive years.....	264
Figure 7.10. The effect of pollen source on the survival rates of seedlings produced by <i>A. ligulata</i> plants within two recipient stands in Kinchega National Park over two consecutive years.....	265
Figure 8.1. <i>A. carneorum</i> seed on plants in the Middle Camp stand.....	272
Figure 8.2. Tests of the fitness of <i>A. carneorum</i> seed produced through outcrossing and selfing on 10 plants from within the Mallee stand.....	276
Figure 9.1 National Parks ranger cutting down recently deceased <i>A. carneorum</i> trees in Kinchega National Park to attain material to carbon date their age.....	289

Figure 10.1. Attempts to propagate <i>A. melvillei</i> , <i>A. loderi</i> and <i>A. ligulata</i> plants from cuttings at Mt Annan Botanic Gardens.....	315
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## Chapter 1: General Introduction

### 1.1 Threats to plant populations world wide

The International Union for Conservation of Nature (IUCN)'s assessment of the world's biodiversity in 2004 calculated that the rate of species extinction had reached 100 to 1,000 times the rate suggested by fossil records before humans, and could reach 10,000 times this rate in two decades. This equates to dozens of species going extinct every day with predictions of as many as 30 to 50% of all species possibly heading toward extinction by 2050. With the expansion of urban and agricultural areas, habitats have been lost for many species. Where species have remained, many now exist as a reduced patchwork of disconnected populations within a fragmented landscape placing further pressure on their health (Diamond, 1989; Pimm & Raven, 2000). Altered fire and grazing patterns, changed drought and flood patterns, and the introduction of weeds, feral animals and diseases have also affected the survival of many plant species. Moreover, habitat destruction leads to an "extinction debt," whereby plants that appear dominant will disappear overtime because they aren't able to disperse to new habitat patches (Hanski & Ovaskainen, 2002; Jackson & Sax, 2009; Kuussaari et al., 2009 and Tilman et al., 1994). Global warming is likely to substantially exacerbate this problem by causing rapid and dramatic changes in the range and distribution of plants around the world (IPCC 2013).

Many plant species are ecosystem engineers providing the backbone for entire ecological communities, whilst others provide critical ecosystem services from stabilization of soils and the creation of microclimates and habitats to providing food and shelter to animals (Tilman, 1988; Schlesinger et al., 1990; Fore et al., 1997). Loss of these species can be disastrous and lead to extinction cascades and loss of ecosystem function that will directly affect human well-being.

The persistence of plant populations relies on the health of individual plants for reproduction, as well as the health of the environment for the long term survival of recruits. Given the many anthropogenic pressures that plant populations face and their diminished habitat across much of their range, the consequences of natural threats are likely to be amplified, increasing local extinction risks to degrees well beyond those likely to have ever occurred prior to anthropogenic disturbance (Hoareau & Arico, 2010; Spierenburg, 2012; Danielsen et al., 2014; *Díaz* et al., 2015). Given this reality, the science of measuring the health of plant populations and predicting their future prospects is crucial and requires an understanding of the many ways in which plants can be affected by natural and anthropogenic

pressures, as well as an understanding of species tolerance to pressures on their physiology and mating systems. Armed with this information, managers can be in a much better position to tailor conservation strategies to be as effective and efficient as possible.

While there are a great many threats that can befall plant populations that place them at a higher risk of extinction, the severity of the threat will depend ultimately on the type of threat, the speed at which it is established, the duration of the threat, and a species' resilience to it. Threats can broadly be split into stochastic naturally induced threats such as disease, herbivory, fire and flood, and anthropogenically induced threats such as the removal of trees for agriculture and fire wood, the poisoning of plants by herbicides and the consumption of plants by introduced grazers. Whilst most natural disturbances are short lived and species have evolved to survive them, anthropogenic disturbances such as those that permanently reduce the size / quality of populations can have lasting and compounding consequences that lead to local extinctions (Lande, 1993; MacNally et al., 1997; Young & Clarke, 2000; Cushman, 2006). Young and even established plants that remain in anthropogenically altered populations can suffer the consequences of harshened local conditions directly and perish (Kapos, 1989; Jules & Rathcke, 1999; Meiners & Pickett, 1999). Even if plants persist, their reproductive health and capacity can be permanently impaired by either physiological stress or else as a result of significant changes to their mating systems associated with their reduced population size or harshened surroundings (Allee, 1931; Allee and Rosenthal, 1949; Allee, 1951; Crow & Kimura, 1970; Aguilar et al., 2006; Collinge, 2009).

### **1.1.1 Assessing threats to plant species and populations**

The health of plant species as a whole and their future prospects have been traditionally measured by assessing the distribution of populations, the size of populations and the stability of their size over time. Restricted species ranges, small population sizes and even reproduction / recruitment failure leading to contracting population size can, however, be poor predictors of population health for some species. Indicators of poor health in one species may simply represent natural variation or fluctuation in another species. Indeed, it is not unusual for there to be a great natural range in the size of populations given differences in geographic and environmental conditions across a range (Hartley et al., 2004). While this can be a consequence of differences in the range of local topographic features that support populations, it can also be the result of differences in the age of stands with smaller stands perhaps representing recent founder events through long distance seed dispersal, rather than

something more sinister. Some species that populate rarer topographical features in the landscape can also be restricted by the size of these features and display smaller population sizes. Judgements about the health of populations in such species can therefore be difficult if we compare to stands in other areas.

The observation of drastic reductions within plant populations at any given point in time, may or may not signal a reduction in population health, depending on the species and environment it exists within. For species that survive in harsh environments such as arid areas, populations can contract naturally during drier (drought) periods and halt reproduction in times of unfavourable conditions, before expanding again when water returns (Büsgen & Münch, 1929; Davies, 1976; Norton & Kelly, 1988; Fenner, 1998; Letnic & Dickman, 2006; Wardle et al., 2013). Other species may experience periodic attacks by grazers such as a periodic swarming by locust plagues, which over time they have adapted to cope, but which leaves populations temporarily diminished. These species may use tactics such as depositing large numbers of seed into a soil stored seed bank prior to the attacks to rejuvenate the population after its decimation (Honnay et al., 2008). In such cases, population size, reproductive output, or even the observation of severe contractions of populations could be poor predictors of population health, and serve to underestimate their health, or overlook other real threats.

Judgements about the health of populations of long lived plants based on levels of reproduction in any given year, or even over a number of consecutive years, can be misleading. Complicating matters further, for plants that can reproduce both sexually and asexually, a naturally low level of reproduction via either mode of reproduction, or a total lack of one form, can be incorrectly interpreted as reproductive failure. It is not uncommon that seed set can be absent or low in certain years within populations of species that are capable of using asexual reproduction to persist (Auld, 1993). Any study assessing the importance of sexual and asexual reproduction in the reproductive strategy of a species capable of both, is often complicated by the temporal variance of this importance, with assessments during a period of low resource availability for example, resulting in gross underestimates. Such errors can be especially likely when the mode of reproduction employed by plants differs even between populations of the same species at the same point in time, as a function of different environmental conditions across a range, or a lack of connection between stands (Douglas, 1981; Sartor et al., 2011; Hardion et al., 2015). Indeed, differences in reproductive capacity between stands can be plastic, but these differences may be fixed, such as when the sexes are separated between different stands of plants (Bierzychudek & Eckhart, 1988). In the later case,

if the focal stand happens to have only male flowers, the inability to set fruit may wrongly appear to be reproductive failure. Moreover, the presence of sexual reproduction in some species may not reflect a stable mating strategy but a remnant of an evolutionary shift from sexual to asexual reproduction if a formerly dynamic environment shifts towards a climatically / environmentally more stable one (Kearney, 2003; Honnay & Jacquemyn, 2008; Vallejo-Marín et al., 2010). This has been the case in many arid zones where sexual reproduction can be reduced or lost in favour of a reliance on asexual reproduction in order to maintain successful local adaptations (Kearney, 2003; Honnay & Jacquemyn, 2008; Vallejo-Marín et al., 2010).

The health of populations and their likelihood of persisting long term can be underestimated even if reproduction and recruitment levels are optimal. Offspring can be produced in large numbers but still suffer fitness consequences resulting from altered levels of gene flow, synonymous with fragmented populations of many species (e.g., inbreeding depression) (Kolreuter, 1761; Darwin, 1868, 1876; East and Jones, 1919). Complicating assessments further, both inbreeding depression and outbreeding depression, may only become evident when conditions harshen, or in later generations (Barrett & Kohn 1991; Widen, 1992; Mitton 1993; Oostermeijer et al., 1994). Even if a loss of genetic diversity is not accompanied by a loss of offspring fitness, where gene flow is restricted, a loss of genetic diversity is necessarily associated with a loss in adaptive capacity that is likely to make populations more vulnerable to stochastic events than they otherwise would be (Anderson et al., 2012, O'Connor et al., 2012, Gonzalez & Bell., 2013). Moreover, if local environments are in poor shape then even fit offspring may struggle to survive long term, making assessments of local conditions as important as population structure / health (Blondel, 1980; Templeton et al., 1990; Lamont et al., 1993; Heinken, 2009). As such, managers should not jump to conclusions about resilience in populations based solely on fecundity or recruitment levels.

Without a clear idea of how populations are structured prior to suspected disturbances, it is difficult to gauge their true state at any given moment in time, their reproductive strategy, mating systems and the ways in which these can temporally and spatially vary between populations. As such it is crucial that we judge the health of populations armed with information about the ecology of each species, before we make judgements about their current states. New sophisticated protocols for assessing the conservation status of plants, that take into account more than just the numbers and ranges of plant populations, have been developed and utilized by government bodies more recently. One of the Global Conservation System widely used protocols that incorporates such information into its assessments of risk is the NatureServe Conservation status protocol (<http://www.natureserve.org/conservation->

tools/conservation-status-assessment) which uses a triage system to weigh key categorical predictors of species and population decline, so as to gain a more quantifiable final measure of extinction risk to a species or ecological community. The ‘IUCN red list of threatened species’ criteria ([http://www.iucnredlist.org/static/categories\\_criteria\\_3\\_1](http://www.iucnredlist.org/static/categories_criteria_3_1)) is another system which is widely used. Both systems take into account as much knowledge about historical disturbance and distribution of plants as available, as well as the nature of the ecological impacts being felt. As these protocols are adopted worldwide, and become mainstream, it will be possible for researchers and land managers to compare and contrast the risks to different species and ecological communities. Most importantly however, more nuanced methods such as these, can far better address the way in which common anthropogenic threats such as fragmentation may be affecting different species over the long term rather than during a relatively short period of observation. This will mean that more cryptic threats to populations that would remain undetected using traditional methods can be discovered, such as a loss of genetic diversity and adaptive capacity as a result of restrictions to mating systems in fragmented environments (Richards, 2007; Sartor et al., 2011; Hardion et al., 2015).

Population models have been used by ecologists for many decades now to predict the reproductive health and extinction risk of plant populations as well as guide their management (Menges 2000a,b; Burns et al. 2010). Matrix projection models combine multiple vital rates and the possible effects of changes in these rates into integrative measures of population dynamics. More complex population models can also accommodate mating system parameters, genetic structure and the demographic structure of populations and neighbouring populations to make more specific predictions about the size, structure of populations (Gauzere et al, 2013). Nevertheless, population models may fall short of making accurate predictions if key ecological or mating system information about a species is missing from the model. A key road block to better modelling of threats to plant populations is the lack of knowledge about temporal variation in the reproductive strategies and mating system parameters, as well as other temporally rare or cyclical ecological factors that fluctuate over time. A better understanding of the reproductive strategies, mating systems, and the way in which the ecology of populations and their reproduction interacts with their environment, make assessments of population health far more accurate, as well as allowing land managers to better target their limited resources for conservation.

## 1.2 Plant reproductive strategies and their importance to population health and persistence

The reproductive strategy of plants consists of the size at which reproduction starts, the subsequent frequency and regularity of reproduction, the amount of resources allocated on each occasion, the size and number of the seeds produced, the modes of reproduction utilized and when to stop reproducing. Several of these aspects of reproduction are mutually antagonistic (e.g. allocation level vs. frequency, seed size vs. number), so that the outcome is likely to be the result of a series of simultaneous compromises. The basic trade off between vegetative growth and reproduction, in conjunction with evolutionary pressure placed upon plants by a changing climate, grazers, and competition from other plant species for space and resources, shapes a plant's reproductive strategy and drives the great diversity of reproductive strategies that species utilize.

There are a wide range of reproductive strategies employed by different plant species. The life span of a plant is often indicative of its reproductive strategy. In highly disturbed dynamic environments where adult mortality is high, selection favours smaller shorter lived species such as annuals that reproduce quickly (Semelparous plants). Short life cycles and early maturity are also associated with small adult size (Kozlowski & Wiegert, 1986). Conversely, in less disturbed habitats, selection tends to favour longer lived perennial plants of large adult size (Iteroparous / polycyclic plants) that devote more resources to competing with their neighbours. In such a situation we would expect vegetative growth, as well as anti herbivory structures to be resourced preferentially over reproduction. Where juvenile mortality is especially high, selection in favour of long-lived individuals that have repeated opportunities for reproduction during their lifetime will be strong. These two contrasting plant types represent two extremes of a continuum, corresponding to the classic categories of r- and K-selected plants (Gadgil & Solbrig, 1972; MacArthur & Wilson, 1967).

While the time of death is predictable in annual and biennial plant species, the life span of longer lived perennial plants is less certain and often varies between plants depending on their genetic make up and whether the local environment they exist within is favourable. Such plants usually go through a period of senescence before death whereby reproduction is reduced or stops some time prior to death (Leopold, 1961). The reproductive strategies open to long lived plants are far more varied than annual plants, given they have leeway as to when, and to what degree they will reproduce. Ultimately, an interaction between the life history and the environment that a species has evolved within will determine the 1. Reproductive effort

(fecundity and seed size) 2. Modes of reproduction and 3. Timing of reproduction / germination that together characterise a species reproductive strategy.

### **1.2.1 Reproductive effort (fecundity and seed size)**

From an evolutionary perspective, the level of allocation to reproduction is thought to be driven largely by the level of local disturbance, with plants in highly disturbed environments likely to allocate more energy to vegetative reproduction than to sexual reproduction. For example, in environments subject to periodic, unpredictable events such as landslides, floods, fire, burrowing by animals, ploughing by humans or grazing, a plant exposed to damage will have to devote resources to mechanical repair or producing anti herbivory structures / chemicals at the expense of resources needed for reproduction (Gutschick, 1999). Competition from other plants also drives the amount and size of seed produced. In competitive environments, a plant's survival may require a high level of resource allocation to vegetative expansion rather than to reproduction so as to adequately compete for resources such as light, water and space (Lovett Doust, 1989; Reekie, 1999). Poor competitors may escape this difficulty by producing more seed with better dispersal ability, which conversely costs energy that could otherwise be put into growth (Venable & Brown, 1988; Rees, 1993; Dalling & Hubbell, 2002).

### **1.2.2 Modes of reproduction**

Allocation of sexual or asexual reproduction by plants is largely driven by biotic and abiotic signals such as density, clone age, resource limitation, and habitat fragmentation (Piquot et al., 1998; Kudoh et al., 1999; Rossetto et al., 2004). Sexual reproduction affords a survival advantage in changing environments by allowing plant populations to maintain higher levels of genetic diversity and therefore adaptive capacity (Harper, 1977; Stearns, 1987; Bernstein et al., 2013; van Kleunen et al., 2001). At the same time, plants can colonize new and favourable environments through seed dispersal, to take advantage of ideal conditions or to escape worsening conditions within their founder populations (Harper, 1977; Stearns, 1987; Zobel, 2008). Additionally seeds that accumulate in soil seed banks can often withstand a much wider range of environmental extremes such as fire, flood and drought than the adult plants (Ooi, 2012). Different stands can also be naturally structured into male and female only stands to encourage outcrossing (Bierzychudek & Eckhart, 1988).

Many species, especially herbaceous perennials in environments that are hostile to seedlings, are reliant on vegetative reproduction which allows a plant to perpetuate well adapted genotypes that maintain local adaptation in stable environments, spread quickly dominate a site when environmental conditions are stable (Jurik, 1985; Muir, 1995; Alvarez et al., 2005). Forms of asexual reproduction such as suckering provides support for new recruits through a direct underground connection to its parent, ensuring a level of resilience and regenerative capacity not afforded to seedlings (Piquot et al., 1998; Honnay & Bossuyt, 2005). In environments where feral animals place immense grazing pressure on new recruits, suckering may one day become the favoured strategy of reproduction as it (Klinkhamer et al., 1997). Indeed, it is common for arid species to favour asexual reproduction over sexual reproduction, so as to increase likelihood of offspring survival and maintain local adaptations in relatively harsh and unchanging environments (Silander, 1985; Caraco & Kelly 1991; Stuefer et al., 1996).

Tradeoffs for plants employing asexual reproduction include local crowding (Nishitani et al., 1999) and a lack of genetic diversity preventing the plant from adapting to selective pressures. It is therefore advantageous for plant species to employ both sexual and asexual modes of reproduction. Indeed the majority of plants with clonal growth also produce seeds (Salisbury, 1942; Ronsheim & Bever, 2000). Many plants that utilize sexual reproduction are hermaphroditic and can self-fertilize, but they typically still reproduce by outcrossing or mixed mating. It has been estimated that predominant outcrossing occurs in about 65% of hermaphrodite plants (Igic & Kohn, 2006) with a mixture of outcrossing and selfing occurring in about 24% plants (Goodwillie et al., 2005; Igic & Kohn, 2006; Jarne and Auld, 2006), leaving only a minority of hermaphrodites that predominantly self-fertilize.

For species capable of both modes of reproduction sexual reproduction, complex patterns of sexual and asexual reproduction can be employed. For these plants, sexual reproduction may have little to do with maintaining population size as the asexual component of their reproductive strategy can often provide this (Silander, 1985; Caraco & Kelly, 1991; Stuefer et al., 1996). In such species, sexual reproduction can be utilized for the purposes of maintaining genetic diversity or colonizing other areas (Harper, 1977; Stearns, 1987; van Kleunen et al., 2001; Zobel, 2008). As mentioned above, species can also transition between sexual modes over time as a consequence of adaption to changing environments, such as from mainly sexual reproduction to mainly asexual reproduction in environments that become steadily more arid (Richards, 1997; Sartor et al., 2011; Hardion et al., 2015; Hough et al., 2013). For longer lived species that can employ both sexual and asexual modes of reproduction,

modes of reproduction can even vary plastically over the life time of the plant in response to periodic and stochastic environmental changes (Douglas, 1981; Cheplick, 1995; van Kleunen et al., 2002; Vallejo-Marín et al., 2010). Few studies however have looked at the broader evolutionary consequences of shifts in the modes of reproduction. Given the predictions of climate change and the worsening conditions expected for many species, understanding whether they are transitioning between the modes of reproduction is key knowledge for a proper assessment of their expected persistence.

### **1.2.3 Timing of reproduction /germination**

In environments with harsh seasons where mortality is high, earlier reproduction is often favoured so that new plants can be recruited prior to the adults being lost and the optimal strategy is to be annual (Kroner, 2003). If adult survivorship is good and conditions for seedling establishment are poor, then it is generally considered advantageous to be a perennial and commit a fraction of photosynthate to reproduction every year.

Annual plants typically wait out winter in the soil as dormant seed, germinating when temperatures increase in spring. Flowering and seed formation are carried out in spring, summer and autumn, with seed dispersal occurring in autumn and plants dying when winter returns. In contrast, perennial plants need to be able to persist through adverse seasons, such as freezing winters in alpine areas and dry, hot summers in arid areas. In temperate climates, annual cycles are generally attuned to seasonal changes, with a necessary period of vegetative and reproductive hiatus during winter (Rutberg, 1987). In many tropical climates a dormant period may be due to other environmental factors like rainfall (Leck et al., 2008). In arid environments where rain can be absent for many years in a row, cycles of reproduction are often tightly linked to rare cyclical large scale rain events (Büsgen & Münch, 1929; Davies, 1976; Norton & Kelly, 1988; Fenner, 1998; Letnic & Dickman, 2006; Wardle et al., 2013). In these environments, ill timed reproduction would be disastrous with climatic conditions often too harsh to support recruitment and survival of seedlings during their vulnerable early stages (Rathcke & Lacey, 1985; Pilson, 2000; Sandring et al., 2007; Schemske et al., 1978, Kudo et al., 2008). Short lived plants get around this by creating dormant seeds that contribute to soil stored seed banks during the harsher periods, whilst long lived plants refrain from investing in reproduction until sufficient rain returns (Letnic & Dickman, 2006; Wardle et al., 2013).

The timing of germination of seed is as important for the persistence of populations as the timing of reproduction. When unfavourable local conditions persist, germination must be avoided or minimised, whilst germination during favourable conditions must be maximised. In environments with generally stable, favourable conditions for recruitment all year round, such as in rainforests, germination as soon as possible is favourable so as to avoid seed predation, grow as quickly as possible and compete for light (Leck et al., 2008). In contrast, in environments where conditions suitable for recruitment and survival of young seedlings are rare or short lived, such as in arid environments, germination might best be delayed to avoid seedlings all dying off in sub optimal conditions. For these plants, the capacity to store seed in a dormant yet viable state within the soil, ready to germinate immediately conditions are right again, is crucial for population persistence (Ooi, 2012). The creating of seed with differing periods of dormancy can buffer against short bursts of favourable conditions that do not last long enough for seedlings to establish. In arid environments where reproduction is closely aligned with rare rain events, such strategies are particularly important in order to avoid the desiccation of recruits after shorter periods of intense rain followed by extreme heat and dry conditions (Grice & Westoby, 1987; Jurado & Westoby, 1992; Phillipi, 1993; Auld, 1995; Honnay *et al.*, 2008).

### **1.3 Plant mating systems and their importance to plant population health and persistence**

A plant's mating system describes the way in which a population is structured in relation to its sexual behaviour. The primary mating systems of plants are 1. Outcrossing (cross-fertilization), 2. Autogamy (selffertilization) and 3. Apomixis (reproduction without fertilization) although many plants employ a mixed mating system whereby plants utilize a combination of two or all three of these mating systems (Brown 1990; Kearns and Inouye 1993).

The level of outcrossing produced by plants, given a randomised pollen pool, provides the clearest indication of the mating system 'preferred' by the plant (Richardson *et al.*, 2000). The 'realized' or actual mating system that will be observed, however, will vary as a function of; i) pollinator types, behaviour, and effectiveness; ii) the consequent quantity and diversity of pollen received by a plant (Stephenson and Bertin, 1983; Snow, 1986; Ayre *et al.*, 1994; Carthew *et al.*, 1996); and iii) variation in the ability of the maternal plant to discern pollen

quality or the fitness of seed through mechanisms of ‘mate choice’ (Wilson and Burley, 1983; Ayre and Whelan, 1989; Becerra and Lloyd, 1992; Barrett, 1995).

Within a given plant population, it can rarely be assumed that mating is occurring in a random fashion. The mating system of any hermaphroditic plant is influenced by many different factors such as the spatial and temporal variance in pollinator behaviour, distribution of male and female floral parts, plant density and the plant’s preferred mating system. Along with stochastic factors, such as pollinator behaviour which a plant has no control over, incompatibility mechanisms as well as maternal mate choice mechanisms (pre zygotic and post zygotic) provide plants with a degree of control over its offspring’s future success by actively preferencing matings that produce progeny of high quality (Uyenoyama, 1986; Holsinger, 1988; Becerra and Lloyd 1992; Barrett 1995). Plants exhibit mating patterns including: a) regular inbreeding and self-fertilisation systems, b) effective inbreeding due to clustering of related individuals occurring in a small area (neighbourhood) and c) negative assortative mating due to various kinds of incompatibility mechanisms. If environmental conditions remain constant, then we would expect the fitness of offspring to be optimal given evolutionary theory regardless of the specifics of a plants’ mating system (Kolreuter, 1761; Darwin, 1868, 1876; East & Jones, 1919).

Disturbance to the mating systems of plant populations can be detrimental if they result in offspring having reduced levels of fitness. For long term conservation of these plants it must be ensured that fitness levels are not reduced by an increase in the frequency of ‘suboptimal’ matings. To do this we must be able to estimate the actual (‘realised’) mating system and the ‘preferred’ mating system for comparison, and ultimately be able to determine which types of matings yield the most fit seed / offspring (Waser & Price, 1989).

The level of gene flow within and between plant populations is important from a conservation point of view and should be characterised, especially if there are concerns about gene flow being restricted. This is of concern where populations have become isolated through land clearing or where pollinators have been affected, lost or new pollinator species take over. In such instances, genetic diversity can be further eroded due to exacerbations in genetic drift (Spears 1987; Menges, 1991; Karron, 1989; Ellstrand, 1992; Lamont et al., 1993; Heschell and Paige, 1995; Agren, 1996; Fischer and Mathews, 1998) combined with reduced gene flow as levels of inbreeding increase and outcrossing rates decrease (Crow & Kimura, 1970; Severns, 2003; Quesada et al., 2004; Hensen et al., 2005; Aguilar et al., 2006).

### 1.3.1 Inbreeding and outbreeding depression- a consequence of mating system disruption

Levels of inbreeding or outbreeding that are too extreme can be associated with loss of fitness in the resulting offspring (Kolreuter, 1761; Darwin, 1868, 1876; East and Jones, 1919). The level of gene flow that is optimal depends on the plant. Some species which have evolved in small isolated populations under conditions where gene flow has been restricted, can persist in small population sizes by removing the deleterious alleles through selection, termed purging (Frankham *et al.* 2002). Such populations may prefer genetically similar pollen. Other species which have evolved under conditions of obligate outcrossing may be self incompatible. If gene flow becomes restricted in the latter case, fecundity may become significantly reduced, or else successful matings between individuals that would otherwise be selectively rejected, may increase (Charlesworth & Charlesworth, 1987; Templeton *et al.*, 1990). Fitness reductions in the offspring produced by these matings is termed ‘inbreeding depression’ (Kolreuter, 1761.), and has been found to affect many plant species around the world (Appendix 1.1). For plants that prefer genetically similar pollen, any movement of new genes into a population may serve to disrupt local adaptation, resulting in offspring with undesirable traits. This process is termed ‘outbreeding depression’ (Waser and Price, 1989; Fenster, 1991; Ellstrand, 1992). Whilst there are many reports of outbreeding depression across many species of plants in the literature (Appendix 1.2), reports of true outbreeding depression are far less common than that of inbreeding depression.

Inbreeding depression has been shown to be mostly caused by dominance and over-dominance at fitness loci (i.e., recessive lethal genes are expressed more often in the homozygous state) (East & Jones, 1919; Crow, 1948), but ecological factors such as resource competition among inbred siblings might also contribute (Price & Waser, 1979, 1982; Charnov, 1987; Schmitt & Ehrhardt, 1990). It has been suggested that outbreeding depression comes from either genetic or ecological mechanisms (Price & Waser, 1979; Shields, 1982). The genetic mechanism associated with outbreeding depression come about as a result of hybrid breakdown through the loss of co-adapted gene complexes or intrinsic co-adaptation (Fenster & Galloway, 2000; Templeton, 1986; Roff, 1998, Fenster & Galloway, 1997; Hufford and Mazer, 2003; Edmands, 2007). The ecological mechanism involves the dilution of locally adapted genotypes to produce genotypes maladapted for survival in either environment, compared to the parental genotypes (Waser & Price, 1989; Frankham *et al.*, 2002). A less well reported way in which outcrossing between highly divergent populations can result in suboptimal offspring results from matings between populations with differing chromosome number (Bruneau & Anderson, 1988).

Inbreeding and outbreeding depression can be expressed either pre or post zygotically (Crow & Kimura, 1970; Oostermeijer et al., 1995; Fisher & Matthies, 1998; Aguilare et al., 2006). Inbreeding depression is expressed in a variety of plant traits, including germination (Sheridan & Karowe, 2000), biomass (Vange, 2002; Galloway et al., 2003), survival (Charlesworth & Charlesworth, 1987), and reproduction (Husband & Schemske, 1997). The expression of inbreeding depression is predicted to differ between traits of different developmental stages because of differential selection (Husband & Schemske, 1996).

Whilst histocompatibility mechanisms can prevent pollen tube growth directly after pollen is deposited on flowers, later acting forms of inbreeding depression include abortion of seed that begin to initiate, or seed that is structurally formed but inviable. In even later acting forms of inbreeding depression, seeds that germinate may germinate late giving them a survival disadvantage, or seed may be small and seedlings lack size and vigour leading to slower growth rates and increased mortality (Crow & Kimura, 1970; Oostermeijer et al., 1995; Fischer & Matthies, 1998; Hendrix & Kyhl, 2000; Goverde et al., 2002; Aguilare et al., 2006; Peterson et al., 2008; González-Varo et al., 2010). Even later acting forms of inbreeding depression have been noted after offspring are fully formed and sometimes present as a lack of reproductive vigour / sterility rather than any loss of fitness in the plant itself (Oostermeijer et al., 1995; Aguilar et al., 2006). Negative effects might not be expressed until the F2 generation or later (Frankham et al., 2002; Tallmon et al., 2004; Becker et al., 2006; Edmands, 2007). This is often the case with outbreeding depression where hybrid breakdown is masked by heterosis (dominance or over dominance) in the first (F1) generation but becomes more obvious in the second (F2) and later generations when homozygosity increases (Lynch, 1991).

It must also be noted that inbreeding depression or outbreeding depression, that might otherwise present earlier in the life cycle of a plant, may be masked by unusually favourable environmental conditions and have little consequence (ie, avoid mortality), but then become evident in the phenotype under harsher conditions when the organism experiences higher levels of stress (Crow & Kimura, 1970; Oostermeijer et al. 1995; Fischer & Matthies, 1998).

### 1.3.2 Uncovering the mating system of plants

Due to the wide range of pollen and seed dispersal techniques employed by different species (Collins & Rebelo, 1987), the methods of study of plant mating systems are extremely diverse. With advancements in genetic technology, population genetic analysis and paternity

analysis have become the most commonly used methods to assess the mating system of organisms. The genetic structure of populations contains information about the modes of reproduction utilized by plants, as well as the types of matings that have occurred historically. Population genetics can reveal the degree to which sexual and asexual reproduction is being used, the level of outcrossing that has occurred within populations and between populations and the distance that pollen is being transported by pollinators as well (Douglas, 1981; Bierzychudek & Eckhart, 1988; Cheplick, 1995; van Kleunen et al., 2002; Vallejo-Marin et al., 2010). Whilst population genetics can tell us about general historical trends in the reproductive strategy and mating system of plants, paternity analysis can be used to give us direct information about the current realized mating system. Indeed, conservation of plant populations is greatly aided with knowledge about the genetic structuring of populations as well as information about the current mating system via paternity analysis. Comparisons between the two sets of data can inform us about any alterations in gene flow that may have occurred in recent history, as well as provide hints to the nature of such an alteration.

More complex mating systems may not always be easy to interpret from genetic data alone. Whilst obligate asexual reproduction might be inferred from clonality, this precludes the possibility that it came about via parthenogenesis. Moreover, a high level of genetic diversity within a population could be interpreted as the result of high levels of gene flow but it may be that mate choice mechanisms are selecting for outcrossed pollen amongst a sea of locally transferred pollen. This has been illustrated in previous studies of Australian native plants (Proteaceae), where pollinator observations have inferred the complete opposite to what genetic analysis of progeny have indicated. Specific examples include recent work studying pollinator movements within the genus *Banksia*. These have revealed that most pollen transfer may occur within inflorescences, or among inflorescences, on the same plant (Carthew, 1993; Vaughton and Carthew, 1993). From these observations we would expect that for these plants, the ‘normal’ mating system would be one with high levels of self-fertilisation. In contrast it was found that they display a great capacity for mate choice, which can lead to almost obligate outcrossing in some species as shown by allozyme studies of progeny arrays (Scott, 1980; Carthew et al., 1988; Vaughton & Carthew, 1993). Indeed, the process of genetic transmission can be complex, with outcrossing rates alone not enough to confidently infer the process that lead to the structure and matings we detect. Indeed, outcrossing rates tell us nothing about 1) events that may take place prior to the production of seeds, 2) pollinator behaviour which ultimately influences and can alter outcrossing rates, 3) any other environmental variable that may influence the outcrossing rates of a plant population, 4) origins of outcrossed pollen, and

5) whether there is a specific type /s of outcrossed pollen that is preferred over others. To ascertain a comprehensive and holistic picture of the dynamic mating system of hermaphroditic plants, a combination of techniques must therefore be employed together.

A comprehensive assessment of the mating system of a plant and its health, should include a combination of; observations of pollinator foraging behaviour to predict levels of gene flow and outcrossing (Brown et al., 1975; Scott, 1980; Shaw and Allard, 1982; Ayre et al., 1994); analysis of floral morphology to predict what types of pollinators should service them; pollen-style incompatibility and embryo abortion studies (Scott, 1980) to look for mechanisms of mate choice; and manual pollination experiments which provide plants with a range of outcrossed pollen from various sources to determine what matings are favoured (preferred mating system) (Price & Waser, 1989). Used in combination, these methods can provide researchers with a detailed account of the means by which matings are taking place within a system, as well as estimating whether current matings are seemingly optimal or suboptimal (Brown et al., 1975; Scott, 1980; Shaw & Allard, 1982; Waser & Price, 1989).

For plant populations, genes are moved primarily by either pollinators or by seed dispersal, with some plants being wind pollinated. A variety of pollen visitors may result in a range of pollen types of different origins being available to a given plant. While the 'realised' mating system will then reflect both the quality of pollen available to it and the capacity for it to discriminate among the pollen types (Wilson & Burley, 1983), ultimately the quality of offspring produced is reliant on the types of movements pollinators make and their effectiveness at transferring pollen. Different pollinators should have different effects on the patterns of pollen movement between plants due to their varying modes of foraging behaviour (Faegri & van der Pijl, 1979). Knowledge of the different types, relative numbers and foraging behaviours of pollinators can allow us to predict to some degree the movement of pollen between plants within a population and whether we might expect distant populations to be connected. Confirmation of key pollinator species lost from populations or the detection of disruptive introduced pollinators can be especially useful for assessing the health of mating systems (Taylor & Whelan, 1988; Vaughton, 1996; England et al., 2001). Moreover, as there can be seasonal variation in pollinator movements, knowledge of pollinator behaviour over several seasons may need to be attained, to truly explain the way populations are genetically structured (Copland & Whelan, 1989).

Manual pollination experiments do not only provide us with the capacity to uncover the level of self compatibility of stands, and detect and study the nature of any mate choice

mechanisms present, they can also provide us with a broad picture of the ‘preferred mating system’ for a plant species / population. Given the ‘preferred mating system’ may differ significantly from the ‘realized mating system’ where gene flow is restricted in modified environments, manual pollination experiments also provide us with a tool to detect such disparities. Given that evolutionary theory predicts that without disturbance natural matings should provide pollen of optimal quality, experiments that are capable of producing more and/or fitter offspring by providing outcrossed pollen, infer a disparity between the optimal ‘preferred’ mating system and the currently operating ‘realized’ mating system. For example, Australian plants which in general display a broad suite of associated pollinators and a great capacity for mate choice (Ayre & Whelan, 1989), typically show limited seed set with mean fruit set ranging from 0.1% to 7.2% for 18 species (Collins & Rebelo, 1987), even though plants in healthy populations rarely appear to be pollen limited. This means that we expect there to be clear contrasts between a plant’s ‘potential’ mating system (determined by pollinator foraging behaviour / movements), its ‘preferred’ mating system (revealed through hand pollinations), and its realised mating system (reflecting the effects of mate choice and revealed by genotyping plants).

## **1.4 Reproduction and recruitment failure in plants**

Failure to reproduce is used as one of the key processes for identifying populations at threat of extinction. Reproductive failure in plants can happen for many reasons. While it can be a consequence of temporally restricted natural stochastic phenomena (e.g., climatic events, outbreaks of disease, or spikes in local grazing pressures), reproductive failure that is anthropogenically driven is of particular concern to managers. The effect on the health and persistence of unnaturally fragmented populations will depend ultimately on the intensity and duration of the underlying disturbance, as well as the tolerance and life history of the species in question. While natural disturbances can be intense, some may not persist as long as anthropogenic disturbances such as landscape modification (e.g., fragmentation) or ongoing impacts from introduced species (e.g., feral grazers). Habitat loss and fragmentation, combined with climate change, environmental degradation through pollution, the invasion of exotic species and overexploitation, are considered the largest threats to biodiversity worldwide (Diamond, 1989; Pimm & Raven, 2000). Understanding the joint effects of land use and climate change specifically on biodiversity and the provision of ecosystem services has become a critical concern for land managers, highlighted by the United Nations’ Intergovernmental

Platform on Biodiversity and Ecosystem Services (IPBES) (Hoareau & Arico, 2010; Spierenburg, 2012; Danielsen et al., 2014; Díaz et al., 2015).

As discussed earlier (in **1.1.1**), assessing reproductive health of populations of long lived plants can be especially difficult given that plants display complex life histories and reproductive strategies. Reproduction in long live plants in particular may be rare and sporadic leading to underestimates of the reproductive health if observations are undertaken over inadequate temporal scales, outside specific reproductive periods (Fenner, 1998; Letnic & Dickman, 2006; Wardle et al., 2013). Complicating assessments further, the importance / role of sexual reproduction in plants which also reproduce asexually can also vary between species and even between stands of the same species (Douglas, 1981; Cheplick, 1995; van Kleunen et al., 2002; Vallejo-Marín et al., 2010). Moreover, shifts in the reproductive strategy of plants can mean that either sexual or asexual reproduction is lost from populations and thus further confound estimates. This can happen gradually as an evolutionary shift with changing climatic / environmental conditions (Silander, 1985; Caraco & Kelly, 1991; Stuefer et al., 1996; Piquot et al., 1998; Honnay & Bossuyt, 2005), or as a plastic response in some species to sudden changes in local conditions (Douglas, 1981; Cheplick, 1995; van Kleunen et al., 2002; Vallejo-Marín et al., 2010). Many clonal organisms however display rare episodic sexual reproduction (e.g., *Actina*) and some species even switch between sexual and asexual reproduction to make use of the different advantages afforded to both modes under different conditions (Douglas, 1981; Cheplick, 1995; van Kleunen et al., 2002; Vallejo-Marín et al., 2010).

While estimates of reproductive success in trees are usually based on seed production (Fuchs et al., 2003; Schoen & Stewart, 1986; Herrera & Jovani, 2010), this method of assessing reproductive health of plant populations can also be deceptive. An individual has not successfully reproduced, from an evolutionary or population dynamics perspective, until its offspring are themselves of reproductive age (Primack & Kang, 1989). Given mortality at the seed-to-seedling transition is generally high (Harper, 1977), potential tradeoffs between seed number and seed quality (Primack & Kang, 1989) as well as for density dependent mortality beneath maternal crowns (Janzen, 1970) exist, seed number is not necessarily a good proxy for lifetime reproductive success. Even initial reproductive success does not guarantee long term survival of recruits. If environmental conditions are suitable for asexual or sexual reproduction (fruit set) but unsuitable for recruitment, or long term survival of these recruits, then populations will suffer in the same way over the long term, as if seed were not set. Such a situation may occur simply if seed are consumed by a plague of insects or killed by pathogens, or else if a short rain event in an arid environment allows for seed set but does not continue

long enough to nurse seedlings through the most vulnerable first stage of their establishment (Slatkin, 1974; Seger & Brockman, 1987; Philippi & Seger, 1989; Stearns, 1992; Roff, 2002; Evans et al., 2007). While asexual reproduction via such forms as suckering can be more resilient to such a change than newly established seedlings (Piquot et al., 1998; Honnay & Bossuyt, 2005), their survival would still likely require more favourable external conditions than required to initiate them.

In environments under pressure such as those that are anthropogenically disturbed by clearing for urbanization and agriculture, the chances for successful long term recruitment of offspring are generally diminished (Blondel, 1980; Templeton *et al.*, 1990; Lamont *et al.*, 1993; Heinken, 2009). If maternal plants are starved of resources and particularly stressed but still manage to recruit, these recruits may lack the level of vigour required to persist long term or through a period of high climatic stress even in the short term (Kapos, 1989; Matlack, 1993, 1994, Malcolm, 1998; Jules & Rathcke, 1999; Meiners & Pickett, 1999). Even when reproduction is achieved by resilient parent plants, harshened local conditions can mean that the relatively less resilient seedlings perish before contributing to the next generation (Lamont *et al.*, 1993). If the mating system of plants in disturbed populations is affected, resulting in loss of genetic fitness of offspring (inbreeding depression), the chance that recruits persist is likely to be even further reduced (Crow & Kimura, 1970; Oostermeijer *et al.*, 1995; Fischer & Matthies, 1998). In plant populations where recruitment is reduced for extended periods, the age structure of populations will inevitably be skewed towards older or senescent plants which may in turn lead to further reduced reproductive capacity (Jones, 1945; Rohner & Ward, 1999; Brand, 2002; Garcia, 2003; Zavaleta *et al.*, 2007; Li, 2012).

In the medium term, populations that struggle to recruit can persist within a highly modified environment with an extinction debt; a condition in which populations still present in a habitat patch are expected to go deterministically extinct (Tilman *et al.*, 1994; Hanski and Ovaskainen, 2002; Jackson & Sax, 2009; Kuussaari *et al.*, 2009).

Overstory trees in pasture which exist as either lone ('paddock') trees on farmland or small isolated stands of trees kept for shading livestock which have generally been found to have reduced seed production, reduced outcrossing rates and reduced recruitment rates compared with trees in continuous forest (Aldrich & Hamrick, 1998; Burrows, 2000; Dick, 2001; Cascante *et al.*, 2002; Fuchs *et al.*, 2003; Hanson *et al.*, 2008) provide some of the best examples of stands with an extinction debt. Unlike animals, plants lack the mobility to relocate other than through dispersal of propagules, making the potential consequences of habitat

modification more devastating. Indeed, an increasing number of species face risk of extinction because they occur in very restricted or highly fragmented areas, and have a reduced number of populations or individuals (World Conservation Union 1994).

## **1.5 The threat of fragmentation**

Fragmentation in landscape ecology is the disintegration of large areas of natural habitat into smaller, isolated patches (Saunders et al., 1991; 1995) which results in the emergence of discontinuities in an organism's preferred habitat (Young, 1996; Young & Clarke, 2000). It also encompasses habitat size and area, habitat subdivision, creation of edges, altered connectivity among patches and changes in habitat quality (Ewers and Didham, 2007). Regardless of whether fragmentation of ecosystems and populations comes about by natural or manmade disturbances it has been shown to be a major cause of local population and species extinctions (Lande, 1993; MacNally et al., 1997; Young & Clarke, 2000; Cushman, 2006).

Plant populations can become fragmented because of direct threats such as natural disasters (floods, fires and landslides) (Dechaine & Martin, 2004), diseases, (Patz et al., 2004; Hilty et al., 2006), outcompeting by invasive species (Kearns et al., 1998; Wiser et al., 1998), climate change (Reed & Frankham, 2003; Frankham, 2005; Bradshaw et al., 2006) and more recently by anthropogenic means such as land clearing for agricultural / urbanization purposes and grazing by agricultural and introduced feral animals (Auld, 1993; 1995). The nature of the threat will determine the extent, shape and density of stands within the fragment (Levin & Kerster, 1974; Fumier et al., 1987; Govindaraju, 1988). Stochastic events may alter or disrupt ecological communities immediately but are often short lived such as fires and floods (Levin & Paine, 1974; Delcourt et al., 1983; Pickett & White 1985) allowing stands to regain their natural structure afterwards (Hilty et al., 2006).

In some cases species can have naturally fragmented distributions (Harris, 1988; Begon et al., 1990; Laurance & Yensen, 1991; Murcia, 1995; Ford et al., 2001; Llorens, 2004). Some species may exist within a landscape where resources are distributed in a patchy manner, such as in arid environments where water availability can be restricted to small springs dotted throughout a large expanse. Other species may be exclusively associated with certain geological features similarly distributed, such as sand dunes. These species will display a permanently fragmented distribution which should not be confused with other fragmented

populations that have shrunk (Forman & Godron, 1986; Turner, 1987; Turner, 1989; Weins et al., 2004).

Human induced fragmentation is generally more long term and has served to permanently affect landscapes around the world on a scale rarely achieved by natural processes (Jennersten et al., 1992; Vitousek, 1994; Sih et al., 2000). It is recognized as the principle driver of biodiversity loss and species extinctions (Burgess & Sharpe, 1981; Wilcox & Murphy, 1985; Noss & Cooperrider, 1994; Frankham et al., 2002). The introduction of domestic grazing has been a primary driver of fragmented landscapes (Llorens, 2002) and in Australia, an estimated 473 million hectares (62% of the continent) is used for agricultural purposes (Australian Natural Resources Atlas-Australian Government). Further to this the rapidity and scale of change associated with human induced fragmentation fails to allow time for organisms to acclimate to the altered physical structure and processes in the distorted habitat (Hilty et al., 2006).

Plant populations can be particularly susceptible to fragmentation as their immobility means they are unable to avoid negative effects by relocating to less stressful areas, as many animals can (Blondel, 1980; Templeton et al., 1990; Lamont et al., 1993; Heinken & Winkler, 2009). Fragmentation and loss of habitat are recognised as the greatest threats to plants worldwide with over 40% of the world's land used for agriculture (Young & Clarke, 2000). Remaining natural vegetation in these areas often exists in a mosaic of fragmented patches, often totally disconnected from one another (Murcia, 1995; Young & Clarke, 2000; Laurance et al., 2009). There have been many studies on how habitat size and the degree of spatial isolation affect plant health, plant reproduction and long term population persistence (Appendix 1.3).



Figure 1.1: Arial picture of Australia's fragmented landscape.

### 1.5.1 The effects of fragmentation

The fragmented state of populations has been shown to bring about a myriad of changes to local abiotic and biotic processes (Appendix 1.4) that can serve to further degrade populations even once the 'direct' cause such as land clearing has ceased (Lamont et al., 1993; Lande, 1995; Menges & Dolan, 1998). Indeed much literature exists on the physical and genetic stresses on plants associated with fragmentation resulting from direct and indirect effects on plant physiology and plant mating systems (Steffan-Dewenter & Tscharntke, 1999; Harris & Johnson, 2004; Steffan-Dewenter & Westphal, 2008; Gonzalez-Varo et al., 2009; Jakobsson et al., 2009).

Changes to local abiotic conditions might be viewed as the most direct pressure on the health of biotic processes within fragmented populations (Laurance, 2009). An increased edge to unit area ratio means that a higher proportion of the habitat may experience greater variations in abiotic conditions such as light intensity, temperature, humidity, soil moisture and wind, (Harris, 1984; Kapos, 1989; Parsons, 1991; Murcia, 1995; Jose et al., 1996, 1998a; Ozanne et al., 1997; Laurance et al., 1998; Vitt et al., 1998; Newmark, 2001; Pohlman et al., 2009), often referred to as 'edge effects'.

Unfavourable alterations to local abiotic conditions as a result of edge effects can limit resources acquisition for fruit and seed development (Stephenson, 1981; Lee & Bazzaz, 1982; Lee 1988; Campbell & Halama, 1993), make conditions unfavourable for recruitment when seed are set (Laurance, 2009), increase susceptibility to pathogens (Jones, 1976; Jennersten et al., 1983) and increase competition for resources from invasive species better suited to the altered conditions or allowed access for the first time (Kearns et al., 1998; Wiser et al. 1998; White et al., 2002; Yates et al., 2004). Furthermore, the increased proportion of accessible edges for larger herbivores coupled with often easier access to the interior of less dense vegetation can serve to increase the pressure of generalist herbivores on fragmented plant communities (MacGarvin et al., 1986; Warren, 1987; Donoso et al., 2004; Terborgh et al., 2006). This threat to populations is only intensified with the introduction of agricultural livestock and feral grazers that accompany the fragmentation of native vegetation in agricultural (Moore, 1962; Lange & Graham, 1983; Auld, 1990, 1993, 1995; Westbrooke et al., 2001; Denham & Auld, 2004). A reduced size and capacity of fragmented populations to buffer these environmental processes also makes them more vulnerable to stochastic events such as floods and fires (Kery et al., 2000; Hobbs & Yates, 2003).

Pressures imposed by fragmentation can also have effects on the reproductive strategy of some plant species. For plants that can utilize both sexual and asexual forms of reproduction, a less well known effect of habitat degradation on such plants is the capacity for the disturbance to shift the balance between reproductive modes (Vergeer et al., 2003; Díaz-Almela et al., 2007; Arnaud-Haond et al., 2010). A lack of sexual reproduction may be a plastic response that is countered by an increase in asexual recruitment rather than reproductive failure in the deleterious sense of the term (Douglas, 1981; Cheplick, 1995; van Kleunen et al., 2002; Vallejo-Marín et al., 2010). Even if populations display resilience to the pressures of harsher local abiotic conditions, and increased herbivory, many fragmented plant populations can still suffer damaging alterations to their mating systems as a simple consequence of isolation and reductions in their densities (Charlesworth et al., 1987; Ellstrand & Elam, 1993), or as a result of negative impacts on mutualists they rely on for reproduction (Howe, 1977; Sih & Baltus, 1987; Jennersten, 1988; Byers, 1995; Dewenter & Tscharntke, 1999; Cunningham, 2000; Cordeiro & Howe, 2003).

The effects of fragmentation on the mating systems of plants have been the focus of many studies (Appendix 1.5). Firstly, through simple scarcity, fragmentation reduces the number of breeding individuals within the population and consequently the number of available mates thus effectively reducing the chances of a successful mating (Allee, 1931; Allee

& Rosenthal, 1949; Allee, 1951; Groom, 1998). The diminished physical structure of fragmented populations can also have ‘indirect’ negative impacts by affecting other organisms that interact with a particular species, and which are key to their reproduction and persistence. Namely, habitat fragmentation, which can negatively impact on the interaction of plants with their animal pollinators (Jennersten, 1988; Byers, 1995), seed dispersers (Howe, 1977; Sih & Baltus, 1987; Cunningham, 2000; Cordeiro & Howe, 2003), mycorrhizae fungi (Peay et al., 2010), insect herbivores (Cagnolo *et al.*, 2009) and parasites (Valladares *et al.*, 2006), all of which may affect the long-term viability of plant populations.

The effects of fragmentation on the abundance and behaviour of pollinators and seed dispersers have been found to have particularly devastating effects on the persistence of populations, and this has been extensively studied in many systems around the world (Appendix 1.5 & 1.6). Plants in small isolated populations may be less attractive to pollinators and seed dispersers than plants in large patches, or simply harder for them to locate leading to pollen limitation and limited dispersal of seed (Sih & Baltus, 1987; Jennersten, 1988; Mustajärvi *et al.*, 2001; Groom, 1998; Llorens, 2004). Fragmentation may also cause pollinators to become locally extinct resulting in the potential for reproductive failure in plant species (Buchmann and Nabhan, 1996; Washitani, 1996; Allen-Wardell, 1998; Cunningham, 2000). Especially in agricultural regions, the loss of natural areas, food sources and the use of insecticides can lead to selective losses of those native pollinator species that are less tolerant to such changes (Meyer *et al.*, 2007; Johnson *et al.*, 2004). In some cases these pollinators might be key to providing a specific plant species with much of the genetic diversity on which they rely (Lysenkov, 2009; Lepais *et al.*, 2010; Wikelski *et al.*, 2010; Finger *et al.*, 2014).

Fragmentation has been shown to alter foraging behaviour once pollinators have located a fragmented patch, because many pollinators actively avoid edges and are forced to remain within isolated fragments for longer periods of time (Laurance *et al.*, 2009). Insect pollinators may also be unable to travel the vast distances required between stands in fragmented environments, effectively reducing outcrossing and increasing the proportion of matings between related neighbours, or as a result of selfing. These restrictions should serve to reduce genetic diversity as well as increase the chances populations will suffer the effects of inbreeding depression (Goverde *et al.*, 2002; Aizen & Feinsinger, 2003; Peterson *et al.*, 2008). Ultimately, in fragmented populations the disturbance to pollinator services can result in a reduced number of matings (Goverde *et al.*, 2002; Aguilar *et al.*, 2006; Peterson *et al.*, 2008), restricted gene flow (Fischer & Matthies, 1998; Ghazoul, 2005; Leimu *et al.*, 2006; Bowman *et al.*, 2008), inbreeding depression (Crow & Kimura, 1970; Nei *et al.*, 1975; Charlesworth &

Charlesworth, 1987; Young & Clarke, 2000), and in severe cases total reproductive failure (Crow & Kimura, 1970; Oostermeijer et al., 1995; Fischer & Matthies, 1998; Hendrix et al., 2000). A lack of seed dispersers can result in reduced recruitment and altered genetic structure also (Howe, 1977; Handel, 1983; Chacoff, 2007). At the very least, if genetic diversity is lost through increased levels of inbreeding, populations will lose adaptive capacity and will be more vulnerable to climatic shifts such as global warming (Whitlock, 2002; Roze & Rousset, 2003; Theodorou & Couvet, 200).

The effects of fragmentation on gene flow can be exacerbated by the introduction of foreign pollinators with differing foraging behaviours, such as the effect of introduced honeybees (*Apis mellifera*) on the mating systems of many native Australian plants. Many studies have found that honeybees decrease levels of outcrossing and increase levels of inbreeding as a result of their efficiency and restricted foraging behaviours (Taylor and Whelan, 1988; Vaughton, 1992; Vaughton, 1996; and England et al., 2001), however only recently has the need to conserve pollination interactions been realised (Inouye et al., 1998).

Some studies of small fragmented populations have failed to find evidence of reduced reproductive capacity despite drastic changes to the pollination of plants (Costin et al., 2001; Leimu et al., 2006). Some plant species can persist in small population sizes by removing (purging) the deleterious alleles through selection (Frankham et al., 2002). The Wollemi Pine which contains no genetic diversity at several hundred loci, is one such example (Frankham et al., 2002). Species such as these might even be susceptible to outbreeding depression rather than respond positively if mates are too genetically divergent (Fischer & Matthies, 1997; Edmands, 2007). Pollinator numbers can also be boosted in isolated populations if there is little vegetation in the surrounding region and they are forced to target these remaining stands for food (Stouffer & Bierregaard, 1995; Mustajarvi et al., 2001; Murren, 2002). Several studies have also shown large increases in pollen dispersal distances by pollinators motivated to forage over larger distances between isolated patches and this may counteract the negative effects of fragmentation on reproduction (Dick, 2001; White et al., 2002; Dick et al., 2003, Byrne et al., 2008; Hanson et al., 2008; Ottewell et al., 2009; Cörtes et al., 2013). In these cases isolation may have encouraged pollinators to move pollen between plants in stands that would not normally be connected thereby increasing natural levels of gene flow and putting susceptible species at danger of outbreeding depression (Waser and Price, 1989; Fenster, 1991; Ellstrand, 1992).

In some species outbreeding can result in inter specific pollen flow and hybrid production by more abundant congeners, which can dilute the gene pool of pure-bred genotypes not suited to local conditions (Levin et al., 1996; Wolf et al., 2001). This highlights the importance of judging species / populations with a fragmented distribution case by case. Nevertheless, for the many species that are vulnerable to fragmentation, managers are keenly aware of bolstering or at least maintaining genetic diversity within populations, to increase or conserve their adaptive capacity in preparation for the worst effects of global warming (Tallmon et al., 2004; Byrne et al., 2011; Miller et al., 2012; Whalley et al., 2013). This type of conservation strategy is often referred to as ‘genetic rescue’.

Whilst much literature about the negative effects of fragmentation on plant reproductive capacity has been published (revisit Appendix 1.3), at present there are fewer studies looking in detail at the structural and genetic thresholds for these negative effects. There are also a lack of studies following new recruits to evaluate the consequences on their fitness and hence the potential for stands to persist over time. For practical reasons, studies documenting the effects of fragmentation on reproduction also generally focus on short lived species in temperate environments. Studies of long lived species or species that grow in extreme environments where reproduction and recruitment can be sporadic, opportunistic and infrequent are less common.

### **1.5.2 Assessing vulnerability to fragmentation**

Studies have shown considerable variation in a species’ response to increased fragmentation and its effects (Lamont et al., 1993; Aizen et al., 2002; Aizen & Feinsinger, 1994; Lamont et al., 1994). The specific effects of fragmentation on a given plant species and degree to which they will be affected will depend on an interaction with the nature of the fragmentation (spatially), the environment within which species exist, the species specific tolerance to such a disturbance and the ongoing altered conditions. Depending on the size, level of isolation, density and shape of stands after land has been cleared, the consequences for reproductive health can range from nothing to reductions in successful matings, reductions in recruitment of offspring, or reductions in the long term survival of offspring (Lamont et al., 1993; Aizen & Feinsinger, 1994; Lamont et al., 1994). Whilst a relationship between smaller stands and reduced outcomes is generally expected (Ellstrand & Elam 1993; Richards, 2000), many studies have found that the level of isolation between plants / stands is a greater determinant of reproductive health in some species than the size of an individual stand (Abeli

et al., 2015). The severity of edge effects has also been shown to be influenced by the fragment shape and area (Saunders et al. 1991).

The effects of fragmentation may be minimal if fragmented populations are surrounded by a matrix that is still permeable to pollinators and seed dispersers for plant species (Frankham, 2005). If a fragmented stand is surrounded by other flowering plants, pollinators may be more likely to traverse between fragmented stands than if the surrounding land is unvegetated, or populated with agricultural crops. Crops that are flowering at the same time as fragmented native plants however, may attract generalist pollinators that might service the adjacent fragmented population to a degree that may not occur if surrounded by other native species with different flowering patterns (Nicholls & Altieri, 2013). Surrounding vegetation can also change temporally, especially with the changing of seasons. Nowhere is this more obvious than in arid and alpine environments where hot summers and freezing winters restrict the presence of many plant species that occupy a space within cooler and warmer months respectively. In arid environments, where reproduction is often associated with large scale rain events, when a large flush of understory ephemeral vegetation also emerges to flower, isolated stands of plants that are usually disconnected during prolonged periods of drought may be partially connected by this ephemeral vegetation across which pollinators are more likely to traverse. As such, any estimates of population connectivity should take into account such temporal dynamics existing in different environments.

The difference in the way in which populations of two different species will respond to the same level of fragmentation will be determined by their physiological tolerance to local conditions, as well as their reproductive strategy and mating system specifics (Aguilar et al., 2006). Levels of fragmentation that constitute a threat to one species may be overcome in another by differences in their reproductive strategy and / or differences in key mating system parameters (Ellstrand & Elam 1993; Hobbs & Yates 2003). For instance, reductions in gene flow and fitness consequences of inbreeding depression are not felt equally, if at all, by all species (Bacles and Jump, 2011), with impacts depending on the specific pollen vectors utilized by plants and their level of selfcompatibility (Sork and Smouse, 2006; Eckert et al., 2010; Breed et al., 2012). As such, investigations into the reproductive health of plants must be done species by species, although we might expect species with similar life cycles to respond more similarly than those with vastly different life cycles.

The mode of reproduction utilized by plants is expected to have significant consequences for their capacity to withstand both the isolation of existing within a fragmented

population as well as the associated harshened local abiotic and biotic conditions. Generally speaking, asexual reproduction is thought to be more resilient to fragmentation than sexual reproduction, at least in the short term (Silander, 1985; Caraco & Kelly, 1991; Stuefer et al., 1996 Matesanz, et al., 2015). For species that do not rely on pollen dispersal, the effect of isolation which is so often damaging to sexual species is irrelevant. Secondly, asexually derived recruits such as suckers that maintain a connection and thus support from the parental plant, are generally more resilient to desiccation and grazer damage than vulnerable seedlings (Piquot et al., 1998; Honnay & Bossuyt, 2005).

In more dynamic environments, plants are generally more likely to employ sexual reproduction to introduce genetic diversity to stands and increase adaptive capacity as well as provide increased dispersal capacity (Harper, 1977; Stearns, 1987; van Kleunen et al., 2001; Zobel, 2008; Bernstein et al., 2013). Sexually reproducing plants can also have the advantage of dispersing over large distances to colonize more suitable environments than the ones they currently occupy. While this capacity does not provide local resilience to populations, when these environments are fragmented this can provide the opportunity for seed to escape local harsh conditions into adjacent land that may be of a better condition (Richards, 2007; Sartor et al., 2011; Hardion et al., 2015). The production of genetically diverse offspring may also allow populations that find themselves in harsher local conditions to adapt to their surroundings, however, the speed at which anthropogenically induced fragmentation of populations occurs may be too fast to expect such adaptation to occur. Another advantage to sexual reproduction in harsh environmental conditions is the capacity for generating a dormant soil stored seed bank. Resilient long lived soil stored seed banks can persist during harsh conditions above ground and provide a buffer against prolonged periods of harsher environmental conditions (Ooi, 2012). Species with a persistent seed bank may also be buffered for some time against genetic erosion, as seed banks act as a reservoir of genetic diversity (Templeton and Levin, 1979; Honnay et al., 2008).

For plants that utilize both sexual and asexual reproduction, the degree to which they utilize each mode of reproduction should affect the overall resilience of such species to their fragmented environments also. In general, sexual reproduction increases when stressful conditions prevail to increase adaptive capacity (Richards, 2007; Sartor et al., 2011; Hardion et al., 2015), whilst asexual reproduction is employed to take full advantage of favourable / stable conditions (Kearney, 2003; Honnay & Jacquemyn, 2008; Vallejo-Marín et al., 2010). However, if harsh climatic / environmental conditions are stable in region, such as in arid and semi arid regions, plants may favour forms of asexual reproduction such as suckering which is more

resilient to desiccation damage from herbivores than seedlings are (Piquot et al., 1998; Kearney, 2003; Honnay & Bossuyt, 2005; Honnay & Jacquemyn, 2008; Vallejo-Marín et al., 2010). In such cases ex may be maintained as a secondary method of reproduction, so as to provide populations with some capacity to adapt and colonize (Richards, 2007; Sartor et al., 2011; Hardion et al., 2015).

In general, plants with more mobile pollinators that traverse unvegetated parts of the landscape (matrix) are expected to fare better than those species whose pollinators typically forage over shorter distances and do not move across gaps in vegetation in the landscape (Templeton & Levin, 1979; Cabin, 1996; Eriksson, 1996; England et al., 2002). Typically bird and some mammal pollinators have the capacity to link fragmented populations of plants over far larger distances than insect pollinators, although some insect pollinators are more mobile than others (Lysenkov, 2009). This mobility allows pollinators better scope to locate isolated plants within the landscape meaning the negative effects of inbreeding (inbreeding depression), so often associated with fragmented plant populations, are also more likely to be avoided (Templeton & Levin, 1979; Higgins & Richardson, 1999; Ouborg et al., 1999; Frankham et al., 2002). Plants that have highly specialized and obligate interactions with their pollinators are also thought to be most susceptible to habitat fragmentation effects, as any impact on the pollinator would directly affect the plant and *vice versa* (Bond, 1994; Johnson & Steiner, 2000; Ghazoul, 2005).

The degree that reduced levels of outcrossing within fragmented populations will effect plant reproduction and survival in populations, depends greatly on the species specific level of self-compatibility (Lamont et al., 1993). Gene flow levels that cause inbreeding depression in one species may be preferred by another (Lamont et al., 1993; Aizen & Feinsinger, 1994; Barrett & Harder, 1996; Theodorou & Couvet, 2002). For self incompatible species, the effects of increased inbreeding can be relatively swift and potentially devastating (Schemske, 1983; Lamont et al., 1993; Aizen & Feinsinger, 1994), whilst a self compatible population that has purged itself of deleterious alleles may be more sensitive to outbreeding depression (Schemske, 1983; Byers & Waller, 1999; Hedrick & Kalinowski, 2000; Frankham et al., 2002).

Plants which face the challenge of being bombarded with too much undesired self pollen, as a result of restrictions to pollinator foraging distances, employ mate choice mechanisms, selectively aborting seed that come about through selfing and consequently increase the proportion of outcrossed seed being matured in relation to the number of outcrossing events that occur (Macarthur & Wilson, 1967; Charlesworth & Charlesworth, 1987;

Tempelton et al., 1990; Mills & Smouse, 1994). These species are more likely to maintain higher levels of genetic diversity within seed cohorts when gene flow is restricted in fragmented stands, than a species that does not. Moreover, if a high level of genetic diversity already exists within fragmented stands this loss of diversity in such stands is likely to be slow. Conversely preferentially outcrossing species with populations that display more local genetic structuring, should more rapidly become homogenized by increases in inbreeding (Theodorou & Couvet, 2002).

Predicting how species in these patches will respond is of great interest to land managers. It can be difficult to discern whether reproductive failure in fragmented stands is a result of pressures on the physiology of plants, restrictions to their mating system, a product of anomalous environmental conditions, or simply a dormant period and natural part of their reproductive strategy. While common garden experiments can be used to control environmental factors for annuals and shorter lived perennial species, this is not practical for many larger, long lived species of plants which often take many years to reach maturity. For these plants, investigation must be in the field, but in a time of optimal environmental / climatic conditions in order to get a true picture of their reproductive response, unconfounded by plant physiological or local environmental suboptimalities. Given these conditions cannot be guaranteed at the time of study, fewer studies for these species have been carried out. Moreover, the few studies that have looked at the effects of fragmentation on long lived plants have focused on species existing within temperate environments, most of which are also obligate seeders. For practical reasons, even fewer studies have focused on long lived species native to arid or alpine environments, or species which display a more complex reproductive strategy, such as those that reproduce both sexually and asexually. Moreover, most studies of longer lived plants have focused on European and North American species, with few studies on species in extreme environments, or in the southern hemisphere.

## **1.6 Threatened Acacia of semi arid NSW-A model system to investigate the effects of severe fragmentation on the functioning and persistence of long lived arid plants.**

Arid and semiarid ecosystems occupy 40% of the Earth's land surface (Millennium Ecosystem Assessment, 2005) and are among the systems predicted to be most sensitive to future changes in climate (IPCC, 2001). Many plant species within Australia's highly degraded

arid and semi arid zones show greatly reduced reproductive success, and are also likely to be experiencing the combined effects of habitat fragmentation and loss or disturbance of pollinators altering genetic connections (Auld, 1995; Porteniers, 1998; Auld & Denham, 2001; Porteniers, 2001). If left in their current state, a combination of declining population sizes and loss of adaptive potential is likely to significantly elevate the risk of local extinction of many plant species in western NSW (Ellstrand & Elam, 1993; Fischer & Matthies, 1998; Frankham et al., 2002; Reed & Frankham, 2003). Indeed, intense grazing regimes have clearly led to a drastic skewing of the age structure of these remaining populations, such that the majority of plants have been reported to be either senescent or at least in the later stages of their life (Auld, 1990; Porteniers et al., 2001). No plant populations in the region have suffered the effects of the combination of the highly modified landscape and an intense and unnatural grazing in the semi arid region of NSW more so than those of several semi arid overstory *Acacia* species (Auld 1993; Cohn & Bradstock, 2000; Auld & Denham, 2001). The remaining stands of these *Acacia*, therefore provide ideal model systems to investigate the way in which long lived arid plants reproduce and persist within an acutely fragmented condition, under heavy grazing pressure.

The genus *Acacia* is one of the most specious in Australia, containing in the order of 1,000 species (Orchard & Wilson 2001). Currently 76 species are listed as vulnerable, endangered or critically endangered under the Australian Commonwealth *Environmental Protection and Biodiversity Conservation Act, 1999* (EPBC Act). These long lived *Acacia* species, capable of sexual and asexual forms of reproduction, have been monitored closely over the last 20 years and have been found to be contracting as old plants die and are not replaced by new recruits, which are voraciously consumed by feral and domestic grazers when they appear even via asexual suckering (Auld 1995; Davies, 1995; Ayers et al., 1996; Cohn & Bradstock, 2000; Auld & Denham, 2001). Whilst almost identical scenarios of recruitment failure have also been reported for foreign *Acacia* species (Wiegand et al., 2004), and many other plants around the world (Rohner & Ward, 1999; Brand, 2002; García, 2003; Zavaleta et al., 2007; Ottewell et al., 2010; Li, 2012), *Acacia* in this region are thought to be particularly vulnerable to these grazing pressures (Auld 1993, Cohn & Bradstock, 2000, Auld & Denham, 2001). Particularly in agricultural regions, *Acacia* populations are now typically restricted to small, linear populations along roads and railways, or isolated patches of remnant vegetation surrounded by cultivated land. Several species in the region are listed as endangered ecological communities or vulnerable and threatened species under the *NSW Threatened Species Conservation Act* given their dwindling population sizes and lack of regeneration.

It is likely that simple surveys of semi arid Acacia populations in western NSW stands greatly underestimate their perilous state as adults are often extremely long lived and populations may now consist of largely senescent plants (Porteniers, 2001). For species such as *A. carneorum*, death of the last remaining extremely old plants is imminent. It is widely accepted that conservation needs to happen before these plants senesce and die (Auld, 1993, 1995; Porteniers, 2001). Of further concern is that little to no sexual reproduction has been observed in many populations across the region for over two decades despite constantly flowering, making observations of seedling recruitment even more unlikely (Auld 1993, 1995, Porteniers, 2001). The lack of seed set in these populations is unexplained, and if truly unnatural is another significant obstacle to the recovery and persistence of these aging populations. Moreover, it eliminates the possibility of using seed to grow up seedlings for restocking contracting populations as a management strategy in the future.



Figure 1.2. Senescent *A. melvillei* plants at Mungo National Park in far western NSW.

### 1.6.1 Competing hypothesis for the prolonged lack of sexual reproduction

Several competing theories have been put forward to explain the lack of sexual reproduction in these populations centred on the age structure of remaining stands in isolation or in combination with their highly fragmented condition (Batty & Parsons, 1992; Auld, 1995). It might indeed be likely that several factors in combination are responsible for the current, documented reproductive failure in these species (Auld, 1995; Porteniers, 2001). Given the reduced size, isolation and generally poor condition these populations are now in, it could be argued that from a conservation standpoint a thorough investigation specifically into the

effects of fragmentation on biotic and abiotic processes is most critical (Jennersten, 1988; Menges, 1991; Ouborg et al., 1991; Petanidou et al., 1991; Van Treuren et al., 1991; Lamont et al., 1993).

One of the more popular theories for the lack of seed set in the overstory *Acacia* stands of western NSW, centres around the suggestion that age structure of current populations is drastically altered. As a result of prolonged, intense feral and domestic grazing removing both sexually and asexually derived *Acacia* recruits, populations left with largely old and senescing plants may be incapable of sexual reproduction. Indeed this phenomenon has been observed world-wide, especially in regions used for agricultural grazing (Jones, 1945; Janzen, 1986; Rohner & Ward, 1999; Brand, 2002; García, 2003; Zavaleta et al., 2007; Li, 2012). Even plants which are not quite old enough to be senescing may succumb to reduced reproductive vigour if local environmental stresses increase beyond a given threshold, as can happen with the cumulative effects of prolonged drought or climate change (Ritchie & Bolitho, 2008). Almost identical scenarios of recruitment failure have been reported in some foreign *Acacia* species with old, thinning and senescing populations predicted to go extinct (Wiegand et al., 2004). Alternatively, it has also been suggested that the lack of fruit / seed set in these isolated stands is a potential consequence of alterations to the mating system, lack of genetic diversity within stands consisting of incompatible clone mates, leading to pollen limitation or the deposition of incompatible pollen on flowers as discussed in detail above (Porteniers, 2001). It is also possible that this lack of seed set is simply a consequence of the natural reproductive strategy of these species, with reproduction being naturally rarer in these long lived species than we have imagined. It is also conceivable that the lack of sexual reproduction over such an extended period is explained simply by the fact that the period of observation coincided with an unusually prolonged and severe drought (Figure 1.4). The continued reproductive success of other co-occurring *Acacia* species such as *A. ligulata* and *A. victoriae* (Auld & Denham, *unpublished observations*) during this drought may point to differences in their reproductive strategies / mating systems.

There are huge gaps in our knowledge of these species reproductive ecology, making hypotheses about why they have not set seed for so long simply educated guesses / speculation. We know little about their mating systems including; what age these plants typically reproduce to, all associated pollinators serving each species, foraging behaviour, the capacity of pollinators to move pollen between isolated stands, fitness consequences of increased inbreeding, population genetic structure (genetic potential), levels of gene flow, levels of self compatibility, or capacity to recruit. Moreover, during drought, any attempts to

delineate between such hypotheses would be confounded given our inability to observe reproductive potential under what are likely to be more favourable climatic conditions or reproduction (Letnic & Dickman, 2006; Wardle et al., 2013). Studies of these species to date have uncovered some baseline information about the reproductive strategies and mating systems of several of these species; including that they can reproduce both sexually and asexually by suckering (Auld, 1990), they produce long lived soil stored seed banks (Auld, 1995) and they are almost certainly pollinated exclusively by insect pollinators (Gilpin et al., 2014). Most studies however have focused on documenting the contraction of populations and the effects of climate and unnatural grazing regimes on recruitment (Auld, 1995; Batty & Parsons, 1992), the fire ecology of these species (Hodgkinson & Oxley, 1990) and the effects of climate change on vegetation in the region (Appendix 1.7).

## **1.6.2 Conservation considerations**

Conserving the biodiversity of vegetation in this semi arid zone is, of course, of ecological importance and enshrined in legislation under Objective 1.7 of Australia's National Biodiversity Strategy 'Maintaining evolutionary potential' (Greening Australia undated; Mortlock, 1999; Brown et al., 2003). Given these *Acacia* plants are some of the only overstory species in this area, they are undoubtedly important ecological engineers in the region. Benefits of conserving these plants extend to the varied critical roles they play in this environment such as, providing shade trees for fauna, nesting sites for birds, a source of food, microclimates for smaller species of flora (Morton & Davis, 1983; Recher & Davis, 1997; Sharp, 1997; Facelli & Brock, 2000; Singh & Rathod, 2002) and role in stabilizing soils and preventing desert spread (Singh & Rathod, 2002). Nevertheless, only 3.47% of the NSW Western Plains is currently protected (Benson et al., 2006) as National Park and these areas also have long histories of agricultural grazing (Auld, 1995b). Damage to vegetation populations in protected areas may already be too great for them to fare significantly better than populations on unprotected agricultural land, especially when considering the continuing grazing pressures from rabbits and feral goats, even within local National Parks (Auld & Denham, 2001).

To date, official conservation recommendations, including those in the most recent report to the NSW National Parks and Wildlife Service regarding their current management of the Mungo National Park populations by Porteniers (2001), are centred on reducing grazing pressures on new recruitment (Auld & Denham, 2001; Davies, 1995; Ayers et al., 1996). Management measures being undertaken by government agencies include the development

and implementation of a suitable fire management strategy for particularly vulnerable populations, raising awareness of these species within the local community, protecting historically known seed sources, fencing off populations from grazing or other impacts by rabbits, stock, goats and kangaroos (Figure 1.3), and putting into place abatement plans for competition and land degradation by feral rabbits and goats. Without any current seed production however, this may be far from sufficient. More drastic measures may need to be considered so as not to hang the hope of recovery on these potentially aging trees producing seed once again. If the mating system of populations are in some way compromised, then active genetic rescue may be the only strategy to achieve true conservation of these populations and ensure optimally fit offspring are created. This has been recognised by the NSW government through their research priority actions which include the recognition that seed germination and / or vegetative propagation trials need to take place to determine the most efficient way to actively restore depleted stands.



Figure 1.3. *A. carneorum* stands fenced to exclude grazers (namely rabbits and goats) at Kinchega National Park in western NSW.

### 1.6.3 La Niña rain event provides a golden opportunity

The breaking of a decades long drought in the arid and semi arid regions of far western NSW with a prolonged La Niña driven rain event beginning in January of 2010 and lasting through 2012 (Figure 1.4), provided a timely opportunity to look at the reproductive response of these threatened *Acacia* populations for the first time, under seemingly optimal climatic conditions. The collection of seed and seedlings resulting from this rain provides a rare opportunity to collect novel information on fecundity levels, seed viability, recruitment

capacity, seedling growth rates and seedling mortality rates as well as a chance to make assessments about mating system health based on the fitness of offspring produced.

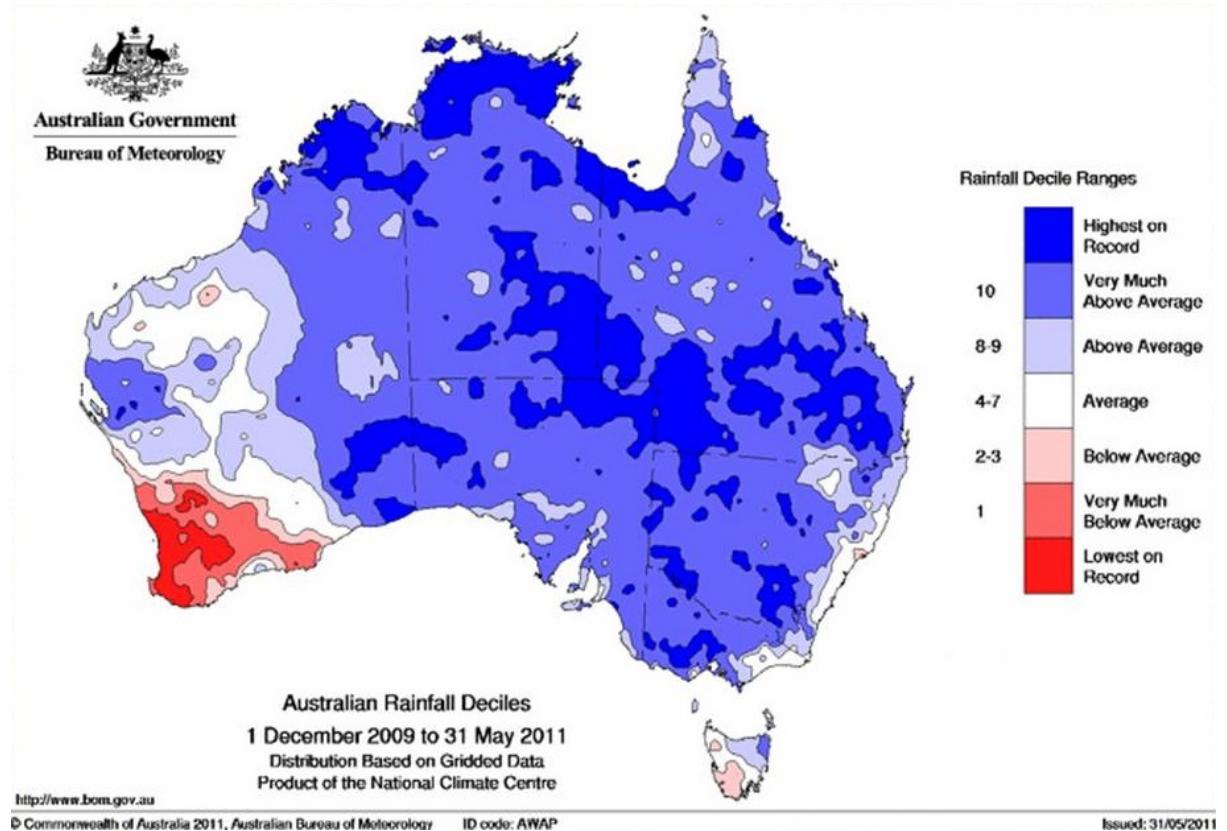


Figure 1.4. Australian Rainfall Deciles 1 December 2009 to 31 May 2011 (Australian Bureau of Meteorology).

#### 1.6.4 Thesis Questions

In this thesis I focus on stands of several threatened overstory *Acacia* species, as well as one species that is not officially threatened but is thought to face the same threats as those that and another that is thriving, all located across western NSW, and employ a multifaceted, multi disciplined and comparative approach, combining quantitative surveys, genetic analysis, and experimental manipulations to attempt to answer the following questions:

1. Are stands old and senescing across their whole range in western NSW?
2. Are stands highly fragmented and in poor condition, or is there important heterogeneity between them?

3. Are stands still capable of sexual reproduction (fruit / seed set) and sexual recruitment given a large scale rain event?
4. Is reproductive success and failure determined by the physical condition of stands, and if so what plant / stands condition parameters or combination of parameters predict reproductive success and failure?
5. Are mating systems of these species currently suboptimal or particularly susceptible to any increased levels of fragmentation?
6. What conservation strategies should managers adopt to conserve these stands?

## 1.7 Study Species

Four species of *Acacia* (*A. melvillei*, *A. homalophylla*, *A. loderi* and *A. carneorum*) which exist within the highly fragmented semi arid regions of far western NSW were chosen for this study. Three are officially characterized as either threatened ecological communities or endangered species (*A. melvillei*, *A. loderi* and *A. carneorum*) in the *NSW Threatened Species Conservation Act* and one which occurs in a relatively more eastern distribution (*A. homalophylla*), is not officially listed as threatened in NSW but is thought to be facing the same pressures. These species were chosen primarily to provide a range of species all experiencing broadly equivocal pressures with respect to their diminished sizes and connectivity as well as high grazing pressures and a lack of sexual reproduction for approximately two decades. A co-occurring thriving *Acacia* species (*A. ligulata*) was also chosen to study in parallel to provide a comparison.

### 1.7.1 *Acacia melvillei* and *Acacia homalophylla*



*A. melvillei* (above left) is an overstory tree species that together with *A. homalophylla* (above right) are commonly referred to as Yarran. *A. melvillei* and *A. homalophylla* are very difficult to separate without their seed pods in which seeds are longitudinally arranged in narrower pods in *A. homalophylla*. *A. melvillei* are reported to hybridize with *A. homalophylla* where their distributions overlap along the eastern edge of *A. melvillei*'s distribution (Chapman & Maslin, 2001). Trees can grow to 15 m high and produce typically globose Acacia inflorescences containing typically 30-50 bright yellow flowers per inflorescence. Plants flower between August and October, and fruit matures between January and February. Plants occur in south-eastern Queensland from Clermont to Stanthorpe and across central NSW, extending to Mildura and Kerang in Victoria. *A. melvillei* in the Riverina and Murray-Darling Depression bioregions has been listed as an Endangered Ecological Community in Part 3 of Schedule 1 of the NSW Threatened Species Conservation Act 1995.

### 1.7.2 *Acacia loderi*



*A. loderi* can grow to approximately 10m high. They produce the same bright yellow inflorescences with 20-30 flowers each. Seed pods are up to 10 cm long, 3–5 mm wide and smooth. Seeds are longitudinally arranged within seed pods. Plants flower between August and October, and fruit matures between January and February. Plants occur in western New South Wales, west of Hillston and north of White Cliffs, extending into South Australia to Oakbank Station and Netley Gap. *Acacia loderi* in the Riverina and Murray-Darling Depression bioregions has been listed as an Endangered Ecological Community in Part 3 of Schedule 1 of the NSW Threatened Species Conservation Act 1995.

### 1.7.3 *Acacia carneorum*



*A. carneorum* grows to 5 m high and produces yellow *Acacia* inflorescences containing typically 35–60 flowers per inflorescence. The heartwood is a striking deep purple colour. Seed pods are rarely produced, but are narrow, oblong and about 2–5 cm long and 8–12 mm wide. Plants flower in January and mature between June and September. Plants are scattered from southwest of Lake Frome and near Peterborough, South Australia to near Tibooburra and Menindee Lakes in New South Wales. *A. carneorum* is an excellent sand stabiliser due to its tendency to grow suckers and forms large mono clonal stands. *A. carneorum* is also listed as vulnerable under Schedule 2 of the *NSW Threatened Species Conservation Act 1995* and under Schedule 8 of the *National Parks and Wildlife Act 1992* (South Australia).

#### **1.7.4 *Acacia ligulata***



*A. ligulata* is a dense shrub or tree up to approximately 4 m high. They produce bright yellow inflorescences containing typically 20–30 flowers per inflorescence. Seeds have a distinct yellow-orange or red aril which is likely to attract seed dispersers. Plants flower between August and October, and fruit matures between January and February. They are widespread in central and southern arid Australia, occurring in all mainland states. Plants usually grow in sand, often in mulga and mallee communities, and are associated with sand dunes which once colonized, they may help to create. *A. ligulata* reproduces only through sexual means and cannot reproduce asexually. They are generally thought to be thriving compared to many of the other longer lived overstory *Acacia* species where they co-occur.

## 1.8 Thesis structure

The data chapters (2-9) of this thesis have been intentionally constructed in scientific paper format, despite maintaining a traditional thesis form where each data chapter builds on the previous one to tell a larger overall story. I have written each data chapter as stand alone manuscripts with the intention of speeding up the process of generating publications from these chapters upon completion of this thesis. In maintaining data chapters as stand alone units, some repetition has been unavoidable in both the introductions and discussions of these chapters, and in the general introduction (Chapter 1) and discussion (Chapter 10). I have grouped all references at the end of the thesis rather than after each data chapter in an attempt to cut back on redundancy. I have also minimized repetition by simply referencing figures (i.e. maps) from previous chapters rather than including them each and every time they are relevant.

As reports of the aging / senescent demographic structure of these *Acacia* stands within western NSW have so far been qualitative in nature, **Chapter 2** importantly begins by testing these observations quantitatively. Specifically, in Chapter 2 I test the hypothesis that stands of *A. melvillei*, *A. homalophylla*, *A. loderi*, *A. carneorum* and *A. ligulata* consist of mainly old and senescent plants across western NSW. To do this I characterize the demographic structure of 133 stands of *A. melvillei*, *A. homalophylla*, *A. loderi*, *A. carneorum* and *A. ligulata* spread across 430, 000 km<sup>2</sup> of western NSW.

As theories for the lack of seed set in these *Acacia* stands have centred around either their poor condition or the impact of a prolonged drought, in **Chapter 3**, I test the hypothesis that the same *Acacia* stands are in too poor a condition to reproduce sexually, even given optimal climatic conditions. To test this hypothesis, I use a subset or all of the stands used in Chapter 2 to survey them for flowering effort, presence of pollen tubes and fruit / seed set after a large scale La Niña rain event across the region. I also look for any evidence that reproductive effort is reduced in the oldest plants making up these stands.

Having found a strong reproductive response to the La Niña rain in the majority of stands surveyed in Chapter 3, a lack of seed set in the majority of *A. carneorum* stands and a minority of stands of the other species surveyed, required explaining. In **Chapter 4**, I test the hypothesis that reproductive (seed set) success and failure in these *Acacia* stands was determined by their relative structure and condition. To test these hypotheses, I measure and compare several structural, plant health, local environmental health and local climatic

parameters between stands, as well as measurements of genetic diversity within stands, to look for evidence that reproductive capacity / effort is reduced in stands that are relatively small and acutely fragmented compared to those in better condition.

Given that high levels of fecundity does not necessarily mean adequate levels of recruitment, especially in highly modified landscapes with intense grazing pressures, in **Chapter 5** I test the hypothesis that these *Acacia* stands are incapable of recruiting seedlings irrespective of their physical structure and condition. To test this, I combined laboratory and field tests of seed and seedling fitness, with region wide surveys of sexual recruitment in the same stands surveyed in Chapters 2, 3 & 4. I also look for any correlations between the structure and condition of stands measured in Chapter 4 and the number of seedlings recruited within stands.

As I could find no evidence to link reproductive success and failure to the physical structure / condition of these *Acacia* stands, but found evidence for a genetic mechanism, I focused on the least and most reproductively responsive *Acacia* species in the region to look for differences in their mating systems that might explain this difference. In **Chapter 6**, I test the hypothesis that there is a significant difference between the pollination of *A. carneorum* and *A. ligulata* plants, as well as between *A. carneorum* stands, with and without a history of setting seed. I test this hypothesis by observing the types, numbers and behaviour of pollinators visiting multiple *A. carneorum* and *A. ligulata* stands as well as assessing the capacity of individual pollinators to carry pollen. I also analyse flowers for the presence of pollen tubes. In **Chapter 7** I test the hypothesis that historic failure to set seed in most *A. carneorum* stands is a function of plants receiving incompatible pollen. I take advantage of rare conditions produced by a region wide rain event, to present *A. carneorum* plants with pollen from a variety of local and distant sources and assess the success and fitness of resulting offspring to test this hypothesis. I also run these experiments on *A. ligulata* simultaneously as a comparison, to assess the effect of pollen source on the fecundity and fitness of offspring produced in a thriving and readily reproducing co-occurring *Acacia* species. In **Chapter 8**, I test the hypothesis that rare *A. carneorum* seed are all a product of selfing or mating with local clone mates. To test this I performed paternity analysis on seed set on several maternal plants from one unusually fecund monoclonal *A. carneorum* stand, to determine the proportion of seed that have come by way of selfing / inbreeding and outbreeding with nearby stands.

Given mine and previous findings that *A. carneorum* stands are almost all genetically monoclonal, and finding zero seed set in most stands despite seemingly optimal climatic

conditions for reproduction, in **Chapter 9** I test the hypothesis that the lack of sexual recruitment predates European influence in the region and therefore could not be a sole consequence of introduced grazers removing seedlings. To test this hypothesis, I carbon date multiple recently deceased *A. carneorum* trees to get an estimate of the age of stands.

Finally, **Chapter 10** brings each of these chapters together in a general discussion of the condition and reproductive health of these *Acacia* stands, and makes predictions about their long term persistence as well as recommending appropriate management.

At the end of this thesis, I present the full manuscript for a paper already published during my PhD tenure (which I have not included as a data chapter) for which I am the first author as well as details for two published papers, one published article and one paper in review in PLoS ONE to which I contributed.

## **Chapter 2: Assessing the demographics of threatened and thriving semi arid *Acacia* in far west NSW.**

### **2.1 Abstract**

The aging and subsequent contraction of populations of overstory tree species in rangelands is well documented worldwide. This largely reflects anthropogenic pressures such as agricultural land clearing, together with increased grazing from livestock and feral grazers leading to increased mortality and reduced recruitment. Without recruitment, stands that may already largely comprise old and senescent plants may soon go locally extinct. In far western New South Wales (NSW), several *Acacia* species subject to intensive grazing by domestic and feral herbivores are claimed to display prolonged recruitment failure but there have been no quantitative range wide demographic surveys. To test the hypothesis that stands now consist of old senescent plants, I characterized the morphology of plants within 133 stands of three such threatened *Acacia* species (*A. melvillei*, *A. loderi*, *A. carneorum*), one potentially threatened species (*A. homalophylla*) and the thriving broadly co-occurring *A. ligulata*, across approximately 430,000 km<sup>2</sup> of western NSW. I found that the vast majority of *A. melvillei*, *A. loderi* and *A. carneorum* stands surveyed across the region were populated only by large mature plants, with the vast majority of *A. loderi* and *A. carneorum* plants within these stands characteristically displaying reduced canopy cover indicative of senescence. In contrast, all *A. homalophylla* and *A. ligulata* stands were comprised of plants of a range of sizes, indicating more consistent recruitment, although this has almost exclusively come by way of intense suckering in *A. homalophylla* stands. *A. ligulata* stands displayed a more even spread among morphological classes and a high proportion of putative recruits. My findings provide the first rigorous support for the claim that almost everywhere, stands of several threatened overstory *Acacia* species are at risk due to senescence and recruitment failure. It remains to be determined to what degree these patterns reflect persistent reproductive and/or recruitment failure, or the episodic nature of recruitment in these species.

## **2.2 Introduction**

Worldwide there are many examples of fragmented plant populations experiencing elevated levels of grazing, displaying little recruitment and now consisting largely of old senescent plants, especially in regions used for agricultural grazing (Jones, 1945; Janzen, 1986; Rohner & Ward, 1999; Brand, 2002; García, 2003; Zavaleta et al. 2007; Li, 2012). Indeed many species that form shade trees on agricultural land in Australia are now nearing the end of their life and recruitment is not occurring even when adults are still fecund (Ottewell et al., 2010). For such populations, even if anthropogenic clearing and grazing pressures were ameliorated or stopped altogether, recovery of these populations would be difficult to achieve or even unlikely to succeed. As such, remaining stands of plants, that have become structured in this way need to be identified as early as possible in order to maximise the opportunity to implement conservation strategies. Given that overstory plant species are expected to provide crucial ecological roles in rangelands, especially when they represent the dominant or only overstory plant species in an ecological community, their loss from landscapes can be devastating for many other species that rely on the services they provide (Morton & Davis, 1983; Recher & Davis, 1997; Sharp, 1997; Facelli & Brock, 2000; Singh & Rathod, 2002). The benefits of conserving overstory trees must, of course, include the retention of critical roles such as nutrient cycling, providing shade for other understory species and fauna (Belsky et al., 1993; Milton & Dean, 1995), homes for vertebrates and invertebrates (Morton & Davis, 1983; Recher & Davis, 1997; Sharp, 1997; Facelli & Brock, 2000), improving soil quality through Nitrogen-fixing (Scholes & Walker, 1993) and stabilizing soils (Singh & Rathod, 2002).

Arid and semi arid ecosystems occupy 40% of the Earth's land surface and are among the systems predicted to be most sensitive to climate change (Hughes & Westoby, 1994; Hughes, 2003). Although the available data is either qualitative in nature or restricted to studies of a small number of stands, it is widely agreed that in far western NSW, natural vegetation cover has declined markedly over the past 150-plus years as a result of agricultural practices involving clearing as well as intense grazing pressures from feral grazers (Batty & Parsons, 1992; Auld, 1995b; Porteniers, 1998; Benson, 1999; Auld & Denham, 2001; Porteniers, 2001; Denham et al, 2014). For a suite of long lived overstory semi arid *Acacia* species capable of both sexual and asexual reproduction, almost complete and prolonged recruitment failure has led to a shift towards older plants and further contraction of their already fragmented distribution (Auld 1993, 1995, Auld & Denham, 2001; Porteniers, 2001). Previous studies

attribute the lack of fruit set and recruitment in these species, in part, to the onset of senescence (Porteniers, 2001), however, this hypothesis has not been rigorously tested.

Here I use a comparative approach for the first time to characterize the morphological structure of stands of five overstory *Acacia* species with apparently contrasting life histories, ranging from highly clonal species to obligate seeders. Specifically I use morphological characteristics as proxy measurements of age to infer the likely age distribution of the remaining trees in multiple stands of four threatened locally occurring *Acacia* species spread across approximately 430,000 square kilometres of western NSW east and west of the Darling River. I then use this data to ask whether the age structure of extant stands appear to be comprised largely of senescent plants devoid of any signs of recent recruitment, as has been reported previously. I also compare the morphological structure of stands of the four threatened species with a co-occurring *Acacia* species that is apparently thriving in the same modified landscape.

## **2.3 Methods**

### **2.3.1 Study species and study area**

This study focuses on three officially threatened overstory *Acacia* species (*A. melvillei*, *A. loderi* and *A. carneorum*), as well as one that is closely related and whilst not yet officially recognized as threatened but faces the same or similar threats that are found within the semi arid region of NSW (*A. homalophylla*) and one co-occurring but thriving species (*A. ligulata*) (Figure 2.1).

*Acacias* are among the few larger overstory tree species native to the arid and semi arid zones of south-east Australia. *A. melvillei*, *A. loderi* and *A. carneorum* are listed as endangered or threatened communities under the ‘NSW Threatened Species Conservation Act’. *A. homalophylla* communities, which together with *A. melvillei* make up the Yarran complex (Pedley, 1978), are not officially listed as threatened, however it is also the dominant overstory species within its ecological community and faces the same threats. As such, throughout this thesis I will group *A. homalophylla* in with *A. melvillei*, *A. loderi* and *A. carneorum* as threatened species, even though it has not been officially listed yet.

These species are difficult to separate unless fruiting. All four of these overstory *Acacia* are capable of both sexual and asexual reproduction, producing suckers as the latter form. While recognized threats to these species are diverse, in all cases they include loss of habitat and lack of recruitment as a result of land clearing and grazing by livestock and feral grazers (see Table 2.1). I also characterize the demographics of stands of the obligately seeding species *A. ligulata*, which in contrast to the threatened species, appears to be thriving and reproducing well. While recognized threats to these species are diverse, in all cases they include loss of habitat and lack of recruitment as a result of land clearing and grazing (see Table 2.1). The varied and critical roles these overstory *Acacia* play as ecosystem engineers in this environment, providing shade trees for fauna, nesting sites for birds, a source of food, microclimates for smaller species of flora and their role in stabilizing soils and preventing desert spread are well known (Morton & Davis, 1983; Recher & Davis, 1997; Sharp, 1997; Facelli & Brock, 2000; Singh & Rathod, 2002).

Table 2.1. **Characteristics of the study species *A. melvillei*, *A. homalophylla*, *A. loderi*, *A. carneorum* and *A. ligulata*:** Life history, environment typically inhabited, geographic distribution across Australia, Sampling distribution across NSW (number of stands sampled), and Legislative status.

Species	Life history	Environment / soil type	Geographic distribution (Nationally)	Sampling area/number of stands (NSW)	Legislative status (NSW)
<i>A. melvillei</i> *	Mainly sexual reproduction, but can reproduce asexually (sucker)	Grows adjacent to Belah ( <i>Casuarina pauper</i> ), Rosewood ( <i>Alectryon oleifolius</i> ), White Cypress Pine ( <i>Callitris glaucophylla</i> ) woodlands, or sand plain mallee ( <i>Eucalyptus socialis</i> , <i>Eucalyptus dwyeri</i> , <i>Eucalyptus morrisii</i> ) in red-brown clayey duplex and sandy loam soils.	Widespread in semi arid and arid NSW, rare in Vic.	Approx. 336,000 km <sup>2</sup> (47 stands).	<i>A. melvillei</i> shrublands is an Endangered Ecological Community
<i>A. homalophylla</i> *	Mainly sexual reproduction, but can reproduce asexually (sucker)	Grows in amongst Belah ( <i>Casuarina pauper</i> ), Rosewood ( <i>Alectryon oleifolius</i> ) and Box gum ( <i>Eucalyptus populnea</i> ) communities, in solonized brown earths.	Widespread in semi arid NSW and Qld.	Approx. 135,000 km <sup>2</sup> (10 stands).	None
<i>A. loderi</i>	Mainly sexual reproduction, but can reproduce asexually (sucker)	Grows adjacent to Belah ( <i>Casuarina pauper</i> ), Rosewood ( <i>Alectryon oleifolius</i> ), White Cypress Pine ( <i>Callitris glaucophylla</i> ) woodlands, or sand plain mallee ( <i>Eucalyptus socialis</i> , <i>Eucalyptus dwyeri</i> , <i>Eucalyptus morrisii</i> ) in red-brown clayey duplex and sandy loam soils.	Widespread in Semi arid and arid NSW and eastern SA, rare in Vic.	Approx. 242,000 km <sup>2</sup> (26 stands).	<i>A. loderi</i> shrublands is an Endangered Ecological Community
<i>A. carneorum</i>	Mainly sexual reproduction, but can reproduce asexually (sucker)	Grows in and adjacent to Belah ( <i>Casuarina pauper</i> ) and Rosewood ( <i>Alectryon oleifolius</i> ) communities in sandy duplex soils and alluvial sands.	Widespread in arid NSW west of the Darling River and in Eastern SA.	Approx. 108,000 km <sup>2</sup> (30 stands).	Vulnerable
<i>A. ligulata</i>	Obligate seeder	Grows in a wide variety of environments in sandy soils and alluvial sands.	Widespread in arid and semiarid Australia (NSW, Vic, SA, Qld, NT, WA)	Approx. 135,000 km <sup>2</sup> (20 stands).	None

\*Information from this table was sourced from <http://www.environment.nsw.gov.au/determinations>

### 2.3.2 Selection of stands

To characterise demographic structure of the five focal *Acacia* species across the vast majority of their distribution (up to 336,000 km<sup>2</sup> of western NSW), multiple stands of each species were selected for survey during a region wide search for remaining stands (see Table 2 for numbers and Figure 2.1 for geographic locations). Fewer stands of *A. homalophylla* were surveyed than the other four *Acacia* species because I did not set out to survey this species originally. Ten of the stands I initially identified as *A. melvillei*, were later identified as *A. homalophylla* after seed were found on these plants. This is the only way to distinguish these plants morphologically. Rather than removing these stands from further analysis I decided to include them despite acknowledging this is a small sample size. GPS readings of each population were taken at each site (Appendix 1.9).

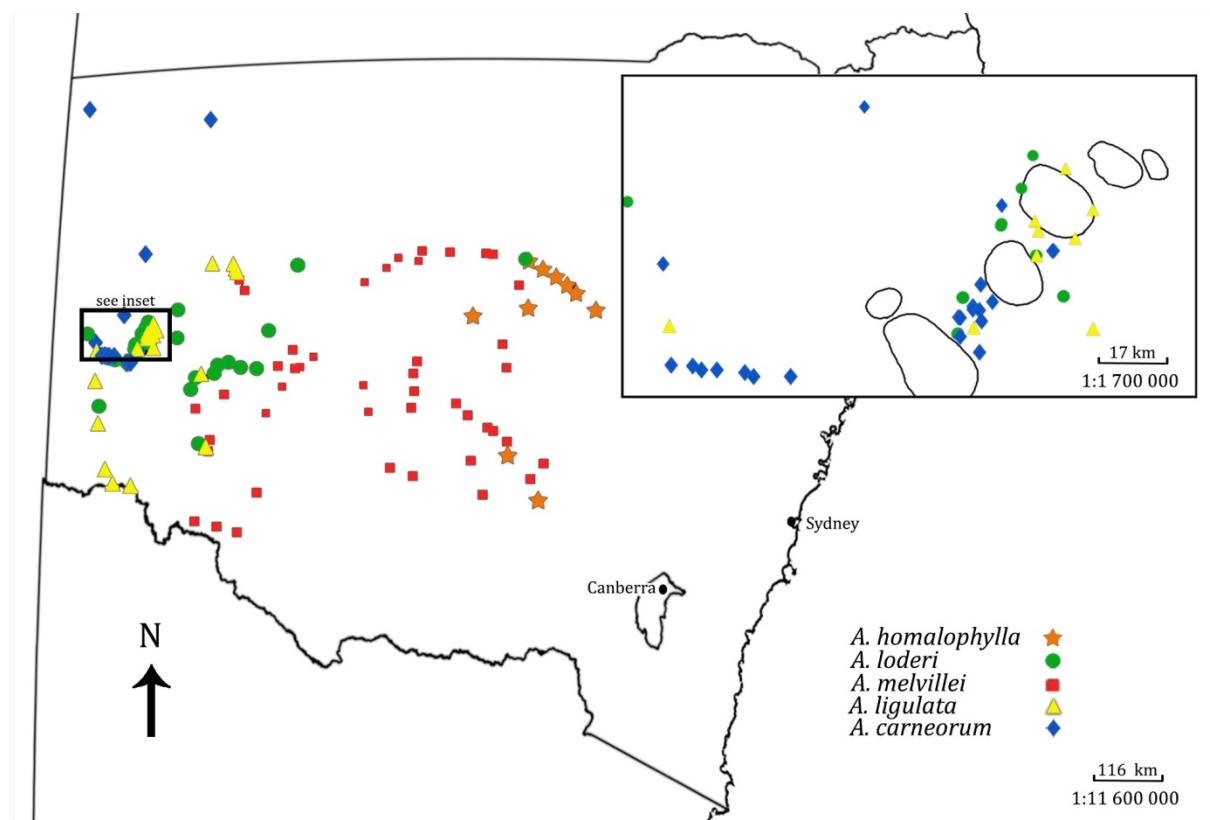


Figure 2.1. Distribution of 47 *A. melvillei*, 10 *A. homalophylla*, 26 *A. loderi*, 30 *A. carneorum* and 20 *A. ligulata* stands surveyed across NSW.

### **2.3.3 Sampling of plants within stands**

At each stand a single belt transect (3m wide) was used to select plants for study. A single ‘independent’ plant (deemed to have no underground root connections with a parental plant), over 2 metres tall (to avoid sampling juvenile plants), located within the approximate centre of each stand, was selected haphazardly as the starting point for a transect. The orientation of the transect chosen was at random from the four major compass directions before I then sampled the first ten non juvenile ‘independent’ plants (over 2 metres) encountered on the transect, as well as all juvenile ‘independent’ plants (under 2 m tall), and suckers that were found along the line of the transect (suckers were classified as plants with obvious lateral roots currently connecting to at least one other plant and were determined by removing top soil to uncover such lateral connections wherever it was not obvious). Ten independent plants were chosen because wider searches of each stand suggested that the plants comprising these stands were extremely homogenous. I was confident that further sampling would not significantly alter the characterization of stand structure and plant health achieved by my sampling effort. Given the limited time I had to conduct field work in the region, it was decided that surveying no more than 10 plants per stand would allow me to travel to and characterise stands across the whole region (sometimes stands comprised of < 10 plants).

For each sampled plant, I measured the height /length (metres), trunk circumference (cm) and percentage of canopy covered by live foliage. I found a strong linear relationship between plant height and trunk circumference for each of the five species ranging from  $r^2 = 0.88$  to  $0.978$  (Appendix 2.6.1), and because plant height and plant circumference were not independent, height was not used in any further analysis of plant morphology.

### **2.3.4 Defining and assigning morphological ‘classes’ of plants for each species**

The relative age of plants within *Acacia* stands was estimated by grouping plants into morphological classes based on their stature and the condition of their canopy, as has been utilized in previous studies (Harper, 1977). Cluster analysis of the morphological data collected above for each plant was performed using the program Primer 6 which uses a ‘nearest neighbour model’ with strict default threshold parameters (95% confidence thresholds) to distinguish between distinct groups of plants based on the trunk widths and canopy cover. Thresholds for distinct morphological classes were identified from the analysis such that plants with a similar trunk circumference (cm) and canopy cover (%) were assigned to a common

morphological class (defined by minimum and maximum trunk width and canopy cover measurements). This approach was also used to distinguish independent plants, and suckers were distinguished from each other within each of these categories. Where a species clearly lacked plants within a morphological range that was flanked by two clearly defined morphological groups of plants, a ‘virtual’ morphological class was assigned so that a total of five comparable morphological classes were assigned for each of the *Acacia* species of interest. In cases where I could not distinguish distinct groupings of plants that spanned a large size range using cluster analysis (ie, where there was continual recruitment), plants were divided up artificially based on their trunk circumference for a total of 5 morphological classes.

I paid special attention to some senescent *A. melvillei*, *A. loderi*, *A. carneorum* and *A. ligulata* plants that were assigned to a morphological class in 2011 but subsequently died in 2012. These particular plants provide a quantifiable morphological profile of trees likely to be at the end of their life span in each of these species, and to provide some circumstantial evidence for the choice of my morphological class 5 which I expect to represent the oldest plants (Figure 2.2). I did not observe any *A. homalophylla* plants die during the course of this study.



Figure 2.2. Example of a typical senescent *A. melvillei* plant within extant stands in Mungo National Park, western NSW that died during the period of study.

### **2.3.5 Stand morphology**

#### **a) Defining and assigning morphological ‘Types’ to stands**

Once each plant was assigned to a morphological class, the morphological structure of each stand was characterised by calculating the percentage of plants per stand that belonged to each of the morphological classes identified above. In line with previous studies that have used J-curves to characterize the relative ages of stands of plants (Drewa et al., 2008; Venter & Witkowski, 2010; Cousins et al., 2014; Peltzer et al., 2014), here I assign stands with a similar proportion of plants of the same age classes into the same stand ‘Type’. All the stands surveyed here of all five *Acacia* species were assigned at stand ‘Type’, increasing from ‘Type 1’ upwards to ‘Type 5’ as the proportion of older plants (plants of a higher morphological ‘class’) within a stand increased.

Type 1 stands are characterized as having > or = to 80% of their plants in groups 1 and 2. Type 2 stands are characterized as having > or = to 50% but < 80% of their plants in groups 1, 2 and 3. Type 3 stands are characterized as having > or = to 50% of their plants in groups 3, 4 and 5. Type 4 stands are characterized as having > or = to 50% but < 80% of their plants in group 5. Type 5 stands are characterized as having > or = to 80% of their plants in group 5.

#### **b) Assessment of the of stand demographics across the region**

For each of the four *Acacia* species, the proportion of all the stands surveyed across the region that were classed as a distinct stand ‘Type’ was calculated to test the hypothesis that stands across the region were homogenously structured.

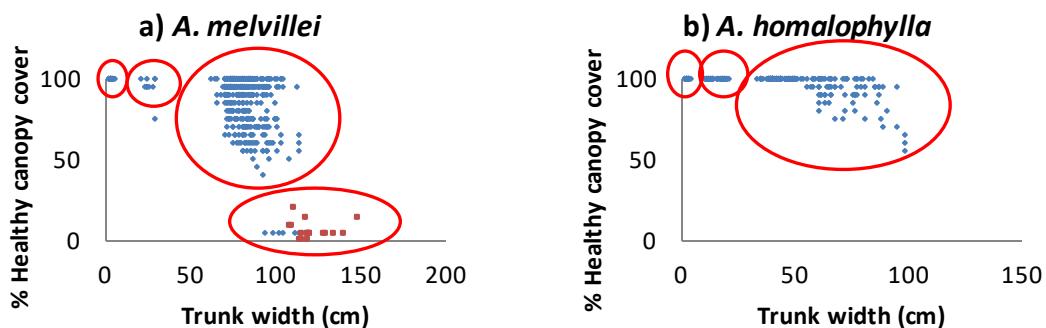
## **2.4 Results**

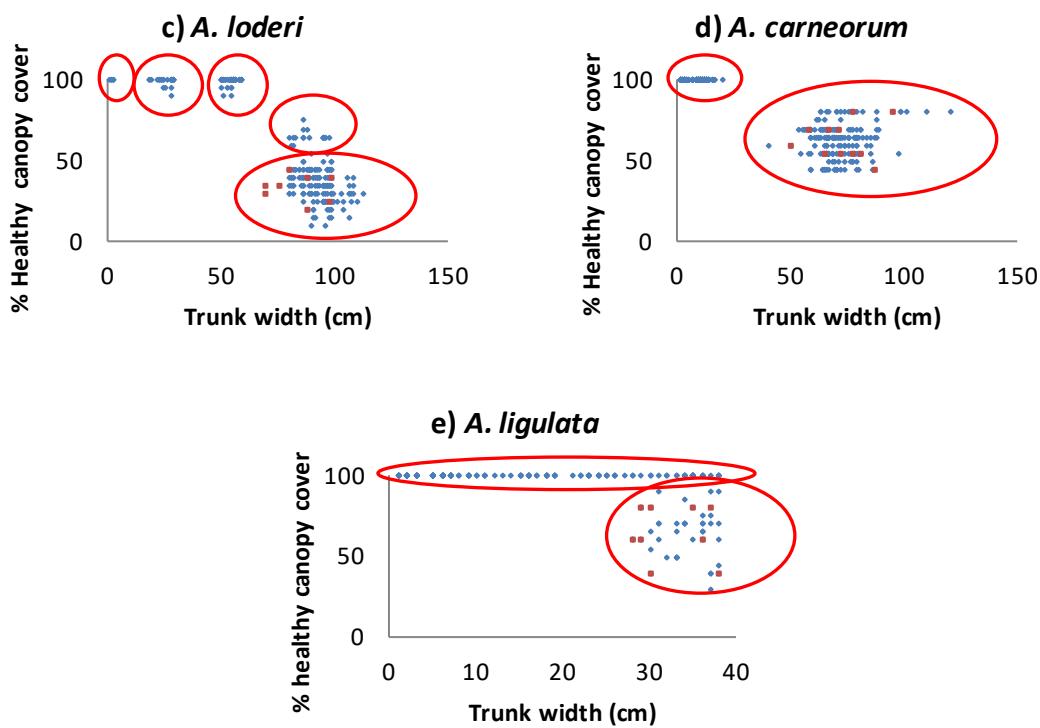
### **2.4.1 Defining and assigning morphological ‘classes’**

Cluster analysis using trunk width and percentage canopy cover as variables to assign plants to distinct groups, revealed 4, 4, 5, 3 and 2 clearly distinct morphological groups of plants for *A. melvillei*, *A. homalophylla*, *A. loderi*, *A. carneorum* and *A. ligulata* respectively (Figure 2.3 a-e). When morphological groups identified by cluster analysis spanning a disproportionate range of trunk width and canopy cover percentages were divided (where less than five morphological groups were identified), a total of 4, 5, 5, 4 and 3 clearly distinct morphological groups of plants for *A. melvillei*, *A. homalophylla*, *A. loderi*, *A. carneorum* and *A. ligulata*.

*ligulata* respectively, were found. 1, 1, 2 and 3 virtual groups were then also assigned for *A. melvillei*, *A. homalophylla*, *A. carneorum* and *A. ligulata* respectively. When suckers were excluded and only independent plants counted only 2, 2, 1, 3, and 3 distinct morphological groups of *A. melvillei*, *A. homalophylla*, *A. loderi*, *A. carneorum* and *A. ligulata* plants were found respectively. For each species the characteristics of each of the five morphological classes are presented in Table 2.2, and an illustration of the general morphology of each class of plants is provided in Figure 2.4. For all five *Acacia* species, plants assigned to morphological classes one to five represented plants that could be thought of as new recruits, young plants, immature plants, mature plants and senescent plants (Figure 2.4).

All the 10 *A. melvillei*, 6 *A. loderi*, 5 *A. carneorum* and 10 *A. ligulata* plants observed to have died within a twelve month period of these surveys had been assigned to morphological class 5, further supporting the hypothesis that morphological class 5 represents the oldest (and senescent) plants (Table 2.2). This represents 52.6, 4.1, 2.6 and 22.7% of all the plants classed as morphological class 5 in the *A. melvillei*, *A. loderi*, *A. carneorum* and *A. ligulata* stands respectively. No *A. homalophylla* plants were observed to die during the period of this study.





**Figure 2.3 a-e. Grouping of *Acacia* plants from each of five species into two to five morphological classes using nearest neighbour model cluster analysis (Primer 6):** clustering made use of data for 470, 260, 200 and 200 plants across 47, 10, 26, 30, and 20 stands of *A. melvillei*, *A. homalophylla*, *A. loderi*, *A. carneorum* and *A. ligulata* respectively. Clustering was based on trunk circumference and the percentage of the canopy covered in healthy foliage. Blue points represent plants surveyed that were alive at least a year after this survey, while red points represent plants that died within a year of being surveyed.

**Table 2.2. Characteristics of morphological classes assigned to *A. melvillei*, *A. homalophylla*, *A. loderi*, *A. carneorum* and *A. ligulata* plants, in 133 stands across western NSW:** Trunk circumference (cm) and percentage of the canopy covered in healthy foliage of plants were used to determine morphological classes for each species, using nearest distance cluster analysis or artificially where no clear clusters were found.

		<i>A. melvillei</i>	<i>A. homalophylla</i>	<i>A. loderi</i>	<i>A. carneorum</i>	<i>A. ligulata</i>
# of morphological classes identified	All plants (independent plants and suckers)	4	4	5	4	3
	Independent plants only	2	2	1	3	3
Method of assigning morphological class	Via cluster analysis	4	4	5	3	2
	Assigned due to lack of plants	1	1	0	2	3
Dimensional cut offs for morphological classes	Morphological class 1 (New recruits)	Trunk circumference (cm)	0.5-5	0.5-5	0.5-5	0.5-8*
		% canopy covered in healthy foliage	100	100	100	100
		Number of plants	311	31	5	70
	Morphological class 2 (Young plants)	Trunk circumference (cm)	6-36	6-17	20-30	6-20*
		% canopy covered in healthy foliage	90-100	100	90-100	100
		Number of plants	10	17	26	30
	Morphological class 3 (Immature plants)	Trunk circumference (cm)	37-59*	18-47	51-60	>20 < 50*
		% canopy covered in healthy foliage	> 40*	100	90-100	< 80*
		Number of plants		153	50	34
	Morphological class 4 (Mature plants)	Trunk circumference (cm)	60-120	48-88	70-100	>50*
		% canopy covered in healthy foliage	40-100	55-100	50-70	> 80*
		Number of plants	427	113	25	34
	Morphological class 5 (Senescenting plants)	Trunk circumference (cm)	90-155	> or = 55*	70-120	50-120
		% canopy covered in healthy foliage	1-25	<55*	1-49	45-80
		Number of plants	19		148	197
Plants that were observed to die within a year of the survey		No. of plants observed to die/ within no. of stands they were found in	10 (4)	0	6(4)	5(3)
		Morphological classes represented	All class 5	n/a	All class 5	All class 5

\*Denotes measurements that have been assigned due to lack of plants within the trunk circumference and canopy cover ranges.

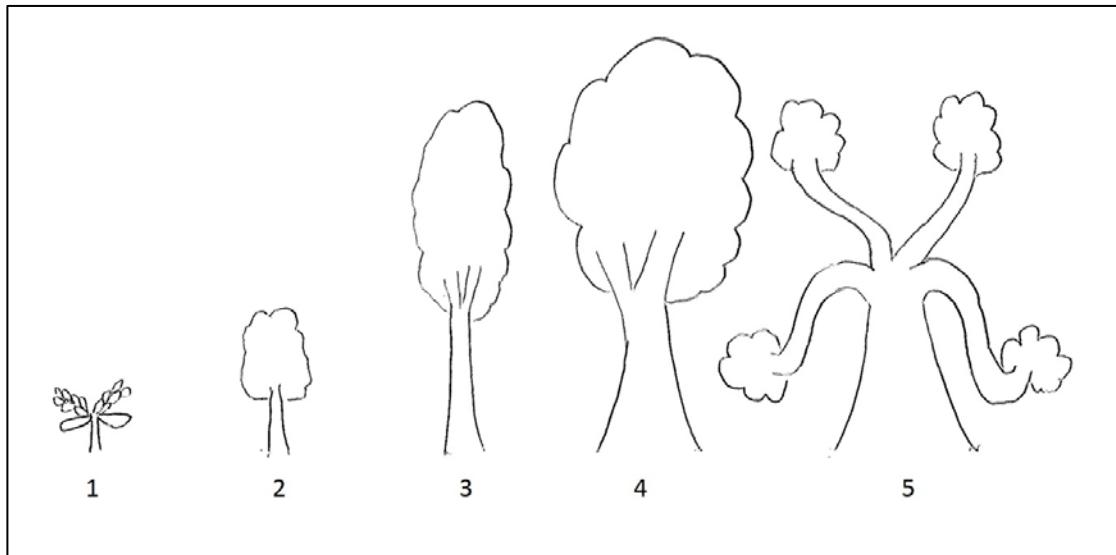


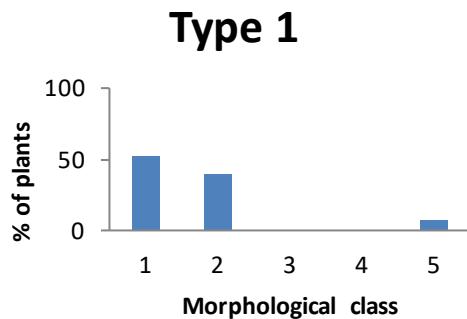
Figure 2.4. **Typical shape of trees in each of the morphological classes that plants were assigned to:** Class 1 represents new recruits, class 2 represents small immature plants, class 3 represents intermediate sized immature plants, class 4 represents mature plants and class 5 represents old senescent plants. \*Note that these illustrations are not to scale.

## 2.4.2 Stand Morphology

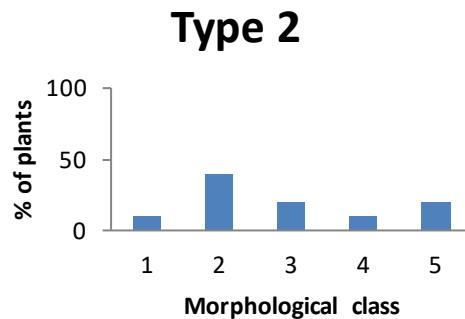
### a) Defining and assigning morphological 'Types' to stands

After review of the morphological structures of all stands for all five *Acacia* species, I qualitatively identified five general structures (stand 'Types'). These stand 'Types' were labelled 1 to 5, with 1 containing the highest proportion of young plants and 5 having the highest proportion of old plants. More specifically, stand 'Type' 1 is characterized by a majority of young plants and few if any older ones; 'Type' 2 stands have an almost even spread of plants across all morphological classes; 'Type' 3 stands are best described by a bell curve shape with the majority of plants in the intermediate morphological class; 'Type' 4 stands, whilst still displaying moderate numbers of plants of most morphological classes, have more older plants than younger ones; 'Type' 5 stands consist mainly of older plants in morphological classes 4 and 5, or just one of these two morphological classes (Figure 2.5a-e). Some photos of stands of these *Acacia* in the region are displayed below (Figure 2.6).

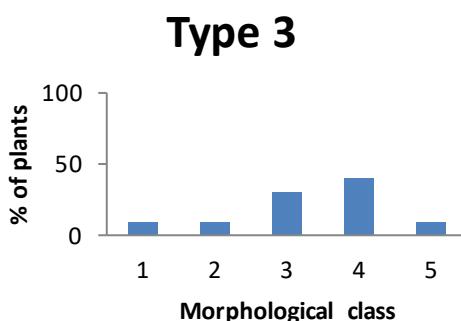
a)



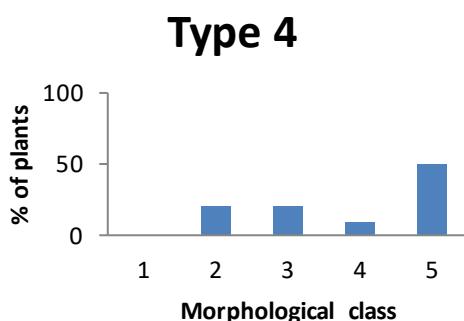
b)



c)



d)



e)

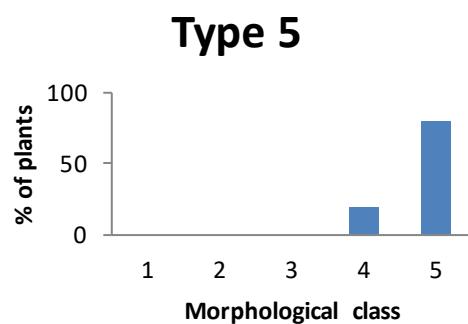


Figure 2.5 a-f. **Defining five stand ‘Types’**: based on the proportion of plants within stands that are classed as morphological class 1 to 5, for *A. melvillei*, *A. homalophylla*, *A. loderi*, *A. carneorum* and *A. ligulata*.



**Figure 2.6. Structure of typically fragmented overstory *Acacia* stands across western NSW:** (clockwise from top left) *A. melvillei* stand at Mungo National Park, *A. homalophylla* stand at Nyngan, *A. loderi* stand on Kinchega National Park and an *A. carneorum* stand just outside Kinchega National Park.

b) Assessment of the morphological structure (age structure) of stands across the region

The vast majority of stands of *A. melvillei*, *A. loderi* and *A. carneorum* consisted of only larger and likely older plants in morphological classes 4 and 5 with none, or with very few, smaller plants (usually suckers) in morphological classes 1, 2 and 3, suggesting a lack of more recent recruitment. In contrast *A. homalophylla* and *A. ligulata* stands consisted of plants which displayed a much more even spread of morphological classes, suggesting relatively recent and continual recruitment. In stands of the obligate seeder *A. ligulata*, all smaller plants were apparently derived from sexual reproduction (seedlings), while for *A. homalophylla* all plants in the smaller morphological classes (1, 2 and 3) were asexually derived suckers. Despite the consistent presence of predominantly larger plants within stands of *A. melvillei*, *A. loderi* and *A. carneorum*, I found some variation in the structure of stands of these three *Acacia* species. While nearly all *A. carneorum* and *A. loderi* stands consisted of only plants in morphological class 5, *A. loderi* displayed more stands with some plants in lower

morphological classes. *A. melvillei* stands, in contrast, were dominated by plants in morphological class 4, with only two stands observed to contain plants in morphological class 5, suggesting a slightly younger overall age structure.

The demographic profile of each species is described in detail below:

#### ***A. melvillei***

The vast majority of *A. melvillei* plants (85%) across all stands were large mature plants, but apparently not senescent as they displayed healthy canopies and none of the 85% were observed to perish during the study. While only 5% of all *A. melvillei* plants surveyed could be considered senescent, there were a very small percentage of younger plants, and most noticeably there were no plants of a middle size (morphological class 3). Importantly all senescent plants were detected within only 3 of 47 stands surveyed, and more extensive searches through a wider area within these stands confirmed that this was the case. Moreover, few suckers were found with suckers detected along the surveyed transects in only 4 of the 47 stands and accounting for between 13% and 57% of the total number of plants within those stands. Once again, more extensive searches within these stands confirmed the general lack of suckers across a wider geographic area than formally sampled. All suckers were assigned to morphological classes 1 and 2, and represented all plants within these categories for all surveyed transects of this species (Figure 2.7 a).

Little variance among stands was observed for this species with 100% of stands surveyed classed as ‘Type 5’ stands with 70% of these stands consisting of older plants in morphological class 4 only (Figure 2.8 a). Rare suckers were observed in a minority (23%) of stands, although interestingly senescent plants were only found in three neighbouring stands in and around Mungo National Park (6% of those surveyed), and in these stands all plants were senescing plants.

#### ***A. homalophylla***

*A. homalophylla* stands in general displayed a far more even spread of plants of different morphological stature than was observed for the other three threatened *Acacia* species. All of the *A. homalophylla* plants surveyed across the region fell into morphological classes 1 to 4 (Figure 2.7 b). In contrast to the other three threatened *Acacias*, I found large numbers of suckers in stands across the region and these were detected in all of the 10 stands surveyed. Suckers accounted for between 29% and 85% of the total number of plants within the

surveyed transects and 73% of all the plants surveyed. Suckers made up all of the plants classed as morphological class 1, 2 and 3 suggesting a persistent lack of seedling recruitment (Figure 2.7 b). Importantly however, when suckers were excluded, I found the demographic profile of this species was similar to the other threatened species, with 98% of all the independent plants surveyed classed as morphological class 4 and 2% as morphological class 3. Moreover, 90% of independent plants in morphological class 3 were detected in just four of 10 surveyed stands (Figure 2.7 b). When suckers were included, 80% of stands were best described as ‘Type 2’ stands with the remaining 20% of stands best described as ‘Type 3’ stands (Figure 2.8 b).

#### **A. *Ioderi***

*A. Ioderi* stands were in general comprised of a relatively high proportion of senescent plants compared with *A. melvillei* and *A. homalophylla* stands. I characterized 67% of all plants surveyed as belonging to morphological class 5 and 20% to morphological class 4 (Figure 2.7 c). I found few suckers in stands across the region, with suckers detected in only 3 of the 26 stands. This accounted for only between 23% and 31% of the total number of plants within those stands where they were found (Figure 2.7 c). While suckers were rare in this species, they made up all of the plants classed as morphological class 1 and some of the few class 2 plants surveyed, highlighting the lack of recent sexual recruitment in this species (Figure 2.7 c). There was little variation between the age structure seen in the different stands of *A. Ioderi* across the landscape, with 69% of the stands surveyed being best represented as ‘Type 5’ stands, consisting largely of the very old trees classed as morphological class 5, with a few recent suckers in some of these stands. The remaining 31% of stands were comprised of seemingly younger plants including 8% ‘Type 4’, 19% ‘Type 3’, and 4% ‘Type 2’ stands (Figure 2.8 c).

#### **A. *carneorum***

For *A. carneorum*, I classified 100% of all independent plants surveyed across the region as morphological class 5. While I found no suckers in most stands (28 out of 30), in the two stands (South Dune and Quarry) where suckers were seen, there were considerably more suckers than independent adult plants (93% of total number of plants at South Dune stand and at the Quarry were suckers). Interestingly, at South Dune stand, 43% and 57% of these suckers were plants classed in morphological class 2 and class 1 respectively, while at the Quarry stand 100% were classed in morphological class 2 (Figure 2.7 d). Again, there was little variation in

the age structure between the different stands surveyed across the region, with the vast number being best classed as ‘Type 5’ (93%), with the remaining 7% of stands where more recent large scale suckering has occurred being best described as ‘Type 1’ stands (see Figure 2.8 d).

#### **A. *ligulata***

In general the morphological structure of stands of *A. ligulata* was more uniform than for the four other *Acacia* species, and is consistent with continuous sexual recruitment (Figure 2.7 e). While there was some minor structural variation, especially between small and isolated stands, 80% of stands surveyed were best described as ‘Type 2’ stands. The remaining stands were all best described as Type 3 stands (Figure 2.8 e).

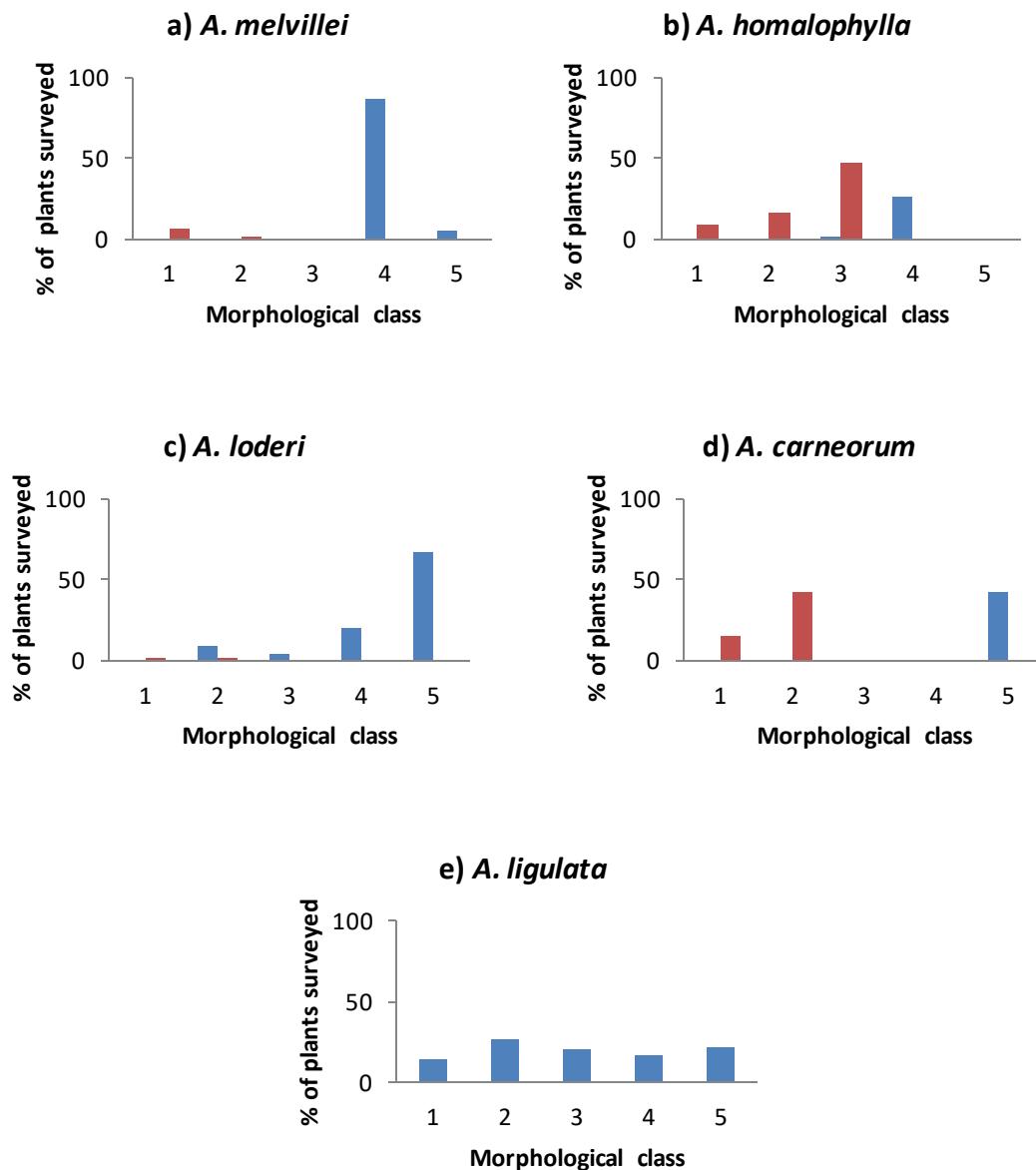
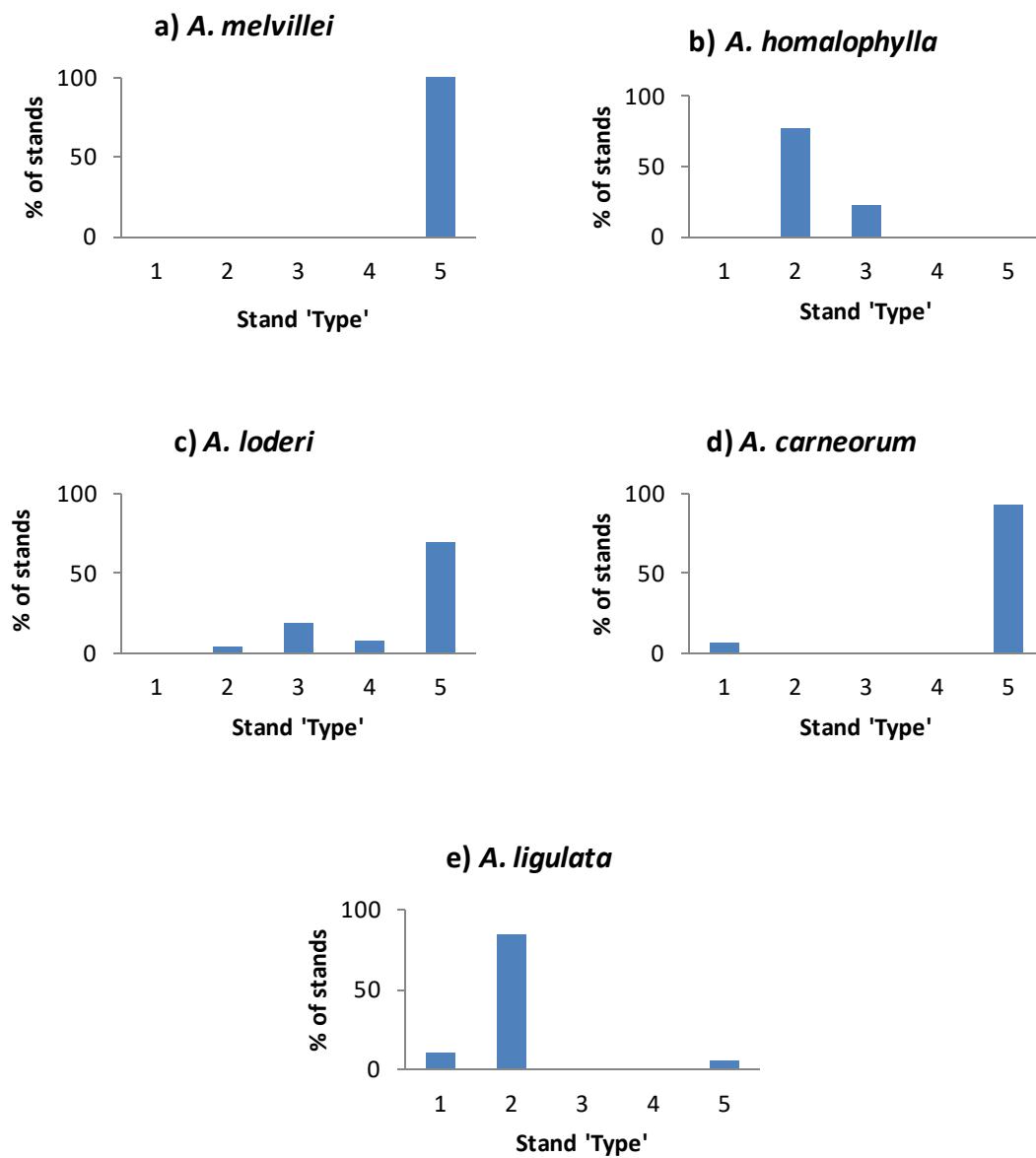


Figure 2.7 a-e. Estimates of the age of *A. melvillei*, *A. homalophylla*, *A. loderi*, *A. carneorum* and *A. ligulata* plants spread throughout the semi arid region of far western NSW: represented as the percentage of 42, 10, 26, 30, and 20 *A. melvillei*, *A. homalophylla*, *A. loderi*, *A. carneorum* and *A. ligulata* stands respectively, that fall within morphological classes 1 to 5. Blue bars represents independent plants and red bars represent suckers.



**Figure 2.8 a-e. Estimation of the age structure of *A. melvillei*, *A. homalophylla*, *A. loderi*, *A. carneorum* and *A. ligulata* stands in the semi arid region of far western NSW:** represented as the percentage of 42, 10, 26, 30, and 20 *A. melvillei*, *A. homalophylla*, *A. loderi*, *A. carneorum* and *A. ligulata* stands respectively, that were classed as stand 'Types 1-5'.

## **2.5 Discussion**

### **2.5.1 Characterizing and comparing the demographic structure of stands**

The demographic profile of the vast majority of stands of *A. melvillei*, *A. loderi* and *A. carneorum*, was consistent with prolonged sexual recruitment failure. Stands were comprised almost entirely of older and often senescent plants. My findings generally support those of both international and Australian studies that have reported high levels of senescence of rangeland stands of several overstory species especially in agricultural regions driven by a lack of recruitment (Rohner & Ward, 1999; Brand, 2002; García, 2003; Zavaleta et al. 2007; Ottewell al., 2010; Li, 2012). My findings also lend weight to previous claims of an imbalance between older and younger plants within stands of threatened *Acacia* species in arid and semi arid NSW (Batty & Parsons, 1992; Auld, 1995b; Porteniers, 1998; Benson, 1999; Auld & Denham, 2001; Porteniers, 2001).

The demographic shift of many plant populations around the globe towards senescent populations has been reported as a dynamic of great concern, and in many cases this phenomenon has been linked to recruitment failure driven by local anthropogenic disturbance (Rohner & Ward, 1999; Brand, 2002; García, 2003; Zavaleta et al. 2007; Ottewell al., 2010; Li, 2012). Intensified grazing regimes are often specifically attributed to such recruitment failure, which has also been exacerbated by the introduction of feral grazers in many systems (Auld 1993; Rohner & Ward, 1999; Cohn & Bradstock 2000; Auld & Denham, 2001; Brand, 2002; Fensham et al., 2010). Whilst the lack of recruitment in these semi arid *Acacia* is likely to be a combination of prolonged seed set failure along with intense grazing regimes and poor ground conditions for recruitment, in many systems it is unlikely that even with high levels of seed set, that sufficient recruitment will occur given harshened local ground conditions in fragmented landscapes (Kapos 1989; Matlack 1993, 1994, Malcom, 1998; Jules & Rathcke 1999; Meiners & Pickett 1999) and the presence of unnaturally intense grazing regimes. Without intervention the remaining stands of semi arid *Acacia* species in NSW are likely to continue to senesce and go locally extinct in the near future, or at the very least consist solely of suckers (Tilman et al. 1994; Loehle & Li, 1996).

While the morphological structure of *A. homalophylla* stands found in this study also suggests a lack of recent sexual recruitment, these stands differed most obviously from the other three threatened *Acacia* species by displaying a more even distribution of plants of different ages. Importantly this was found to be a result of what seems to have been

continuous asexual recruitment into stands through suckering. While the presence of these suckers in *A. homalophylla* stands is of likely consequence to the future long term persistence of this species in the region, it also raises the question of why stands of this species are structured so differently to the other three threatened species. One explanation for this striking difference in stand structure could be the location of most remaining plants within thin road verge strips, where regular clearing of understory for maintenance is more likely to disturb roots and thus initiate dense suckering (Maini & Horton, 1966; Weingartner, 1980; Lavertu et al., 1994; Fraser et al., 2003; Frey et al., 2003). Findings by Batty and Parsons (1992) also support this theory as they found natural suckering levels without disturbance in closely related *A. melvillei* to be low, and suckering in large amounts to be almost exclusively a result of disturbance to the roots of plants by road side or agricultural clearing. For *A. homalophylla*, the presence of these suckers en masse is likely to have major consequences for their continued persistence in the region, extending their window of time for persistence despite ongoing recruitment failure via the sexual pathway.

Whilst the large amount of relatively recent asexual recruitment in *A. homalophylla* stands provides the biggest contrast in the general age structures of the four threatened species, the more subtle differences found between the structure of stands in the remaining three threatened species are also of significance to their chances of long term persistence. The finding that the vast majority *A. carneorum* stands were generally comprised of only senescent plants, confirms previous qualitative assessments by Auld (1993) and represents the most unnatural demographic profile among the *Acacia* species surveyed. With the exception of a few rare stands where more recent suckering has occurred in large numbers, *A. carneorum* stands look to be most obviously susceptible to local extinction. Similarly, the finding that the vast majority of *A. loderi* stands also now consist largely of senescent plants suggests stands are set to contract significantly in the near future, despite a small portion of younger plants existing in some stands. As such, *A. loderi* might be viewed as the next most critically endangered species of the five in the region after *A. carneorum*.

Although *A. melvillei* stands were found to be similarly skewed towards the larger and seemingly older end of the spectrum, the finding that the vast majority of plants were mature, but could not be described as senescent, was a noteworthy contrast to the *A. carneorum* and *A. loderi* stands surveyed. While *A. melvillei* stands are still clearly and dangerously unbalanced in their age structure, the window of time until their local extinction might be longer than for *A. carneorum* and *A. loderi*, so long as their life span is similar. Nevertheless, it is clear that

when the majority of older *A. melvillei* plants eventually die, there will be effectively no plants to replace them.

The even distribution of younger, middle aged and older plants within *A. ligulata* stands found in this survey supports previous claims that *A. ligulata* has been sexually reproducing and recruiting amply each year during the prolonged period of sexual hiatus experienced by the other four *Acacia* species. This may suggest that their reproductive strategy and / or mating system is in some way more resilient to the challenges faced by plants in highly disturbed environment, or simply different. Understanding what these life history and / or mating system differences are, other than the fact that *A. ligulata* are obligate seeders, should be considered of high importance for managers wishing to understand the mechanisms behind prolonged sexual reproductive failure in the threatened overstory *Acacia* of the region.

Plant morphology (size) has been used in many studies of many different plant species as a reliable predictor of age (Harper, 1977; Drewa et al., 2008; Venter & Witkowski, 2010; Cousins et al., 2014; Peltzer et al., 2014). Given that these *Acacia* species are threatened, as well as taking into account the high costs of more precise methods of determining their age such as via carbon dating, the use of morphology as a predictor of age was a preferable one here. Moreover, all the trees of each of the five *Acacia* species surveyed, which died during the period of observation, were identified as likely representing the oldest types of plants (morphological class 5). This finding provides some circumstantial evidence and justification for the choice of this age category as representing plants nearing the end of their life. Nevertheless, caution must be taken when estimating the longevity of other plants based on their morphological features. It is possible that the deaths that I observed may have been a result of stresses not related to age that were limited to those trees, such as disease or local environmental conditions. Indeed, the only *A. melvillei* plants that were observed to die across their whole NSW distribution were located in only two stands in Mungo National Park where all other plants were classed as senescent suggesting that these were either older stands or else another site specific factor influencing their condition.

## **2.5.2 Predicting the fate of existing stands**

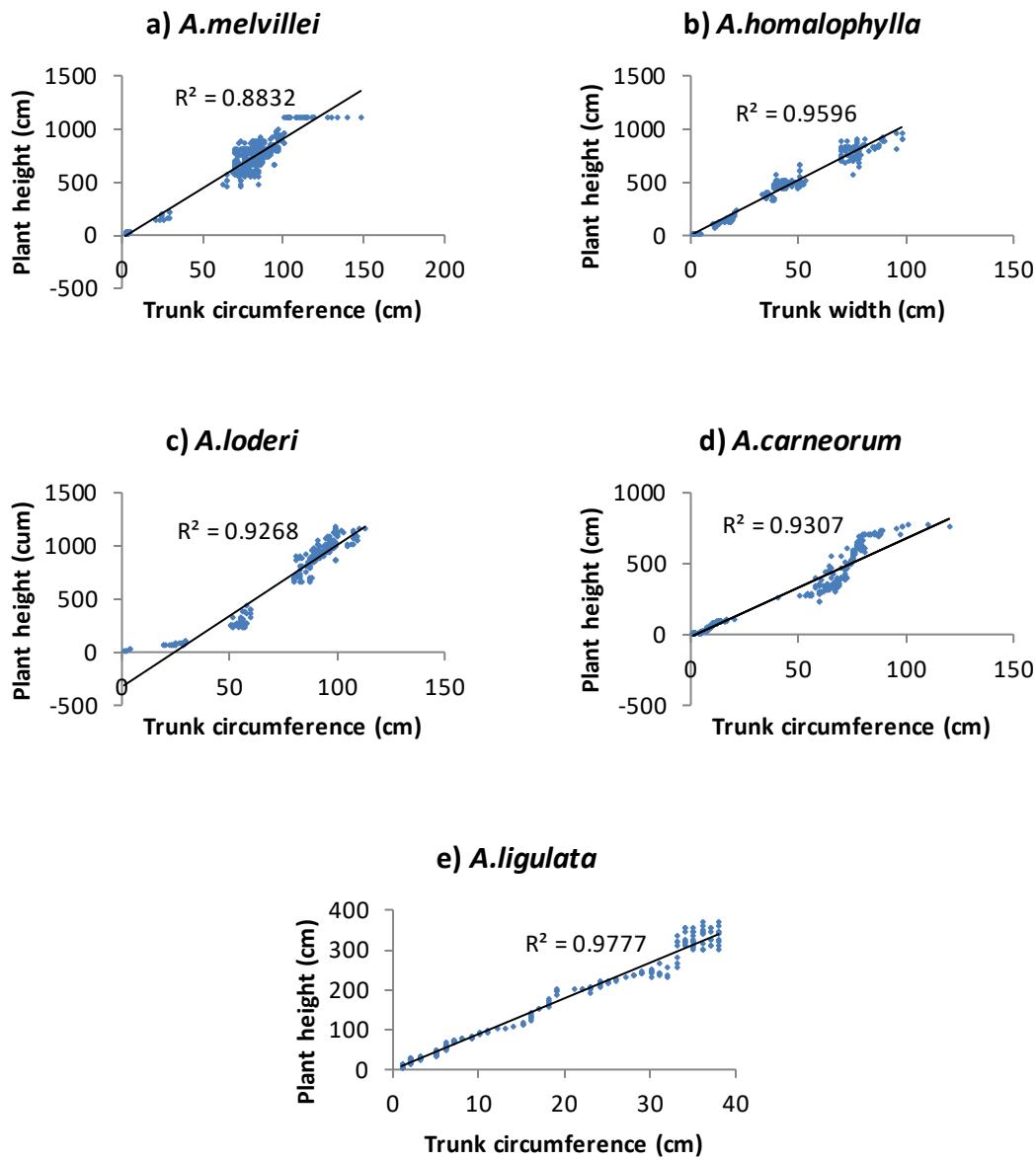
The skewed age structure of *A. melvillei*, *A. loderi* and *A. carneorum* stands is likely to only become more dramatic with time given that their current structure is likely to have been largely driven by feral grazers removing the vast majority of new recruits, together with

persistent reproductive failure over decades (Auld, 1993). Of broader concern is that even with the return of suitable climatic conditions for sexual reproduction and recruitment, it is not clear whether the levels of fruit set or offspring fitness are likely to reflect levels that might have occurred prior to anthropogenic fragmentation of these populations. Given that most remaining plants within stands are senescing, it may be that plants are too old to produce sufficient quantities of fit seed, if any (Laurance & Bierregaard, 1997), or are too unhealthy to reproduce or produce fit offspring (Stephenson, 1981; Lee & Bazzaz, 1982; Lee 1988; Campbell & Halama 1993). Moreover, if the isolation of these stands disrupts pollinator services leading to increased inbreeding, they may suffer inbreeding depression (Ghazoul, 2005; Sork & Smouse, 2006; Lowe et al., 2005; Eckert et al., 2010; Breed et al., 2012). Even if mature plants are resilient to such stresses, it would still be uncertain as to whether their offspring would be resilient enough to expect sufficient levels of recruitment long term. Even when fit seed are produced, harshened local abiotic and biotic conditions within fragmented populations are well known to make ground conditions less suitable, or even too hostile for seedlings to recruit (Harris 1984; Kapos 1989; Parsons 1991; Laurance et al. 1998; Vitt et al. 1998; Newmark 2001; Pohlman et al. 2009).

Taken together, the findings of this study give landscape wide quantitative evidence that these stands are likely to go extinct relatively quickly, without intervention. The lack of young plants by way of sexual reproduction, suggests that even if, or when, sexual recruitment has successfully occurred historically, effective recruitment has not followed for some time. This suggests that natural recovery of these populations is unlikely without intervention, and time is running out. Ironically, it seems *A. homalophylla* stands are faring better due to what is almost certainly high levels of anthropogenically induced suckering observed in all stands surveyed. The abundance of suckers in otherwise ageing stands may replace the mature independent plants when they die and maintain remaining stands for longer than they would otherwise persist. While these stands may fare better than the other species in the short term, the increase in clonality within stands is unlikely to be favourable in the long run. Given that climate change models predict harshening conditions in the semi arid regions of Australia (Hughes & Westoby, 1994; Hughes, 2003), maintaining sexual ability and genetic diversity might become an important key to future survival, even for *A. carneorum* populations where they appear to form naturally clonal stands (O'Brian et al., 2013; Roberts et al., *in review*).

## 2.6 Appendix

**Appendix 2.6.1: Relationship between trunk circumference (cm) and plant height (cm) for 487 *A. melvillei*, 307 *A. homalophylla*, 252 *A. loderi*, 470 *A. carneorum* and 200 *A. ligulata* plants located across 47, 10, 26, 30 and 20 stands respectively across far western NSW.**





## **Chapter 3: The importance of a rare, region-wide rain event for the reproduction of threatened semi arid *Acacia*.**

### **3.1 Abstract**

Understanding the manner in which long lived, iteroparous, overstory plants persist within highly modified landscapes is critical to the conservation of habitats where they occur. Semi arid canopy forming *Acacia* species in far western NSW have been impacted upon by land clearing and intense grazing pressures, leaving populations fragmented. These populations provide an ideal system for studies of long lived species persistence within a highly modified (fragmented) landscape. Previous demographic surveys have failed to detect any fruit set in many *Acacia* stands implying that they are trending to extinction. Hypotheses explaining the failure of these fragmented populations to reproduce sexually have included insufficient genetic diversity, a prolonged drought period, and the widely supported claim that extant plants are senescent. Here I take advantage of a rare La Niña rain event to conduct broad scale quantitative surveys across the region of flowering effort, flower morphology, effectiveness of pollinators and reproductive (sexual) success of 47, 10, 26 and 30 stands of four threatened overstory *Acacia* species (*A. melvillei*, *A. homalophylla*, *A. loderi* and *A. carneorum*) and 20 stands of one co-occurring but apparently thriving species (*A. ligulata*), under conditions where reproduction should not be limited by lack of water. After the rain event I observed high levels of hermaphroditic flowering in a set of 10 stands of each *Acacia* species with an average of between 41% and 100% of the canopy of plants per stand covered in flowers in consecutive flowering seasons. Moreover, between 19% and 31% of flowers on the average *A. melvillei*, *A. homalophylla*, *A. loderi* and *A. ligulata* plant per stand contained pollen tubes growing all the way to their ovules. For the first time, I documented landscape-wide sexual reproductive success in the form of high levels of fruiting/seed set in >=80% of *A. melvillei*, *A. homalophylla* and *A. loderi* stands surveyed, in at least one of the two consecutive years following the *La Niña* rain. While every *A. ligulata* stand surveyed produced seed, only 13% of all *A. carneorum* stands surveyed were found to set any seed, with fecundity levels being extremely low. I also found that the seemingly oldest plants of all five *Acacia* species within these stands were as fecund as younger plants. Together these findings suggest a reliance on occasional large-scale rain events for sexual reproduction to occur in many of these semi arid *Acacia* species in line with many arid species. Persistent reproductive failure within the majority of *A. carneorum* stands, as well as in a minority of *A. melvillei* and *A. loderi* stands however, implies that

reproduction in some stands is constrained by other factors such as sterility or availability of compatible pollen, poor health or the presence of maternally sterile stands.

### **3.2 Introduction**

Reductions in population sizes are often cause for concern, however where population numbers appear healthy, simple counts of individuals within populations may grossly underestimate a species' perilous state. If a plant's physiological health or realized mating system is altered, their reproductive capacity is likely to also be reduced, or in acute cases totally lost, as a result of their fragmentation (Tilman et al., 1994; Loehle & Li, 1996). The latter can be a result of simple isolation from suitable mates, or as a result of inbreeding depression (Crow & Kimura, 1970; Severns, 2003; Quesada et al. 2004; Hensen et al., 2005; Aguilar et al. 2006).

Most studies that investigate the way in which plant populations respond to highly fragmented conditions use short lived plants as model species with high population turnover, given the temporal advantages this affords researchers. Whilst some studies on long-lived species such as European oaks are well known (García, 2003; Zavaleta et al., 2007), such studies are rare especially in species where reproduction only occurs rarely, such as in arid plants. In arid environments, many animal and plant species use asexual rather than sexual reproduction as a more resilient and energy efficient means of reproduction (Kearney, 2003; Honnay & Jacquemyn, 2008; Vallejo-Marín et al., 2010). An example of this is suckering in plants, which is considered an efficient and resilient method of population maintenance and spread in harsh but stable environments such as arid ones (Piquot et al. 1998; Honnay & Bossuyt 2005). Asexual forms of reproduction such as this also serve to maintain local adaptations (Silander, 1985; Caraco & Kelly, 1991; Stuefer et al., 1996; Song et al., 2002; Honnay & Bossuyt, 2005; Silvertown, 2008). Without understanding the roles of sexual and asexual reproduction in plants that utilize both methods, infrequent sexual reproduction can easily be misinterpreted as reproductive failure, particularly when the species may only rely on infrequent sexual input to maintain a small amount of genetic diversity, or for dispersal and colonization of new areas (Harper, 1977; Stearns, 1987; van Kleunen et al., 2001; Zobel, 2008; Bernstein et al., 2013).

Over-story Acacia species in the semi arid region of far western NSW in Australia that consist of largely old or senescent plants, and which have not been observed to set fruit for two decades, provide model systems to study how long lived plants' reproduction is affected by acute fragmentation. Whilst several theories have been put forward to explain this lack of fruit set as a consequence of senescence, pollen limitation or inbreeding depression (Porteniers, 2001), until now none of these theories have been tested. Moreover, a lack of seed

set in any given year of a long lived plant species is not always unnatural. It may be that very infrequent fruit set in these long lived species is all that is required to sustain populations, or that the period of observation coincided with an unusually prolonged and severe drought. Indeed, periods of unusually high rainfall are often required to trigger reproduction in any arid and semi arid plant species (Davies 1976; Norton & Kelly, 1988; Letnic & Dickman, 2006; Wardle et al., 2013), and synchronized reduction in herbivory during such rainy pulses have been key to explaining woody regeneration in Australia (Austin & Williams, 1988).

For this research I take advantage of a rare region-wide La Niña rain event starting in 2010 and 2011 (Wardle et al., 2013) to survey a cross section of the remaining stands of four threatened semi arid *Acacia* species and one thriving, co-occurring comparative species, to assess the reproductive effort and output of these populations under seemingly optimal rain conditions. Specifically, for the same set of stands of the five semi arid *Acacia* species studied in Chapter 2, I test the hypotheses that:

1. Flowering effort and the sexual morphology of flowers among *Acacia* species and stands are equivalent.
2. Flowers are pollen limited or else receiving inviable pollen
3. Extant plants are not capable of setting fruit and seed after a large scale *La Niña* rain event.
4. Reproductive output is not increased by higher levels of flowering effort of plants, the sexual morphology of flowers on plants, the number of flowers receiving viable pollen, or the age of plants.

### **3.3 Methods**

#### **3.3.1 Study species, stands, plants and study area**

I utilized the same set of five co-occurring over-story *Acacia* species, stands and plants from across western NSW selected in Chapter 2 of this thesis (Figure 2.1) as the focus of this study. This comprises three officially threatened overstory *Acacia* species (*A. melvillei*, *A. loderi* and *A. carneorum*), one that is closely related and whilst not yet officially recognized as threatened, faces the same or similar threats (*A. homalophylla*) and one that appears not to be under threat of population decline (*A. ligulata*).

### **3.3.2 Assessment of flowering effort among populations in response to the 2010 La Niña rainfall**

For each of the five *Acacia* species, I randomly selected 10 stands per species from the total number of stands surveyed in Chapter 2 (Figure 2.1), to measure flowering effort. Only the ‘independent’ plants were used (all over 2 m tall). Suckers were not included in these surveys since any failure to flower may simply be due to differences in their biology or immaturity. The percentage of canopy surface covered in fruit within 12 x 0.5m by 0.5m quadrats placed evenly around the canopy of each of the 10 plants selected above (encompassing the lower, mid and upper sections of the canopy, on each of the N, S, E, W sides of the plant evenly) was visually estimated by referring to photos of the canopy of each species covered in flowers, increasing in coverage in increments of 10% to 100%. An average measurement of flowering effort from all 12 quadrats was calculated for each plant. This was done in the two consecutive years directly following the start of the La Niña rain event in 2010 & 2011 for *A. carneorum* and 2011 & 2012 for *A. melvillei*, *A. homalophylla*, *A. carneorum* and *A. ligulata*. The reliability of this visual estimate technique was validated using counts of numbers of flowers (actually inflorescences /flower spikes) over 12 quadrats across the surface of canopies (see Table 3.2 for details & Appendix 3.6.1 for validation).

### **3.3.3 Assessment of the sexual morphology of flowers and pollen tube growth**

On the same plants used to estimate flowering effort, ten flower spikes (inflorescences) from all 12 quadrats within the canopy were collected in both consecutive years, to determine whether seed set might vary because of fundamental differences in flower morphology / functional gender. For each inflorescence on each plant, I chose three flowers that had been fully opened for several days and looked for the presence or absence of anthers and ovaries using a stereo microscope. Each flower was then characterised as either male only, female only, or hermaphroditic (see Table 3.2 for sampling detail). Next, the same flowers examined above were also used to estimate the proportion of flowers with pollen tubes initiating down the styles and the proportion reaching the ovules of flowers. Dissected flowers had their anthers and external structures removed such that only stigmas attached to ovaries of flowers remained. They were then softened in 2M hydrochloric acid over night, rinsed in water and stained the following day with analine blue, then squashed onto a slide with cover slip. I then inspected these slides under a fluorescence microscope using the UV spectrum to visualise pollen attachment and growth of pollen tubes. I determined the percentage of flowers

exposed to pollinators with pollen tubes (a) initiating, (b) reaching the ovary and c) the % of initiated pollen tubes reaching the ovules of flowers (Table 3.1).

### **3.3.4 Assessment of sexual reproductive success in response to La Niña rain**

I assessed the reproductive health of plants in the two consecutive years following the beginning of the La Niña rains in 2010, for all 133 of the stands shown in Figure 2.1 (Chapter 2, thesis). As above, only the ten ‘independent’ plants (all over 2 m tall) were used given potential differences in their natural reproductive capacity / effort compared to that of suckers\*.

*\*The reproductive effort of all suckers surveyed of each of the five Acacia species was found to be considerably less than for associated adults, and in many cases they did not set seed at all.*

#### *a) Presence or absence of fruit*

I recorded the presence and absence of fruit for plants when fruit set considered to be at the peak over two consecutive years in recognition of potential lag effects (See Table 2 for dates). A plant was recorded as having fruited if any fruit were observed after a timed search of 5 minutes per plant (which was ample time to make an accurate decision).

#### *b) Reproductive effort (Fruiting intensity)*

Where fruit was observed, an estimate of the percentage of a plants canopy covered in fruit was also made to estimate the reproductive vigour (effort) of these plants. Pictures of fruit cover density increasing in increments of 10% were used to estimate fruiting intensity. This was validated as an accurate visual estimation method (see Table 2 for details & see Appendix 3.6.1b for validation of this technique). The percentage of canopy, within 12 quadrats placed evenly around the canopy of each plant in the same way as in methods 1 and 2, was determined and for each plant, and an average measurement of all quadrats for each plant was calculated.

#### *c) Estimating the number of seed produced per pod*

As plant fecundity is ultimately dependent on both the number of fruit produced and the number of seed per fruit, the average number of seed per fruit pod was also calculated for all the plants surveyed for fruit set. To representatively sample the whole plant, 100 mature fruit were collected from the same 12 quadrats on the plant described above. The fruit from

each plant was pooled and 200 were selected at random from each of the ten plants and from each of the 10 stands surveyed to get a representative sample for each plant. The number of developed intact seed per fruit was counted to get an average number per plant.

d) *Estimating the average number of seed per mature plant*

Estimates of the number of seed produced on all the plants above were calculated by first estimating the surface area of the canopies of each of the plants using the same quadrats used above placed around the sections of the canopy of plants where healthy foliage was located. Given the irregular shapes of the canopy of these species (see Figure 3.2). I decided this method was superior to alternative methods that model the canopy of plants as spheres to calculate surface area (Morse & Robertson, 1987). The surface area of each plant was then multiplied by the average number of fruit per quadrat found in (b) above, to estimate the total number of fruit per plant. Finally, the average number of seed per plant found in (c) above, was multiplied by the estimates of the number of fruit produced per plant to gain an estimate of the overall number of seed per plant.

**Table 3.1: Assessment of reproductive effort, reproductive success and fecundity of *A. melvillei*, *A. homalophylla*, *A. loderi*, *A. carneorum* and *A. ligulata* in western NSW in response to a large scale La Niña rain event:** Details of the Numbers, dates, measurements and sampling effort per plant used to assess 1. Flowering effort among populations in response to the 2010 La Niña rainfall, 2. Sexual morphology of flowers, 3. Pollinator services and pollen/flower quality, and 4.sexual reproductive success in response to La Niña rain.

Assessment	Response variables	No. of stands separated by >30km in which 10 randomly selected independent plants were surveyed.					No. flowers surveyed per plant	No. of fruit surveyed per plant	Dates of surveys		Measurement	Sampling effort
		<i>A. melvillei</i>	<i>A. homalophylla</i> *	<i>A. loderi</i>	<i>A. carneorum</i>	<i>A. ligulata</i>			All species	<i>A. carneorum</i>	Others	
Reproductive Effort	Flowering Effort	10	9	10	10	10	N/A	N/A	Jan. 2011	Sept. 2010	% of canopy surface covered in flowers	Visual density estimates, using 12 one metre <sup>2</sup> quadrats placed at high, medium and low points at positions north, south, east and west on a plants canopy.
	Flower Morphology	10	9	10	10	10	120	N/A	Jan. 2011	Sept. 2010	% of flowers with female function	360 flowers were selected for characterization by haphazardly collecting 10 inflorescences from all 12 quadrats used above, and haphazardly collecting 3 flowers per inflorescence.
	Pollen availability/viability	10	9	10	10	10	30	N/A	Jan. 2011	Sept. 2010	% of flowers with (i) Pollen deposited on stigma, (ii) Pollen tubes initiated, and (iii) Pollen tubes reaching flowers ovary	The same 360 flowers characterized above for sexual morphology were dissected, stained and viewed under a UV microscope.
	Presence of	47	9	26	20	20	N/A	N/A	June 2011	Jan. 2011 &	% of plants within populations	Visual searches of the canopy were conducted until fruit were detected or

Reproductive success	fruit							& 2012	2012	that sets some fruit	for a maximum of 10 minutes.	
	Fecundity (fruit set)	47	9	26	20	20	N/A	N/A	June 2011 & 2012	Jan. 2011 & 2012	% of canopy surface covered in fruit	*same method as for measuring flowering effort above.
Fecundity (seed set)	No. seed per fruit pod	47	9	26	20	20	N/A	200	June 2011 & 2012	Jan. 2011 & 2012	Average number of seed per fruit pod	Where possible, 100 mature fruit were collected haphazardly from each of the 12 quadrats within the canopy, and the number of mature healthy seed per fruit was counted.
	No. seed per mature plant	47	9	26	20	20	N/A	N/A	June 2011 & 2012	Jan. 2011 & 2012	Number of seed per plant	The number of seed per plant was estimated by combining estimates of canopy surface area, no. of fruit per quadrat, and number of seed per fruit pod.

\*Only 10 populations of *A. homalophylla* were sampled because they were not able to be identified from *A. melvillei* correctly until after fruit set.

### **3.3.5. Effect of plant age on reproductive capacity and reproductive effort (fruiting intensity) after rain event**

I assessed whether senescence reduces the capacity of stands of all five *Acacia* species to set fruit, and their level of reproductive effort. For the same mature, as well as juvenile ‘independent’ plants selected in all 133 stands in Chapter 2 of this thesis, I compared the; a) presence or absence of fruit and b) reproductive effort (fruiting intensity) as measured above, over the same two consecutive years.

### **3.3.6 Statistical analysis of data**

I used two way analysis of variance (2 way ANOVA) with *Acacia* species nested in stands (no species shared actual locations) to compare flowering effort among populations, and proportion of flowers with pollen tube initiating and growing to ovules, between the five *Acacia* species as well as between stands of each species. Where data was collected over two consecutive years, two separate ANOVAs were done. Tukey’s post hoc tests were used to test for differences between each of the *Acacia* species and each of the stands. Whilst the assumptions of ANOVA were not always met as specified by the Shapiro-Wilks test of normality and Levine’s tests of equal variances for all sets of data analysed after several transformations, statistical consultancy advised that this approach was still appropriate given the nature of these data (close to normally distributed). Moreover it has been argued that ANOVA is a robust enough test to deal with large deviations from normality and unequal variances especially if the sample sizes are not very small (less than 5) and the sample sizes are not unbalanced, both of which were not the case for any data set analysed here (Underwood, 1981; Underwood, 1997).

The same comparisons between *Acacia* species and stands, for the percentage of plants within stands that set fruit in response to La Niña rain, and the fruiting intensity of those plants was dealt with using Kruskal-Wallis tests given the non parametric nature of the data. Mann-Whitney U tests were used to test for differences between each of the *Acacia* species and each of the stands against each of the other species and stands.

## 3.4 Results

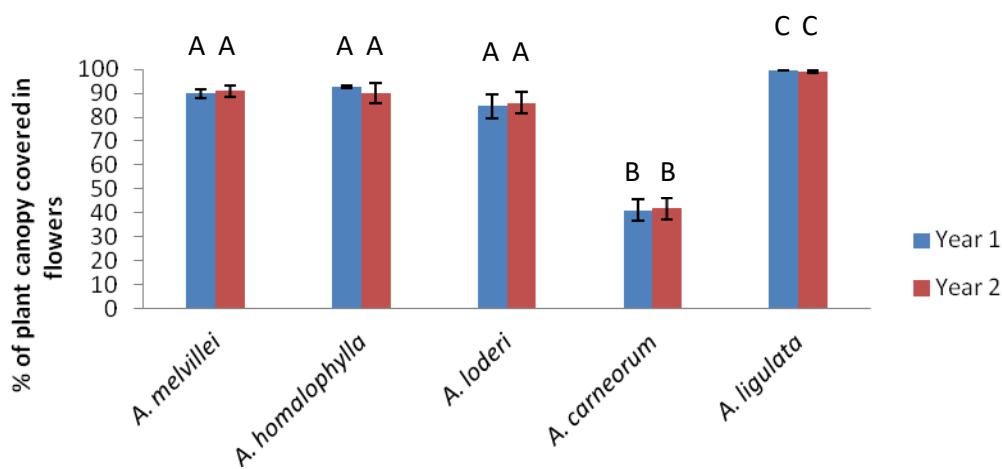
### 3.4.1 Assessment of flowering effort

In each of the two consecutive years, flowering effort was high in *A. melvillei*, *A. homalophylla*, *A. loderi* and *A. ligulata* with average flowering intensities of more than 80% across all stands and little variation among stands of each species (Figure 3.1). In contrast, flowering effort was significantly lower for *A. carneorum* (41%). Although some *A. carneorum* plants were found to have 100% of their canopies covered in flowers as seen on the other four species, it was sometimes as low as 15%.

Two way analysis of variance revealed a significant difference in the flowering effort among the five *Acacia* species surveyed and a significant difference in flowering effort between stands of each species (Table 3.2). Tukey's post hoc tests revealed that in both years, flowering effort for *A. ligulata* (99.6 % SE $\pm$ 0.3 & 99% SE $\pm$ 0.5) and *A. carneorum* (41.1% SE $\pm$ 4.5 & 42% SE $\pm$ 4.5) were significantly higher and lower respectively than the other three *Acacia* species. The average flowering intensities of *A. melvillei* (89.8% SE $\pm$ 1.76 & 91% SE $\pm$ 2.40), *A. homalophylla* (92.7% SE $\pm$ 0.6 & 90% SE $\pm$ 0.6) and *A. loderi* (84.5% SE $\pm$ 5.0 & 86% SE $\pm$ 4.4) plants were found not to be significantly different in consecutive years. Moreover, Tukey's post hoc tests also revealed that two and three stands of *A. loderi* and 10 *A. carneorum* respectively had significantly different ( $p<0.000$ ) levels of flowering effort however flowering effort between any of the *A. melvillei*, *A. homalophylla* or *A. ligulata* stands surveyed, was not significantly different.

**Table 3.2. Statistical results for two way ANOVAs of flowering effort over two consecutive years:** F statistic (F), degrees of freedom (df) and significance (P) for *Acacia* species (*A. carneorum* and *A. ligulata*) nested within stand (four stands of each species) for two consecutive years (2010 & 2011 for *A. carneorum* and 2011 & 2012 for *A. ligulata*).

Factor	Year	F statistic	df	P value
Species	1	446.8	4,45	<0.001
	2	725.5	4,45	<0.001
Stand	1	9.78	9,490	<0.001
	2	22.7	9, 490	<0.0001



**Figure 3.1. Flowering effort of five semi arid *Acacia* species across western NSW :** Percentage of plant canopy surface containing flowers during the peak flowering season (assessed in September of 2010 and 2011 for *A. melvillei*, *A. homalophylla*, *A. loderi* and *A. ligulata* and January 2011 and 2012 for *A. carneorum*). Data represent means ( $SE \pm$ ) for 100 plants (10 per population).

### 3.4.2 Assessment of the sexual morphology of flowers and pollen tube growth

I found that 100% of flowers collected from all populations of each of the five species appeared to have both male and female parts (anthers and stigmas respectively).

In the *Acacia* species surveyed, with the exception of *A. carneorum*, I found a high percentage of flowers with pollen tubes initiated on all plants within all ten stands in both years they were assessed. Noticeably, no pollen tubes were detected on *A. carneorum* flowers in the first year of survey. In the second flowering season however, 13% of all the *A. carneorum* flowers surveyed contained pollen tubes (Figure 3.2).

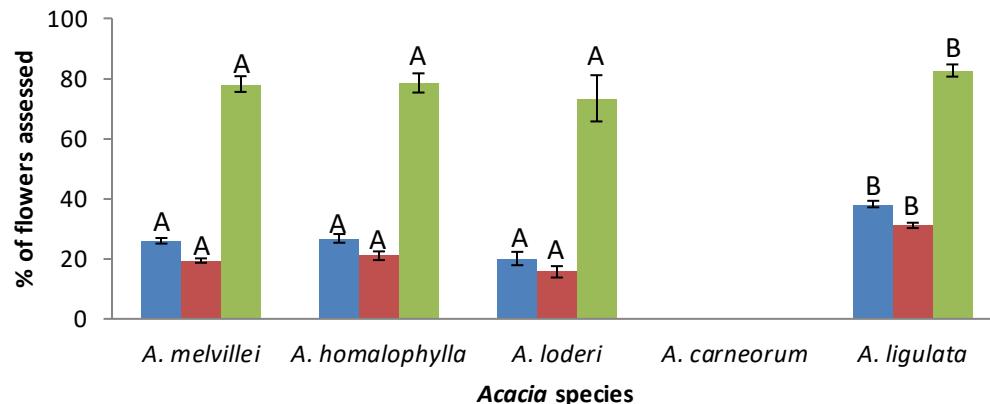
A two way analysis of variance found significant variation among the five species in the mean percentage of flowers with pollen tubes in both years (Table 3.3). However, Tukey's post hoc tests found that the overall variance in the proportion of flowers with pollen tubes, found among the species, was driven by much higher proportions of flowers with pollen tubes on *A. ligulata* (38.2%  $SE \pm 1.1$  & 38.8%  $SE \pm 1.0$  in consecutive years), and significantly less on *A. carneorum* plants 2011 (13%  $SE \pm 1.2$ ) compared with the other three *Acacia* species surveyed. No pollen tubes were found in *A. carneorum* flowers in the 2010 survey. No difference was found in the percentage of flowers with pollen tubes between the ten different stands surveyed, of each of the *Acacia* species, in either of the consecutive flowering seasons (Table

3.3). A lower proportion of the same flowers assessed above had pollen tubes growing all the way to the ovules of the flowers of all five *Acacia* species (Figure 3.2).

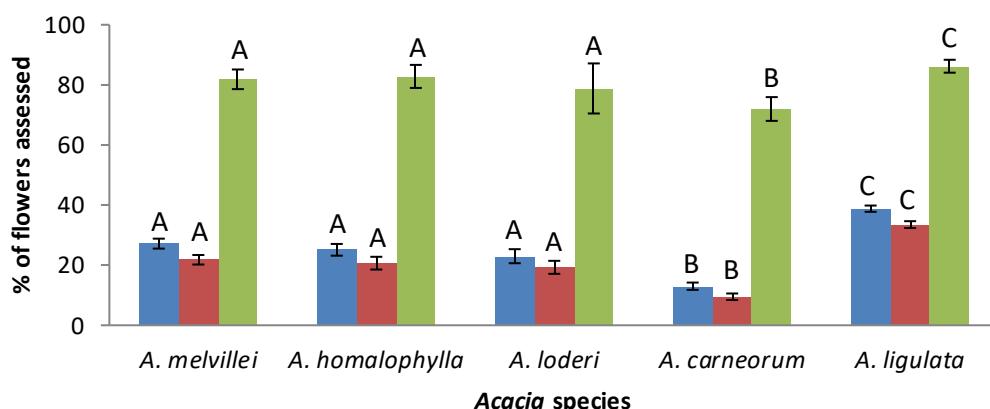
Two way analysis of variance excluding *A. carneorum* in the first year of flowering uncovered a significant difference in the average percentage of flowers with pollen tubes reaching the ovules among the remaining four *Acacia* species between both flowering seasons (Table 3.3). However, Tukey's post hoc tests found that the overall difference between the species in the proportion of flowers with pollen tubes found reaching the ovules was driven exclusively by significantly higher proportions of flowers with pollen tubes on *A. ligulata* (31.1% SE $\pm$ 0.89) in the first flowering season and both significantly higher proportions of flowers with pollen tubes on *A. ligulata* plants (33.5% SE $\pm$ 1.14) and significantly lower proportions of pollen tubes on *A. carneorum* plants (9.5% SE $\pm$ 1.08 ) in the second flowering season, compared with the other three *Acacia* species surveyed. No difference was found in the average percentage of flowers with pollen tubes reaching ovules between the ten different stands surveyed of each of the *Acacia* species in either of the consecutive years (Table 3.3).

Of the flowers of the five *Acacia* species that were found to have pollen tubes initiating within them, a high percentage had pollen tubes that had grown all the way to the ovules of the flower, with little variation observed between plants (Figure 3.2). Two way analysis of variance excluding *A. carneorum* found no significant difference among species for the average percentage of flowers with pollen tubes reaching the ovules in the first flowering season, while a significant difference in the proportion of flowers with pollen tubes was found between all five species in the second year of flowering (Table 3.3). Tukey's post hoc tests revealed this difference to be driven exclusively by significantly lower levels in *A. carneorum* (72% SE $\pm$ 2.1) compared with the other three *Acacia* species. No difference was found between stands of each species in either year (Table 3.3).

a)



b)



- % of flowers pollinated with pollen that initiate pollen tubes
- % of flowers with pollen tubes reaching the ovary
- % of flowers pollinated with pollen where pollen tubes reached the ovary

Figure 3.2: **Presence of pollen tubes in stigmas of *A. melvillei*, *A. homalophylla*, *A. loderi*, *A. carneorum* and *A. ligulata* flowers:** during the first (a) and second (b) flowering seasons for each species after the onset of the *La Niña* rains in January of 2010. (Values are overall means and SEs for 360 collected from 10 plants within each of 10 populations per species. The same letters above bars denote statistical similarity between like colours across species groups).

**Table 3.3: Statistical results for two way ANOVAs of the percentage pollen tube initiation, pollen tube growth to ovules and the proportion of flowers with pollen tubes that reach ovules, over two consecutive years:** F statistic (F), degrees of freedom (df) and significance (P) for *Acacia* species (*A. melvillei*, *A. homalophylla*, *A. loderi*, *A. carneorum* and *A. ligulata*) nested within stand (four stands of each species) for two consecutive years (2010 & 2011 for *A. carneorum* and 2011 & 2012 for the other four *Acacia* species).

Measurement	Factor	Year	F statistic	df	P value
% of flowers with pollen tubes initiating down styles	Species	1	139.8	4, 45	<0.0001
		2	46.5	4, 45	<0.0001
	Stand	1	0.709	9, 490	p=0.701
		2	0.672	9, 490	p=0.734
% of flowers with pollen tubes reaching ovules	Species	1	116.0	4, 45	p<0.0001
		2	55.6	4, 45	p<0.0001
	Stand	1	0.416	9, 490	p=0.927
		2	1.489	9, 490	p=0.149
% of flowers with pollen tubes initiating that also reached ovules	Species	1	0.537	4, 45	p=0.657
		2	3.837	4, 45	p=0.005
	Stand	1	0.225	9, 490	p=0.991
		2	1.216	9, 490	p=0.200

### 3.4.3 Assessment of sexual reproductive response to La Niña rain

#### a) Presence of fruit after rain event (on 'independent' non juvenile plants)

I found that the vast majority of independent plants of *A. melvillei*, *A. homalophylla* and *A. loderi* produced fruit in one or both of the two years following the start of the La Niña rains in January of 2010 (Figure 3.3). Specifically, I found 93% of *A. melvillei* plants, 80% of *A.*

*homalophylla* and 70% of *A. loderi* set some fruit, with 39 out of 47 stands (83%) of *A. melvillei*, 8 out of 10 stands (80%) of *A. homalophylla* and 21 out of 26 stands (81%) of *A. loderi* producing at least some fruit. In contrast, only a minority (12%) of all the *A. carneorum* plants surveyed across the region produced any fruit in either year. Fruiting plants of *A. carneorum* were found within only 4 of the 30 (13%) populations surveyed (Figure 3.4). All *A. ligulata* plants produced fruit.



Figure 3.3. Fruit set on overstory *Acacia* in western NSW after a region wide La Niña driven rain event in 2011: Left: *A. melvillei* fruit. Right: *A. homalophylla* seed.

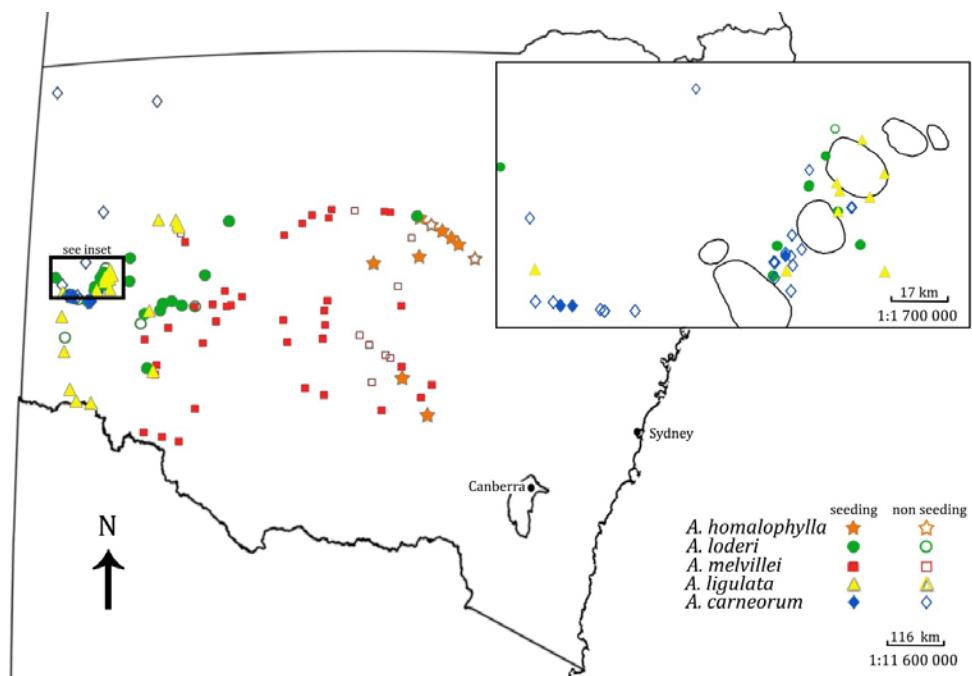
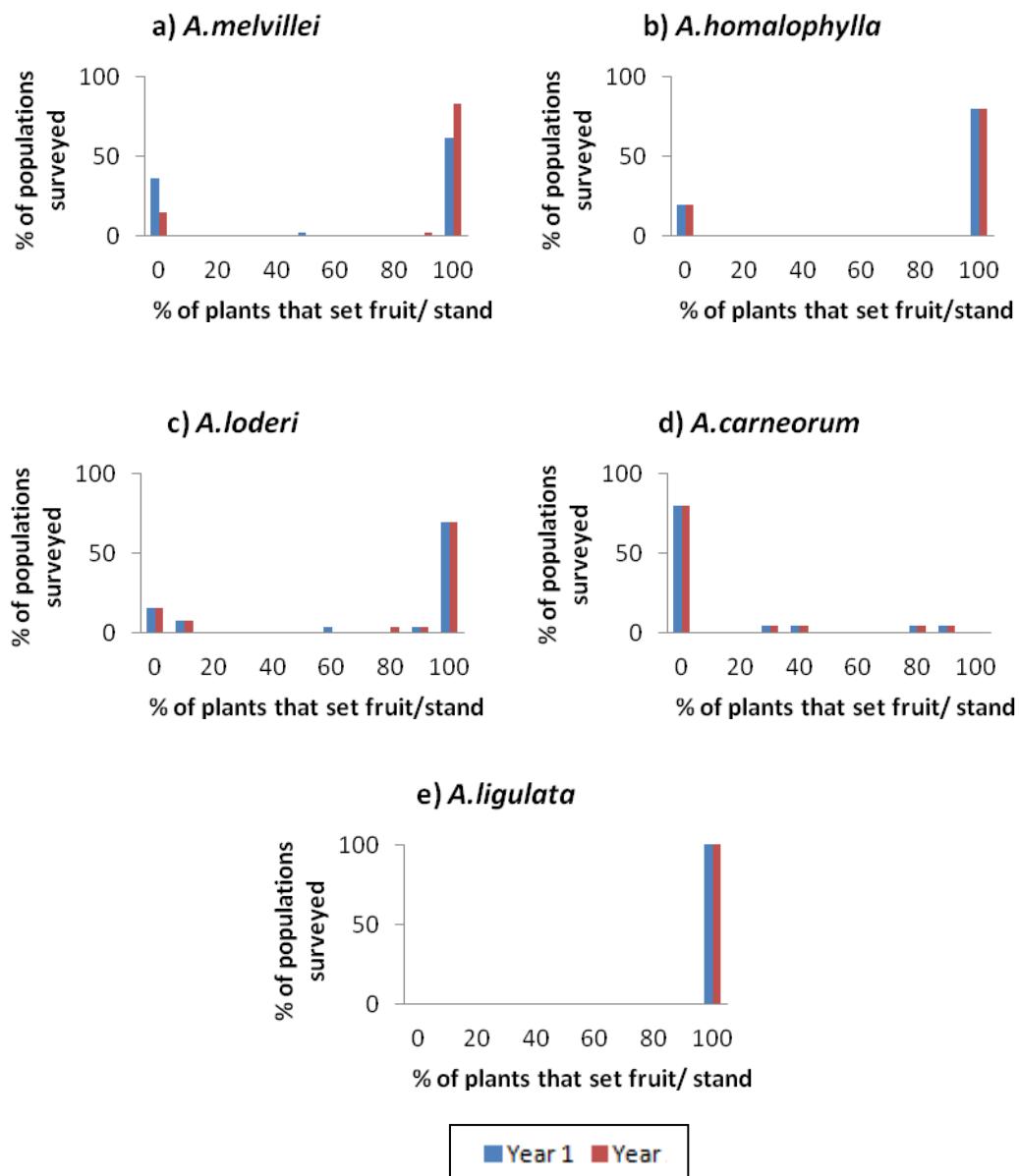


Figure 3.4. Sexual reproductive success or failure of surveyed *Acacia* stands in western NSW: The presence and absence of fruit and seed in 47, 10, 26, 30 and 20 stands of *A. melvillei*, *A. homalophylla*, *A. loderi* and *A. carneorum* in at least one of two consecutive years following the beginning of the La Niña rains in January of 2010. Inset displays Kinchega National Park and the region around the Menindee Lakes.

Where fruit was observed on a plant, it was common that fruit was present on all 10 mature plants surveyed in that population. In *A. melvillei* (79%) and *A. loderi* (81%) stands that set fruit had fruit on all surveyed plants. In contrast, only 50% of the *A. homalophylla* stands that set fruit had fruit on all surveyed plants, and none of the four *A. carneorum* stands that set fruit had fruit on all surveyed plants. Plants that did not set fruit, in either year, largely belonged to populations for which all plants responded in kind. I found that 98% of *A. melvillei*, 95% of *A. homalophylla*, 90% of *A. loderi* and 99% of *A. carneorum* plants that did not set fruit were located in stands where none of the surveyed plants set fruit in either year. Similarly, for all cases where *A. melvillei*, *A. homalophylla* and *A. loderi* plants did not set fruit in the first year but did in the second, I found that all other plants in the stand surveyed responded the same way. Moreover, 82% of *A. melvillei* and 75% of *A. homalophylla* plants in populations that did not set fruit in the first year set fruit in the second year, suggesting a lag effect to the rain event in these populations. Interestingly this phenomenon was not observed for *A. loderi* or *A. carneorum* with 100% of non fruiting plants in the first year failing to set fruit in the second, suggesting that these plants were truly unresponsive to this rain event. In a rare few cases I found a mix of fruiting and non fruiting plants, in either year, within the same population for *A. melvillei*, *A. homalophylla* and *A. loderi*. Specifically, I found a mix of fruiting and non fruiting plants in either year in only 2% of all *A. melvillei*, 11% of all *A. homalophylla*, and 15% of all *A. loderi* stands. In all four stands of *A. carneorum* where fruit was set, a mix of fruiting and non-fruiting plants occurred in both years with 30-90% of plants in those populations setting fruit. Interestingly, for two senescent populations of *A. melvillei* located on Mungo National Park, high intensity fruit set in the first year was followed by a total lack of fruiting in the second year. Furthermore, all of these senescing *A. melvillei* plants in Mungo National Park were observed to die soon after the second survey, suggesting that their failure to reproduce was related to their impending demise.

A Kruskal-Wallis test revealed a difference in the average proportion of plants per stand, over the two years measured, that set fruit among the five *Acacia* species  $\chi^2$  (4, n=133 =68.838, p<0.000). Mann-Whitney Utests revealed that this difference was driven by a significantly higher average proportion of trees per stand that set fruit over the two consecutive years in the *A. ligulata* stands (100% SE±0.0) surveyed, as well as a significantly lower level among the *A. carneorum* stands (9% SE±4.22) compared with *A. melvillei* (73.8% SE±4.50), *A. homalophylla* (72.2% SE±12.1), and *A. loderi* (79.7% SE±8.2) stands which were not found to be significantly different from one another (Figure 3.5).



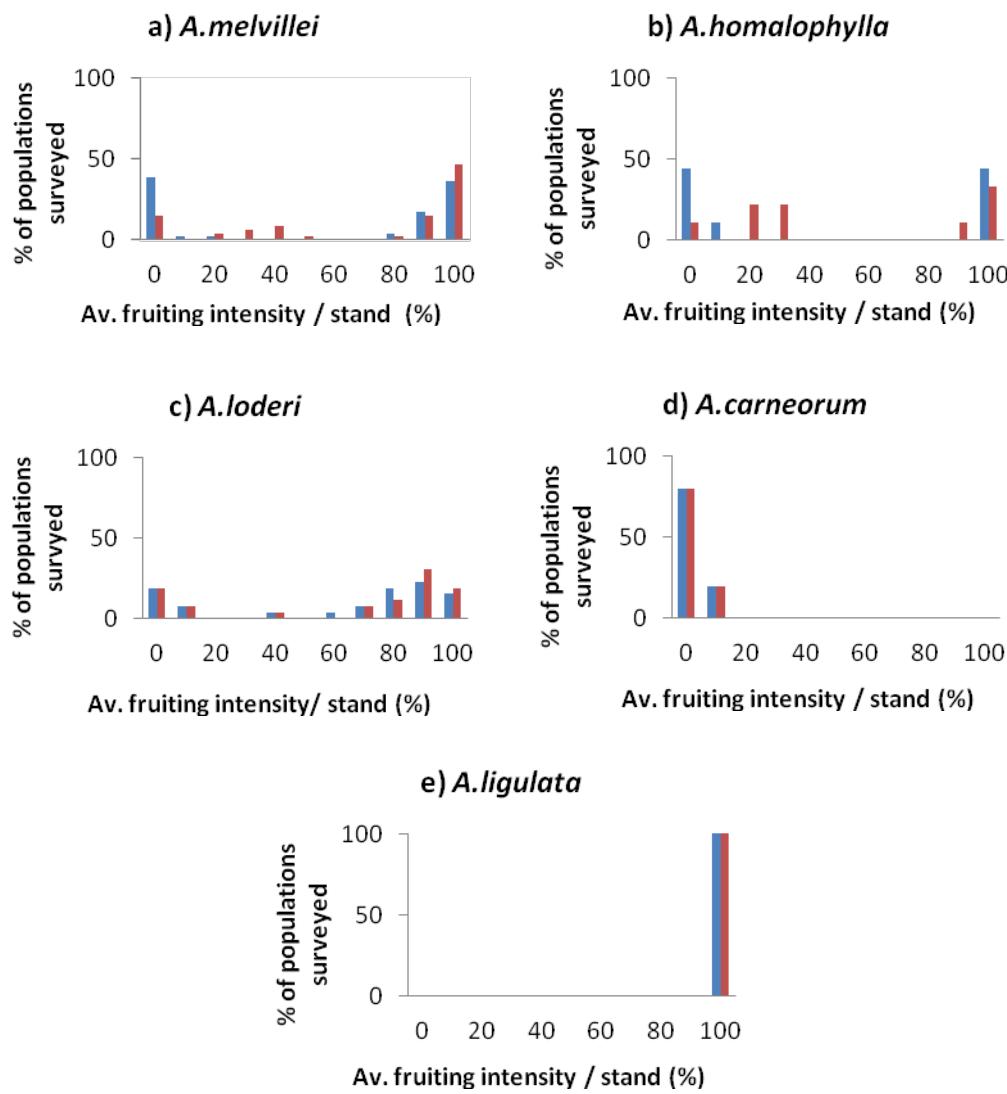
**Figure 3.5. Reproductive capacity of *Acacia* stands:** % of all populations surveyed of a) *A. melvillei*, b) *A. homalophylla*, c) *A. loderi*, d) *A. carneorum* and e) *A. ligulata* that set any fruit in year 1 (2010) (blue) for *A. carneorum* & 2011 (blue) for the other four species) and year 2 (2011 (red) for *A. carneorum* & 2012 (red) for the other four species).

#### b) Fecundity (Fruiting intensity)

While all *A. ligulata* plants displayed the highest average fruiting intensities compared with the other four species, I also found that independent *A. melvillei*, and *A. loderi* plants set fruit at high intensities per unit area of the canopy (Figure 3.6). *A. homalophylla* also produced high levels of fruit on average in year 1 (77% SE $\pm$ 17.30). In contrast with *A. melvillei* and *A.*

*ligulata*, this dropped significantly to (60% SE $\pm$  14.05) in year 2. For the *A. carneorum* plants in the few populations that did set fruit, a drastically lower level of fruit set was observed with no plant estimated to have more than 1% of their canopy covered with fruit. Although the range of fruit set was greater in *A. melvillei*, *A. loderi*, *A. carneorum* and *A. homalophylla* compared with *A. ligulata*, the majority of plants that fruited in all species produced comparably high levels of fruit (Figure 3.6). For each species, plants with similar levels of fruiting intensity were generally clustered within stands, showing that the variation in fruiting intensity among plants is driven by average differences between some stands. I found that almost all *A. melvillei*, *A. homalophylla* and *A. loderi* plants with the lowest fruiting intensities (less than 50% in any year) existed within only a few of the stands of that species that were surveyed (only 10 to 20% of stands surveyed).

A Kruskal-Wallis test revealed a difference in the average fruiting intensity of plants among the five *Acacia* species over the two years measured ( $\chi^2$  (4, n=92) =87.632, p<0.001). Mann-Whitney U tests revealed that this difference was driven by a significantly higher average fruiting intensity among *A. ligulata* stands (99.87% SE $\pm$ 0.09), as well as significantly lower levels among the *A. carneorum* stands (0.03% SE $\pm$  0.01), compared with the other species (*A. melvillei* 59.58% SE $\pm$ 5.25, *A. homalophylla* 46.47% SE $\pm$ 15.01 and *A. loderi* 58.75% SE $\pm$ 7.21) which were not significantly different from one another. A Kruskal-Wallis test also revealed significant variation among stands in fruiting intensity for all species. *A. melvillei* ( $\chi^2$  (46, n=456) =403.367, p<0.000), *A. homalophylla* ( $\chi^2$ (9, n=95) =69.510, p<0.000), *A. loderi*( $\chi^2$  (25, n=243 ) =189.514, p<0.000), *A. carneorum* ( $\chi^2$  (29, n=300) =77.321, p<0.000)and *A. ligulata*( $\chi^2$  (19, n=200) =36.547, p<0.009) over the two years surveyed.

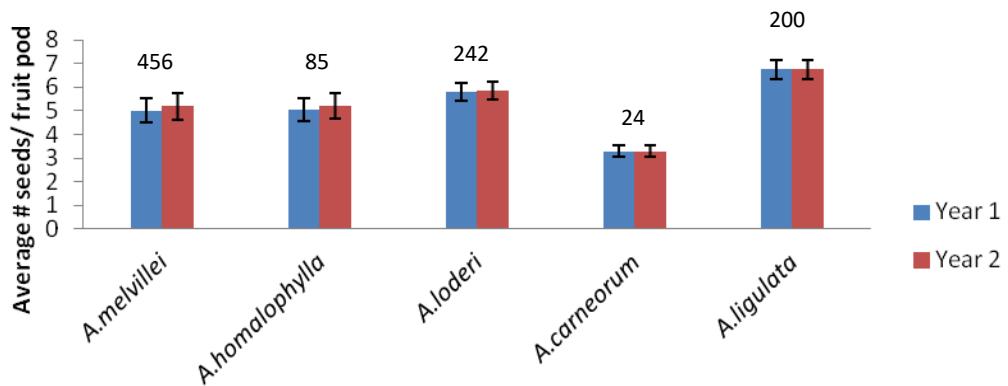


**Figure 3.6: Variance in fecundity (fruiting intensity) across multiple *Acacia* stands:**  
 Percentage of all populations surveyed of a) *A. melvillei*, b) *A. homalophylla*, c) *A. loderi*, d) *A. carneorum* and e) *A. ligulata* within year 1 in (2010 (blue) for *A. carneorum* & 2011 (blue) for the other four species) and year 2 (2011 (red) for *A. carneorum* & 2012 (red) for the other four species).

### c) Number of seed per fruit pod

There was considerable variation in the number of seed per fruit pods among *Acacia* species. In both consecutive years surveyed, *A. melvillei* fruit ranged from 3-7 seed per pod, *A. homalophylla* ranged from 3-8 seed per pod, *A. loderi* ranged from 3-9 seed per pod and *A. carneorum* ranged from 1-6 seed per pod (Figure 3.7). *A. ligulata* consistently had the highest number of seed per fruit (5-12), with *A. carneorum* the least seeds per fruit (1-6). There was on

average little variation in the number of seed per fruit pod between plants of the same species for all five Acacia species (see SE bars on Figure 3.7).

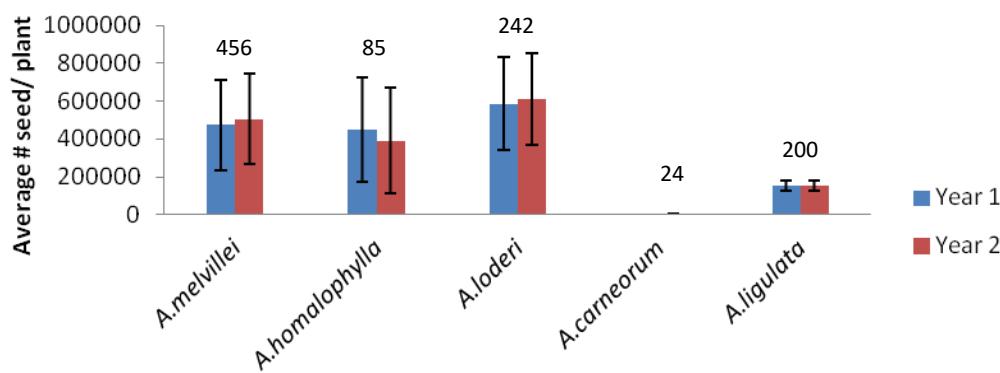


**Figure 3.7. Average number of seed per *Acacia* fruit:** Averages calculated from averages per plant across all plants surveyed in all 39, 8, 21, 4 and 20 stands of *A. melvillei*, *A. loderi*, *A. carneorum*, *A. ligulata* and *A. homalophylla* respectively that set fruit in year 1 (2010 of *A. carneorum* & 2011 for the other four species) and year 2 (2011 for *A. carneorum* & 2012 for the other four species), at peak fruiting season after the beginning of a region wide La Niña rain event beginning in January of 2010. Error bars (SE) represent variation between plants across all stands surveyed. \*Numbers above bars represent the number of plants surveyed for each species in both years.

d) Number of seed produced per plant

For each of *A. melvillei*, *A. homalophylla* and *A. loderi*, high levels of variability in the number of seed produced per plant was found, however this variability was comparable between these species (Figure 3.8). Such variability was considerably lower between plants in the *A. carneorum* and *A. ligulata* stands surveyed (Figure 3.8).

\*In all species, far lower levels of variability in fecundity were found between plants located in the same stand than found between plants from all stands pooled together.



**Figure 3.8. Average (+ SE) number of seed per *Acacia* plant:** across all plants surveyed in all 39, 8, 21, 4 and 20 stands of *A. melvillei*, *A. loderi*, *A. carneorum*, *A. ligulata* and *A. homalophylla* respectively that set fruit in year 1 (2010 for *A. carneorum* & 2011 for the other four species) and year 2 (2011 for *A. carneorum* & 2012 for the other four species), after the beginning of a region wide La Niña rain event beginning in January of 2010. Error bars (SE) express variance in the average number of seed between plants). \*Numbers above bars represent the number of plants surveyed for each species in both years

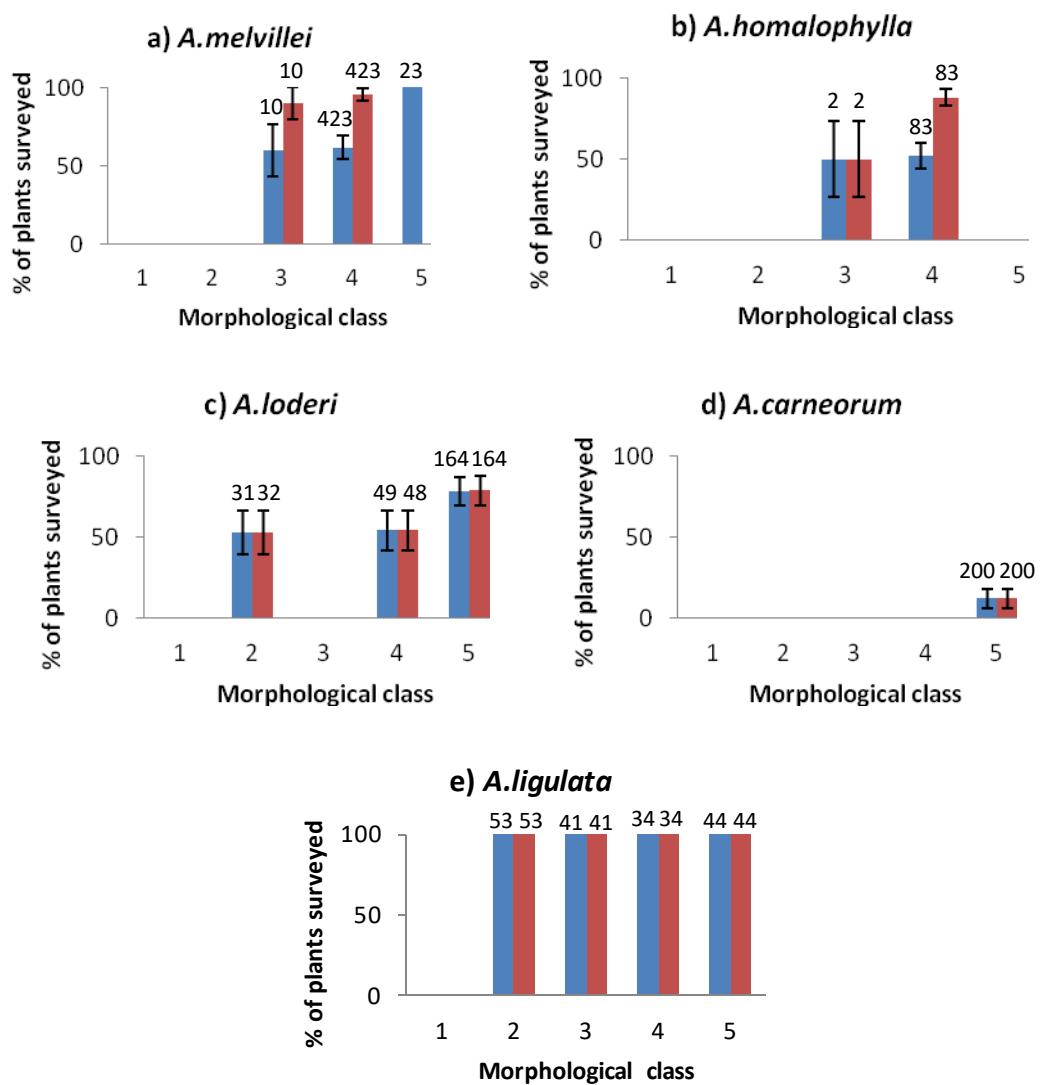
### 3.4.4 Effect of plant age on reproductive capacity and reproductive effort (fruiting intensity) after rain event

I found no evidence that the capacity to reproduce sexually, nor the fruiting intensity of independent plants in any of the four threatened *Acacia* species, was affected by their age. While a lack of *A. melvillei* and *A. carneorum* plants in younger age classes meant comparisons of reproductive success across age classes were limited, I found no evidence that *A. melvillei* plants in morphological class 5, that likely represent the oldest of these plants, reproduced any less vigorously than those in morphological class 4 which likely represent still mature but younger plants. I found that 100% of plants surveyed classed in morphological class 5 produced fruit in the first year while they were still alive (Figure 10), and had on average 99% ( $SE \pm 0.67$ ) of their canopies covered in fruit in year 1 (Figure 3.10). While these plants in morphological class 5 failed to produce fruit the second year of observation, their death soon after in that same year illustrates their capacity to set fruit right up until death.

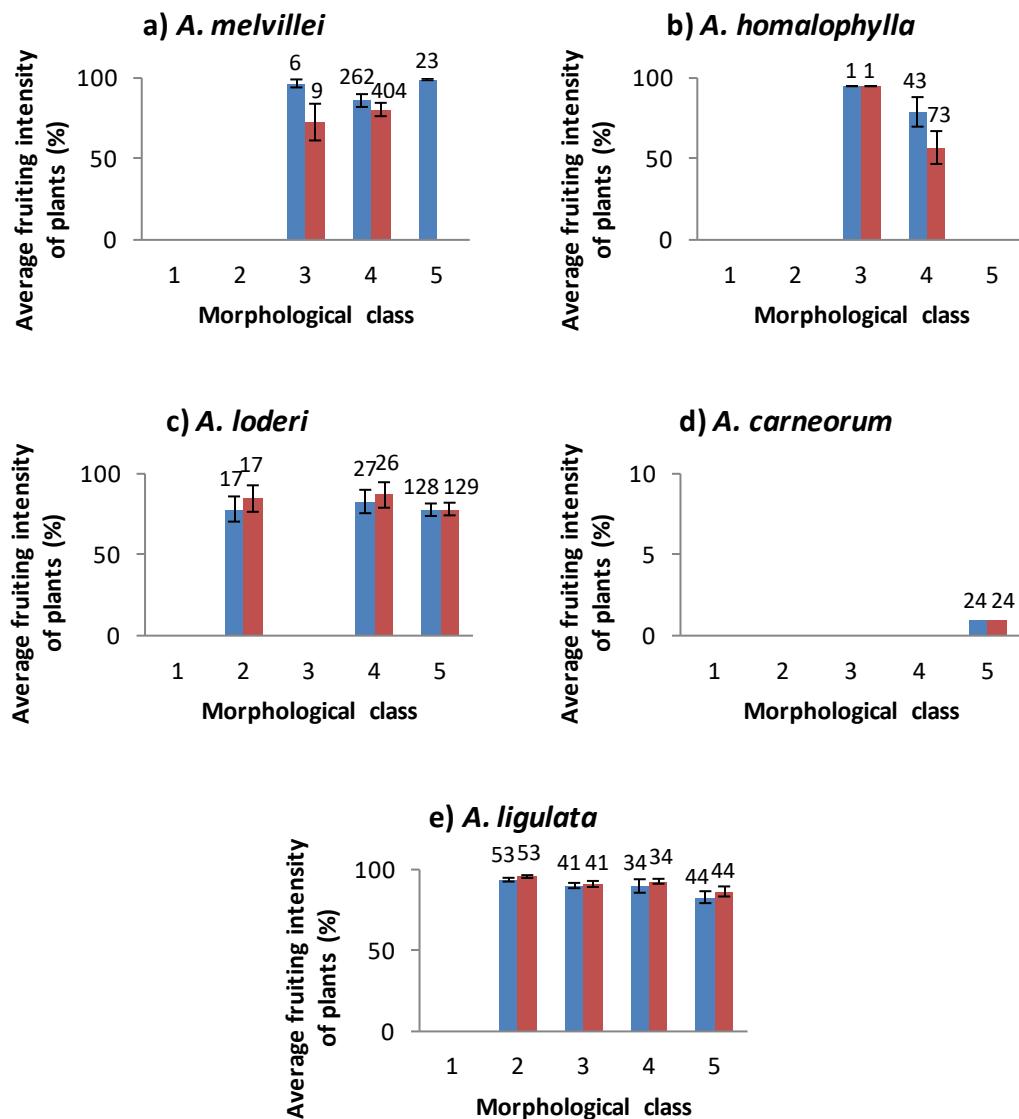
For *A. homalophylla*, whilst noticeably lower proportions of senescent plants set fruit in the first year surveyed after the rain event, a majority still produced fruit in both years (Figure 3.9). Moreover, 38% of senescent and 36% of younger plants were found to have 90% or more of their canopies covered in fruit, highlighting the capacity of older plants in this species to maintain high levels of fecundity per canopy area (Figure 3.10).

While a small drop in fruiting intensity was seen in the oldest of *A. loderi* plants (morphological class 5), fruiting intensity was still nearly as high as on plants in younger morphological classes (Figure 3.10). Moreover, 50% and 76% of morphological class 5 *A. loderi* plants in years 1 and 2 were recorded to have 80% or more of their canopies covered in fruit. An independent t-test found that the average fruiting intensity of *A. loderi* plants in morphological class 5 was not significantly different from the average fruiting intensity of *A. loderi* plants in morphological class 4 ( $t(1,230)=0.540$ ,  $p=0.589$ ). All the independent plants of *A. carneorum* that set fruit that were all characterized as morphological class 5 plants, had only 1% of their canopies covered in fruit. While I could not rule out that younger independent plants would be more fecund than these old plants, I observed no fruit set on any of the younger suckers in the select populations where they were found (Figure 3.10).

Whilst all *A. ligulata* plants were found to set some seed irrespective of their stature / age (seedlings excluded), one way analysis of variance also revealed no significant differences between the average fruiting intensity between plants of each age class  $F (4, 196) = 1.580$ ,  $p=0.180$ .



**Figure 3.9. Effect of morphological class on the capacity of *Acacia* plants to set fruit:**  
 Percentage of *Acacia* plants across all populations of a) *A. melvillei*, b) *A. homalophylla*, c) *A. loderi*, d) *A. carneorum*, and e) *A. ligulata* surveyed, that set fruit in year 1 (2010 (blue) for *A. carneorum* & 2011 (blue) for the other four species) and year 2 (2011 (red) for *A. carneorum* & 2012 (red) for the other four species). \*Numbers above bars represent the number of plants sampled for each morphological class.



**Figure 3.10. Effect of morphological structure on the fecundity of fruit setting *Acacia* plants:** Average fruiting intensity (% of plants canopy surface area bearing fruit of fruit setting plants across all populations) of a *A. melvillei*, b *A. homalophylla*, c *A. loderi*, d *A. carneorum* and e *A. ligulata* surveyed, that set fruit in year 1 (2010 (blue) for *A. carneorum* & 2011 (blue) for the other four species) and year 2 (2011 (red) for *A. carneorum* & 2012 (red) for the other four species). \*Numbers above bars represent the number of plants sampled for each morphological class.

### 3.5 Discussion

#### Evaluating the reproductive response to La Niña rainfall

Studies of reproductive health of long lived plants should encompass appropriate temporal and spatial scales. For long lived arid and semi arid species, unusual rainfall events provide an opportunity to make assessments of populations when not subject to moisture stress. My observations of a substantial sexual reproductive response by *A. melvillei*, *A. homalophylla* and *A. loderi* plants to a large scale La Niña rain event, highlights the importance of such rain events for their sexual reproduction and the need to assess reproductive success of long lived species over longer periods, especially in arid environments. This finding is also consistent with previous studies in arid / semi arid systems which found a similar reproductive response to large scale rain events in a range of other plant species (Büsgen & Münch, 1929; Davies 1976; Norton & Kelly, 1988; Fenner, 1998; Letnic & Dickman, 2006; Wardle et al., 2013). In stark contrast, the lack of any sign of fruit in the majority of *A. carneorum* stands and a minority of *A. melvillei*, *A. homalophylla* and *A. loderi* stands surveyed, shows that factors other than just lack of water must explain the lack of sexual reproduction in these stands. It is clear from findings here however, that lack of flowering effort, differences in flower morphology, pollen limitation, or the morphology of plants does not explain the differences in sexual reproductive capacity found between certain stands.

Access to rarely available water in arid and semi arid environments has obvious physiological benefits important for the production of fruit, as well as possibly facilitating a rapid population increase in pollinators required for plant reproduction in the region (Hawkins & DeVries, 2009; Marín González, 2010). Under a ‘boom and bust’ reproductive regime it is not unusual to see some degree of natural contraction of populations for an extended period. Indeed, such contractions might be expected to have no long term negative effect on the persistence of populations of these semi arid *Acacias*. With added anthropogenic pressures placed upon them however, population contractions have clearly become too acute for species such as *A. carneorum* especially, to expect natural restoration (Batty & Parsons, 1992; Auld, 1993, 1995b; Porteniers, 2001; Auld & Denham, 2001).

Infrequent fruit set in long lived species such as *A. melvillei*, *A. homalophylla*, *A. loderi* and *A. carneorum* is unsurprising given their long life spans and frequent opportunities to reproduce. Indeed, in long lived clonal plants, temporal gaps between years with successful sexual recruitment have been found to be highly variable in length, from zero to thousands of

years (Eriksson, 1989). Nevertheless, the fact that most *A. melvillei*, *A. homalophylla* and *A. loderi* plants set so much fruit, despite representing some of the most extreme examples of fragmented plant populations in the region (Porteniers, 2001), highlights the resilience of these species to the current pressures they face as old trees in a highly modified landscape. Indeed, the long standing theory that these populations largely consist of only older or senescent plants and do not have the capacity to set fruit has been clearly disproven. While resilience to fragmentation must be conferred through physiological tolerance to more stressful local abiotic conditions, a resilient reproductive strategy and /or mating system is also crucial. While the death of the oldest plants at Mungo National Park, directly after seed set for the first time in two decades, might be interpreted as a lack of reproductive health with age, site specific stresses on these plants cannot be ruled out as the cause of their deaths rather than age given no other plants of this stature were found elsewhere.

Suckering is one means by which parental plants can provide recruits with greater access to maternal resources and protection from environmental stress than seedlings (Piquot et al. 1998; Honnay & Bossuyt 2005). For sexually reproducing plants, mating systems can vary in the level of resilience they provide sexually reproducing plants in several ways; 1. Longevity of flowers/long flowering time thus increasing the likelihood of successful pollen transfer to initiate fruit set (Fréville et al., 2007), 2. Reliance on highly mobile pollinators able to bridge the vast gaps between remaining stands (Lowe et al., 2005; Petit et al., 2005; Bacles & Jump, 2011; Kremer et al., 2012; Vranckx et al., 2011), 3. A high level of self compatibility meaning less chance of pollen limitation and inbreeding depression (Kalisz & Vogler & Hanley, 2004; Knight et al., 2005; Brys et al., 2011; Rodger, et al., 2013), or 4. Ability to set fruit via parthenogenesis (Kearney, 2003).

### **Why was there a lack of sexual reproduction in some stands despite ample rain?**

In the context of the strong reproductive response observed in the vast majority of *A. melvillei*, *A. homalophylla* and *A. loderi* stands in response to the La Niña rain, the finding that there were some stands of each of these three species that did not set any fruit, and some that set significantly less fruit than others, was noteworthy. Along with the lack of sexual reproduction found in the majority of *A. carneorum* populations, this lack of fruit set begs explanation, especially from a conservation perspective. Understanding what factors drive sexual reproductive success and failure in these species should be considered of key

importance to managers looking to conserve these stands. Poor flowering effort, male only flowers, lack of pollen deposition on flowers or old age and senescence might be considered as the most obvious reasons, other than death, to explain why a plant capable of sexual reproduction may fail to set seed. However, all stands of all five species of the semi arid *Acacia* surveyed here produced large and equivalent numbers of flowers per unit area of canopy, regardless of whether or not they set fruit. All flowers on all plants surveyed, regardless of reproductive success, were also found to be hermaphroditic. Moreover, none of the five *Acacia* species, including plants in the majority of non fruit setting *A. carneorum* stands, could be described as pollen limited given my findings here. Indeed the majority of flowers of all five *Acacia* species surveyed were successfully pollinated, with a significant proportion of these producing pollen tubes to the ovules of these flowers albeit a relatively lower proportion was found for *A. carneorum*. Furthermore, the lack of evidence to show that old plants comprising these populations suffered reduced reproductive capacity, suggests that historical fruiting failure was also unlikely to have been driven by the age of plants, as has been a leading theory to date. Nevertheless, it must be acknowledged that there is a danger that I could have overestimated the reproductive response of senescent *A. melvillei* plants in particular as all of the senescent *A. melvillei* plants were found in only two stands within the Mungo region. Nevertheless, this region could perhaps be considered the most climatically hostile area of their distribution, and where stands exist in the poorest and most fragmented conditions.

It is not without precedence that hermaphroditic plants which flower readily and which are not pollen limited fail to produce fruit, especially in fragmented populations (Lamont et al., 1993; Steffan-Dwenter & Tscharntke, 1999; Cordeiro & Howe, 2003; Aguilar et al. 2006). While the mechanisms for this can be varied, in the case of these dwindling *Acacia* stands understanding whether such sterility represents natural variation or is a consequence of their altered conditions is worth further investigation. The abiotic and biotic effects that fragmentation can have on the reproductive health of plant populations are well known (Jennersten 1988; Ouborg et al. 1991; Goverde et al. 2002; Steffan-Dewenter & Westphal 2008). Such negative effects can come by way of additional stresses both directly on plant physiology via harsh local abiotic and biotic conditions, and indirectly by affecting elements of the plant's mating system, such as associated pollinators foraging behaviours, leading to pollen limitation or inbreeding depression (Charlesworth et al, 1987; Aizen & Feinsinger, 1994; Jacquemyn et al. 2003; Aguilar et al. 2006).

Plants whose mating strategies are compromised by disruptions to pollinator services, leading to pollen limitation, can fail to reproduce in the most extreme cases (Howe, 1977; Lamont et al., 1993; Steffan-Dwenter & Tscharntke, 1999; Cordeiro & Howe, 2003). While I found that these *Acacia* species were receiving pollen that produced pollen tubes, plants which receive mostly incompatible pollen, or do not receive enough compatible pollen, may also fail to reproduce (Turner et al. 1982; Hedrick, 1985; Charlesworth & Charlesworth, 1987; Levin et al., 2009). A possible explanation, or partial explanation for the lack of fruit set in a minority of *A. melvillei*, *A. homalophylla* and *A. loderi* stands could be that these stands are receiving only incompatible local / self-pollen owing to them being more disturbed / isolated populations compared to the ones that set fruit. If non reproducing stands are isolated from other compatible mates beyond a geographic threshold, then we might not expect to see successful sexual reproduction occur in these plants. The fact that no fruit was initiated on these plants despite pollen tubes growing all the way to ovules in a significant proportion of flowers may suggest such an incompatibility realized by some type of late acting pre zygotic, or post zygotic mate choice mechanism (Allen & Hiscock, 2008; Seavey & Bawa, 1986).

Changes in the quality of pollen being supplied to plants can also come about by local extinction of key pollinator species or the introduction of foreign pollinator species into systems. The introduction of European honeybee (*Apis mellifera*) is a well documented case of an introduced pollinator disrupting Australian native plant pollination systems leading to inbreeding depression (Ramsey, 1988; Taylor and Whelan, 1988; Paton and Turner, 1985; Vaughton, 1992). Irrespective of the mechanism by which gene flow is restricted, populations that have already lost much genetic diversity through genetic drift will generally suffer the effects of increased inbreeding levels more quickly than those that have retained higher levels of genetic diversity. Nevertheless, as long as gene flow remains restricted by continued isolation of populations, which is often the case where fragmentation has come by way of anthropogenic land clearing, even populations with reservoirs of genetic diversity, that serve as a buffer to inbreeding depression, will eventually be eroded by increases in inbreeding, combined with genetic drift (Crow & Kimura, 1970; Nei et al. 1975; Murcia, 1995; Aguilar et al. 2006).

### **Natural explanations for lack of fruit set should not be ruled out**

Whilst a lack of sexual reproduction in species capable of setting fruit may initially resemble reproductive failure, especially under seemingly favourable conditions for reproduction, judgements must be made cautiously. The diversity of reproductive strategies that exist among different plant species, and even sometimes between populations of the same species (Richards, 1997; Sartor et al., 2011; Hardion et al., 2015), means that this lack of sexual reproduction might also be explained naturally. A naturally lower sexual reproductive rate, a lesser role for sexual reproduction in the overall reproductive strategy, a different relationship between fecundity and old age, or a combination of several of these factors, might provide an alternate explanation to anthropogenic disturbances. It is plausible that recruitment by way of sexual reproduction is such a rare occurrence in *A. carneorum* plants, that observing it would be expected to be temporarily and spatially very difficult, even after large scale rainfall events (Eriksson, 1989). Indeed, recent findings that *A. carneorum* plants in the region consist of only very old plants that are genetically clonal (O'Brien et al., 2014; Roberts et al., *in review*) suggests that stands are predominantly the product of asexual recruitment. Moreover, recent carbon dating placed *A. carneorum* plants in these same stands at over 200 years old potentially (Auld & Denham, 2001; Chapter 9, thesis), suggesting that asexual reproduction might have been the preferred method of reproduction in these species even prior to anthropogenic disturbances by European settlement, agricultural practises and introduction of feral grazers in the area.

For the minority of *A. melvillei*, *A. homalophylla* and *A. loderi* populations that failed to set any fruit, naturalistic explanations rather than those depending on anthropogenic pressures, are also worth considering. Unlike *A. carneorum* however, it is clear that sexual reproduction has been important for at least a large proportion of recruitment in these species, as well as maintaining high levels of genetic diversity within many, if not the majority of populations (Roberts et al., 2013; Forrest et al., 2015). Nevertheless, it is possible that the populations that do not set fruit are simply part of the natural variation in maternal reproductive capacity in these species, rather than being unhealthy. It is not unprecedented that different plant populations within a species group utilize different reproductive strategies. This can be simply a consequence of local adaptation (Richards, 1997; Honnay & Jacquemyn 2008; Vallejo-Marín, Dorken et al., 2010; Sartor et al., 2011; Hardion et al., 2015), which is often associated with deteriorating environmental conditions (Eckert & Barrett, 1993); Jacquemyn et al., 2005; Vandepitte et al., 2009). Such responses can also come about as a

result of phenotypic plasticity (Abrahamson, 1975, Douglas, 1981, Cheplick 1995, van Kleunen et al. 2002, Vallejo-Marín et al. 2010), or as an evolutionary mechanism to separate the sexes and avoid inbreeding depression (Bierzychudek & Eckhart, 1988). As habitat fragmentation may negatively affect sexual function, clonal reproduction can be favoured resulting in a degeneration of life-history traits associated with sexual reproduction (Eckert, 2002).

### **Fecundity doesn't always signal reproductive success**

While the high levels of fecundity observed on the semi arid *Acacia* of western NSW, might signal that adult plants are physiologically healthy enough to set fruit, if these plants are very old and less than optimally fit, their mating systems compromised, or a combination of both, high levels of fecundity may mask less than optimally fit seed (Fenner, 1992; Welch, 1995). Whilst inbreeding depression can reduce the fitness of offspring (Charlesworth & Charlesworth, 1987; Menges, 1991; Oostermeijer et al., 1994), it is also well known that increases in self-fertilization within plant populations that have become unnaturally isolated can often reduce the availability of ovules and pollen for outcrossing, and less fit selfed progeny may be produced at the expense of fitter outcrossed progeny (Lloyd, 1992; Herlihy & Eckert, 2002). Moreover, many studies have found that recruitment in plants can be severely limited by decreases in the quality of local ground conditions as a result of edge effects resulting from fragmentation and thinning of populations (Harris, 1984; Kapos, 1989; Newmark, 2001; Pohlman et al., 2009). Harsh abiotic conditions, such as higher ground temperatures and increased desiccation, along with hardening of soils, especially in denuded arid environments, are all likely to reduce the chances of seedling survival and even the chances of seed being able to lodge and reach suitable soil conditions in the first place (Kapos 1989; Matlack 1993, 1994b, Malcom, 1998; Jules & Rathcke 1999; Meiners & Pickett 1999). Grazing pressures also generally increase as stands shrink in size (MacGarvin et al. 1986; Warren, 1987; Burkey, 1993; Greig, 1993).

### **Future studies and conservation**

While my observations of the most significant levels of seed set in over two decades in these *Acacia* species provide some hope for the restoration of these fragmented stands, even optimal levels of seed set may now be far from adequate to expect a sufficient number of

recruits to take hold and survive. Uncovering whether we might expect to see substantial levels of effective recruitment resulting from this seed set event, would ultimately require long term monitoring of any recruitment in the field. At the very least, for some of these threatened species, the presence of seed provides managers with a source of material for restocking of stands and any genetic rescue attempts that are likely to be required in the face of climate change (Huntley, 1991; Easterling et al., 2000; Hughes, 2003; Godfree, 2013).

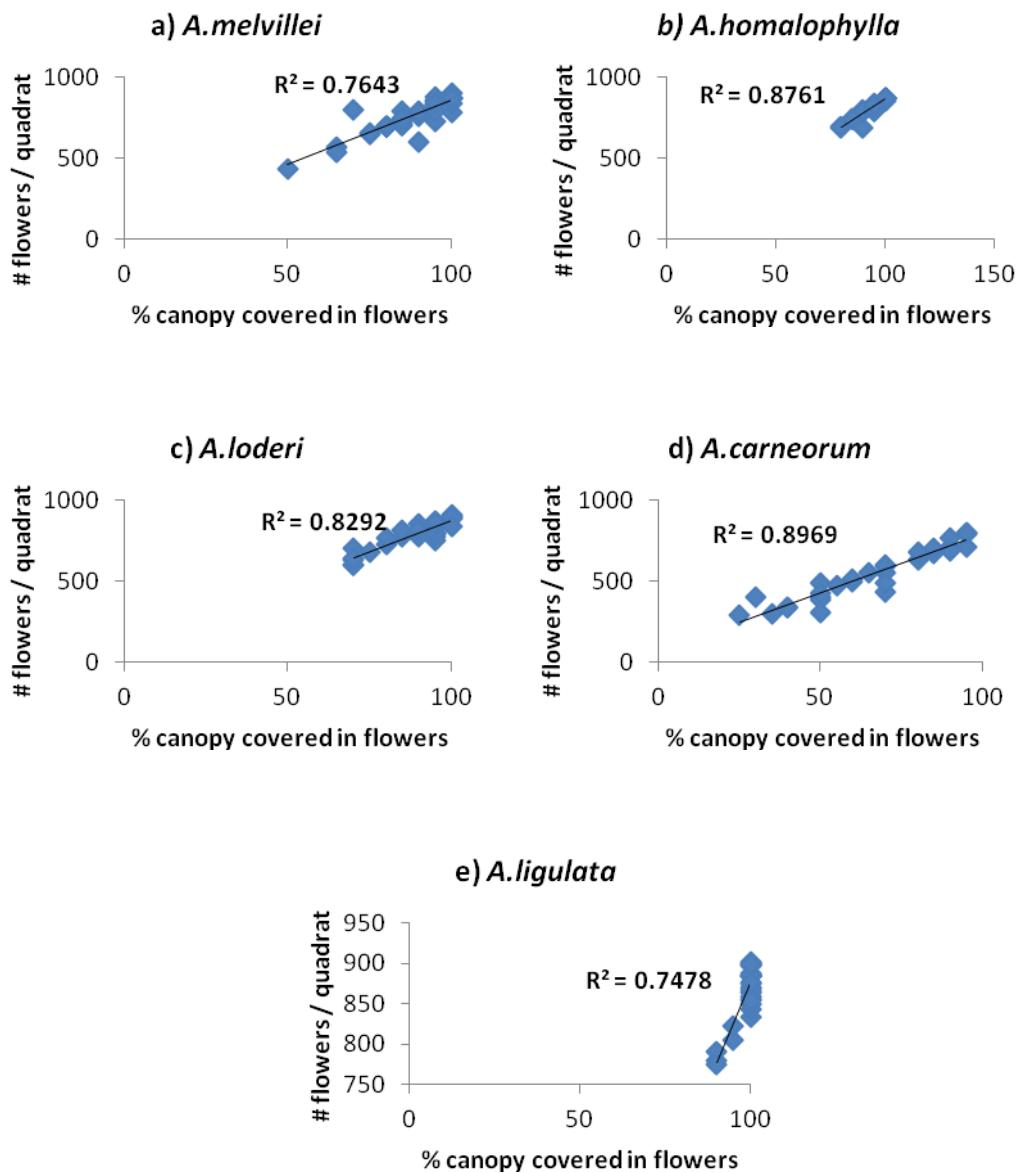
### **3.6 Appendix**

#### **Appendix 3.6.1 Validating the visual method of estimating flowering effort and fruiting intensity**

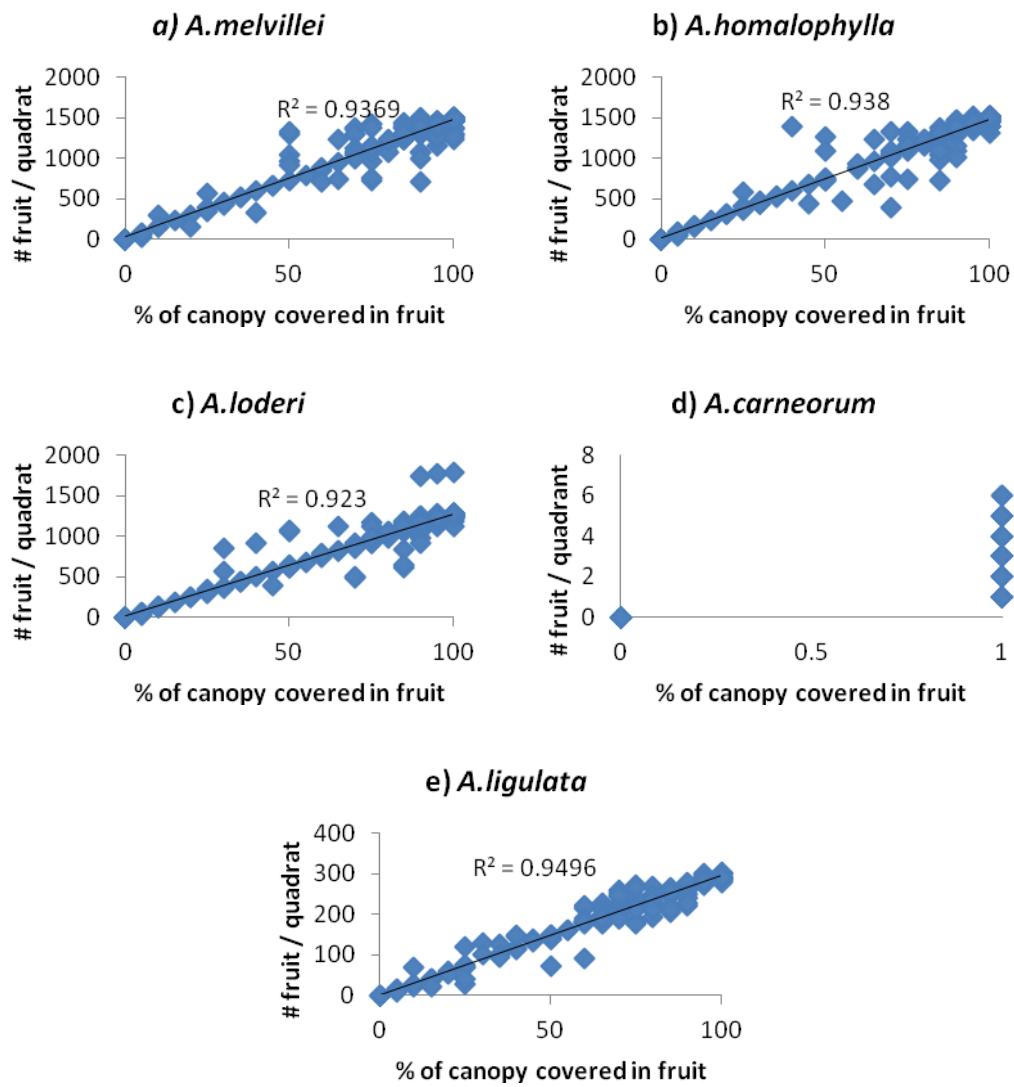
To make consistent and accurate assessments of a) flowering effort and b) fruiting intensity of many populations possible in a short time frame, visual estimation techniques comparing photographs with plants being assessed, to determine the percentage of their canopies surface area covered in flowers and fruit were validated.

Three and ten of the 47, 10, 26, 30 and 20 stands of *A. melvillei*, *A. homalophylla*, *A. loderi*, *A. carneorum* and *A. ligulata* previously selected for this study were randomly selected to evaluate flowering effort and fruiting intensity respectively. For the ten same plants already previously selected within each of these stands, the percentage of canopy surface covered in fruit within 12 x 0.5m by 0.5m quadrats placed evenly around the canopy of each of the 10 plants (encompassing the lower, mid and upper sections of the canopy on each of the N, S, E, W sides of the plant evenly) was visually estimated by referring to photos of canopy of each species covered in flowers and fruit increasing in coverage in increments of 10% to 100%. An average measurement of a) flowering effort and b) fruiting intensity from all 12 quadrats for each plant was calculated. To verify the accuracy of these visual estimates, flowers and fruit were harvested from within these same 12 quadrat areas of the canopy of each plant and were counted. I looked for correlations between flower and fruit coverage values estimated through visual inspection and the number of flowers and fruit counted in the same quadrats, to determine whether they were reliable predictors of the number of flowers and fruit on these. For all five species of plant my visual estimates of flowering effort and fruiting intensity were very accurate and consistent in predicting the actual number of flowers and fruit that were present on plants. As such, the visual technique was verified as suitable for estimating flowering effort and fruiting intensity across the survey area to increase the efficiency of obtaining these data.

a) Flowering effort



b) Fruiting intensity





## **Chapter 4: Looking for drivers of reproductive success and failure in several threatened and one thriving semi arid *Acacia* species in far west NSW.**

### **4.1 Abstract**

In NSW, several semi arid long-lived *Acacia* species have experienced years with little or no fruit set and this has been attributed to habitat fragmentation and senescence. Contrary to expectations I detected high levels of fecundity even in the oldest of plants within stands of several of these species after a region wide rain event in 2010/2011 (Chapter 3, thesis), suggesting factors other than senescence must explain reproductive failure. Here I conduct the first formal surveys of the physical condition of stands and plants in the same 133 stands of three threatened semi arid *Acacia* species (*A. melvillei*, *A. loderi* and *A. carneorum*), one closely related species (*A. homalophylla*) and a thriving species (*A. ligulata*), spread across western NSW. I use this information together with local climatic data and estimates of allelic and genotypic richness at microsatellite loci to explain variation in seed set among stands. I found great intra-specific variance in the physical structure of stands of all five species including stand size, density and proximity to other stands, indicating very different histories of local land use. The majority of stands of *A. melvillei*, *A. homalophylla*, *A. loderi* and *A. carneorum* surveyed were found to consist of fewer than 200 plants, and 47, 89, 73, and 90 % of these same stands are now isolated from the closest neighbouring stand by at least 4 km. In contrast, I found great similarities between all four threatened species and the apparent physical condition of plants measured by the density of healthy canopy cover, presence of observable disease, level of leaf herbivory and epiphyte infection. Reproductive success and failure in these stands could not be adequately explained by these same measures of stand and plant health, or local differences in rainfall and temperature. The lack of genetic diversity found in all five of the 26 *A. loderi* stands that failed to set fruit, despite most stands containing much genetic diversity, may suggest a genetic component to reproductive capacity in this species. This may, however simply reflect a lack of histocompatible mates in neighbouring stands. Taken together, these results suggest that these *Acacia* plants whilst old, are reproductively healthier than suspected to date. Given the depleted local conditions, expecting any long term recruitment may still be optimistic despite the abundance of seed set.

## **4.2 Introduction**

Reproductive failure of plants in highly modified landscapes has to date largely been investigated in the northern hemisphere using short lived temperate species that are obligate seeders with simple life histories. Few studies attempt to explain reproductive failure in longer lived (iteroparous) plants, or plants with complex reproductive strategies. This may reflect the difficulty of conducting long term studies. Given that long lived overstory plants are generally ecosystem engineers within their environments (Morton & Davis, 1983; Recher & Davis, 1997; Sharp, 1997; Facelli & Brock, 2000; Singh & Rathod, 2002), understanding the way they are likely to respond to existing within fragmented patches is crucial for conservation of the many species that rely on them, as well as their own conservation.

In several threatened semi arid *Acacia* species in western NSW, long standing theories that explain the historic lack of sexual reproduction as a consequence of senescence, pollen limitation and inbreeding depression, were largely discarded after a large scale rain event beginning in January of 2010 initiated mass seed set in most stands of most of these species. This confirmed suspicions that water was at least a key limiting factor and confirmed that these species utilize a ‘boom bust’ reproductive cycle, as is the case with many other arid and semi arid species (Büsgen & Münch 1929; Norton & Kelly 1988; Letnic & Dickman, 2006; Wardle et al., 2013). Even though some level of contraction of stands may actually be natural under the climatic conditions during this period, under unnaturally intense grazing regimes, this contraction has clearly become dangerously unsustainable (Auld 1993, 1995, Auld & Denham, 2001; Porteniers, 2001). It has lead to a drastic skewing of the age structure of these remaining stands such that the majority of plants are either senescent or at least in the later stages of their life (Chapter 2-thesis). For *A. carneorum*, death of the last remaining extremely old plants that make up the remaining stands may be imminent. It is widely accepted that any conservation action needs to happen urgently before these plants senesce and die (Porteniers, 2001). Interestingly there were a minority of *A. melvillei*, *A. homalophylla* and *A. loderi* stands and more obviously a majority of stands of *A. carneorum* that did not set fruit after this rain, despite no apparent difference in the age of plants, levels of flowering effort or numbers of pollen tubes reaching the ovary of flowers, when compared to plants in those stands that did set fruit (Chapter 2 & 3, thesis). Checks for fruit set the following year found the same result, discounting a lag effect in these stands. This confirmed that while large scale rain events appear essential for reproduction in these arid *Acacia* species, another factor, or combination of factors is limiting universal fruit set.

Plants that flower well, are not pollen limited and are not senescing can still fail to reproduce for several reasons. In natural stands, low fecundity in individuals can be caused by dysfunctional gametes (Bretagnolle & Thompson, 1993; Otto and Whitton, 2000), influenced by environmental variables (Ramsey & Schemske, 1998) or the deposition of incompatible pollen (Crow & Kimura, 1970; Goverde et al., 2002; Peterson et al., 2008). If plants are isolated enough from one another, associated increases in levels of inbreeding as a result of restrictions in foraging movements of pollinators can serve to reduce, or in extreme cases, halt reproduction as a result of inbreeding depression (Whelan et al., 2000; Mustajärvi et al., 2001; Goverde et al., 2002; Aizen & Feinsinger, 2003; Peterson et al., 2008; Andrieu et al., 2009; González-Varo et al., 2010). Whether this predicted increase in selfing is likely to affect fecundity will depend on the level of self compatibility of these species, or the level of genetic diversity remaining within these stands. To date this is not known for these *Acacia*. While plant species with high levels of self compatibility are likely to be less effected over the short term at least, stands that are preferentially outcrossing, or obligately outcrossing would be expected to suffer reproductive failure if they do not receive sufficiently genetically divergent pollen (Goverde et al., 2002; Aizen & Feinsinger; Andrieu et al., 2009; González-Varo et al., 2011).

While qualitative demographic surveys of these species have been conducted in NSW, (Chapter 2, thesis), more detailed comparative assessments of the physical condition of these stands have never been made. Importantly, the level of variance in the structure of stands and condition of plants of the same species and between species remains unknown, but should be key information for land managers in predicting their future states. Nevertheless, some recent genetic surveys have found both diverse and clonal *A. melvillei* and *A. loderi* stands (Roberts et al., 2013, 2016; Forrest et al., 2015), whilst in contrast all *A. carneorum* stands surveyed so far in the region have been found to be clonal, or at least containing very little genetic diversity (O'Brien et al., 2014; Roberts et al., 2013; Roberts et al., (in press)). It remains to be seen whether the genetic structure of stands has any bearing on their reproductive health. Either way, uncovering the reasons behind reproductive failure and success in these *Acacia* stands is crucial for managers working to conserve these threatened species.

Across the semi arid region of western NSW, I collected data on the condition of plants and stands as well as the genetic structure of the same stands of the four threatened *Acacia* species (*A. melvillei*, *A. homalophylla*, *A. loderi* and *A. carneorum*), and one co-occurring non threatened *Acacia* species (*A. ligulata*) surveyed in Chapters 3 of this thesis, to describe the condition of these stands quantitatively, for the first time. I combined this information on

stand condition with previously collected data on the reproductive response of these same plants to the dramatic drought breaking La Niña rains of 2010-2011 (Chapter 3, thesis), to determine whether the reproductive success and failure observed in different stands appears to be driven by their physical condition, differences in local climate, genetic structure, or a combination of several of these factors.

Specifically I aim to test these hypotheses:

1. Stands of *Acacia* are all in equally poor (fragmented) condition across their range.
2. Plant health will be lower in stands in poorer condition (more highly fragmented).
3. Sexual reproductive success and failure or fecundity of stands of these *Acacia* species is reduced by reduced stand and plant health, lower local rain fall levels, higher average local temperatures, reduced genetic diversity, or a combination of these factors.

## **4.3 Methods**

### **4.3.1 Study species and study area**

The same set of five co-occurring overstory *Acacia* species selected in previous chapters of this thesis (Chapter 2 & 3, thesis) from within a region of approximately 336,000 km<sup>2</sup> across the semi arid regions of NSW in southeastern Australia were again the focus of this study.

### **4.3.2 Selection of plants within stands**

I estimated the reproductive performance of adults using the same 10 haphazardly chosen plants previously surveyed along a single lineal transect through the approximate centre of each stand surveyed in Chapters 2 & 3 of this thesis.

### **4.3.3. Assessing the condition (health) of stands across the region**

The condition of stands of *A. melvillei*, *A. homalophylla*, *A. loderi*, *A. carneorum* and *A. ligulata* was characterised by measuring a suite of readily measurable, demographic, environmental health and individual plant health measures. These are recognized stand and

plant parameters that are often important for the general and reproductive capacity of plants (Jones, 1976; MacGarvin et al., 1986; Kearns et al. 1998; Terborgh et al. 2006; Laurance et al., 2009). The factors measured can be divided into the categories: a) stand condition including (i) stand structure and (ii) environmental integrity; and b) condition of plants. I also looked for any linear relationships between any of the measures of stand condition and plant health.

Table 4.1. **Measures of *Acacia* stand condition and plant health:** subdivided into a) stand condition, (i) 5 stand structure, (ii) 3 environmental integrity and b) 4 plant health measures with the sampling method for each measure.

<b>a) Stand condition</b>	
<b>(i) Stand structure</b>	
1. <i>Size of stands</i>	Each stand was placed into one of four size categories <10, 11-50, 51-200, and > 201 plants.
2. <i>Level of stand isolation</i>	The distance in kilometres to the next stand of the same species was estimated to be <1km, >1km to 3km, >3km to 5km, >5km to 10km and >10km (binoculars were used during a 4WD search across the landscape).
3. <i>Density of mature plants within stands</i>	The density of plants was estimated by determining the average distance in metres of plants to their nearest neighbour of the same species.
4. <i>Average height of plants/ stand</i>	The height of each plant was measured in metres.
5. <i>Average trunk circumference of plants/ stand</i>	The circumference of each plants tree trunk was measured at the very base of the trunk in centimetres.
<b>(ii) Environmental integrity</b>	
1. <i>Density of the understory within stands</i>	Measured using visual estimates of the percentage of the ground covered in understory vegetation. Quadrats (12x 2m <sup>2</sup> ) were placed around each plant at the compass points (N,S,E and W) such that at each compass point 3 quadrats were placed on the ground under, at the edge and 2 metres away from the canopy of each plant. *The visual estimation technique used here was verified as accurate prior to use (See Appendix 4.6.1 a)
2. <i>Density of other overstory species within stands</i>	The density of overstory plants of other species within each stand was measured by counting the number of plants in up to 10 haphazardly chosen 10m <sup>2</sup> plots spread out through the overall area of each stand, or the maximum number of plots that could be fitted into the area of the stand.
3. <i>Land use</i>	The land at each site was classified as (i) Unaltered (National Park, nature reserve or uncleared crown land), (ii) Agricultural land, (iii) Road side verge land.
<b>b) Condition of plants</b>	
1. <i>Density of healthy canopy cover</i>	The percentage of the canopy of plants in each stand that was covered in healthy foliage was measured by visual estimation, using photographs of plants of each species with a full healthy canopy as a reference. An average value of all ten plants was calculated for each stand. *The visual estimation technique used here was

	<i>verified as accurate prior to use (See Appendix 4.6.1 b).</i>
<i>2. Presence of observable disease</i>	The percentage of leaves surface that was affected by any disease (including parasites) was determined by visually estimating the percentage of leaf surface that was discoloured. A total of 50 leaves chosen randomly from a handful of leaves collected on 4 sides (N, S, E, W) of each plant from low, mid and high in the canopy were used. *The visual estimation technique used here was verified as accurate prior to use (See Appendix 4.6.1 c).
<i>3. Amount of leaf herbivory</i>	The same leaves that were inspected for evidence of disease were also inspected for evidence of herbivory and the percentage of the leaf removed by herbivory was estimated visually. *The visual estimation technique used here was verified as accurate prior to use (see Appendix 4.6.1 d).
<i>4. Level of Epiphyte infection</i>	The percentage of plants canopy covered by epiphytes was estimated visually by measuring the surface area of any epiphytes found on plants and the surface area of plant canopies using 1m <sup>2</sup> quadrats. *See Appendix 4.6.1 e for test of method.

#### **4.3.4 Assessing the role of stand condition and climate on sexual reproductive success**

I looked for readily quantifiable predictors of reproductive success in these five semi arid *Acacia* species, *A. melvillei*, *A. homalophylla*, *A. loderi*, *A. carneorum* and *A. ligulata*. Regression analysis was used to look for the presence of linear relationships between the 12 stand and plant variables measured above, as well as the average annual rainfall and average maximum temperature at the closest Australian Bureau of Meteorology weather station (for 2010 and 2011) to each *Acacia* stand and a) the percentage of independent mature trees within stands that set fruit and b) the average fecundity levels of plants (fruiting intensity per unit area of plant canopy), in both consecutive years quantified previously (Chapter 3, thesis). All percentage data was arcsine transformed, and all data obtained through ‘counts’ was square root transformed to fit a normal distribution. In case a combination of these ‘stand, plant and climatic variables best explained the presence and intensity of fruiting rather than a single variable, hierarchical regression incorporating a combination of multiple variables was also performed (Appendix 4.6.3).

#### **4.3.5 Comparison of genetic diversity between stands with and without fruit**

I used microsatellite markers developed previously (Roberts et al., 2013) to characterize the genetic make up and structure of multiple stands of *A. loderi* and *A. carneorum*\*, the species observed in Chapter 3 of this thesis with the most striking reproductive failure. In an attempt to determine whether stands that failed to produce fruit lacked genetic diversity, the levels of genetic diversity and structure of 14 and 12 stands of *A. loderi* and *A. carneorum* respectively that proved highly fecund and three and two stands respectively that did not produce any fruit in the two consecutive years after the 2010/2011 La Niña rainfall were compared. For each plant, juvenile leaf was haphazardly sampled from 30 adult plants spread throughout each stand, or every adult plant within the stand if stands consisted of less than 30 adult individuals and a GPS reading of each plant’s location was recorded. Genomic DNA was extracted for each individual using the standard CTAB method (Doyle and Doyle, 1987). Two separate sets of eight microsatellite markers that I developed together for *A. loderi* and *A. carneorum* (Roberts et al., 2013) were used for genotyping. To PCR amplify loci of interest I used Multiplex-Ready Technology and the standard PCR protocol (Hayden et al., 2008) and multiplexed two to three primer pairs per run. Genotyping was performed on the ABI 3100 fragment analysis machine and scored using the ABI genemapper software version 3.1. We

used the programs Genalix version 6.5 (Peakall & Smouse, 2012) to analyse the set of multilocus genotypes obtained from each stand. Specifically, I characterized the level of genetic diversity as measured by the average number of alleles per locus ( $A$ ), average expected heterozygosities ( $H_e$ ), and fixation indices ( $F_{IS}$ ) in up to six fruiting and non fruiting stands of the *Acacia* species. Because *A. loderi* and *A. carneorum* reproduce both sexually and asexually, I used GenClone v 2.0 to estimate the probability that  $n$  (where  $n = 1, 2, 3...i$ ) copies of a multilocus genotype (MLG) were produced by distinct episodes of sexual reproduction ( $P_{sex}$ ) (Arnaud-Haond and Belkhir, 2007). Where  $P_{sex} < 0.05$  it is improbable that  $n$  MLG copies were derived by sex alone. Genepop version 4.2 was used to perform pair-wise tests for linkage equilibrium.

*\*The genotyping of *A. loderi* and *A. carneorum* stands was done in collaboration with another team member Dave Roberts.*

## **4.4 Results**

### **4.4.1 Assessing the condition (health) of stands across the region**

#### *a) Structure and condition of stands*

I found a high degree of variation in stand size, density and understory cover among stands of all five *Acacia* species surveyed, meaning I rejected my first hypothesis that stands were similarly structured across the region (Figure 4.2). The least isolated stands of each species contained hundreds of plants which were located within 1 km of their nearest stand. In these stands I found nearest neighbour distances between mature stems ranging on average from as low as 1 to 2.2 m and levels of understory ground coverage ranging from as high as 94% to as low as 35%. The most fragmented stands of each species contained less than 10 plants (many lone plants also existed), which were located within 1 km of the next closest stand. Often these stands also had low densities of plants characterized by nearest neighbour distances between mature stems as high as 125 to 6.8 m, and relatively lower levels of understory ground coverage, ranging from as low as 1% to 57.5% among the five species. While the vast majority of remaining stands surveyed of *A. melvillei* (87%), *A. homalophylla* (89%) and *A. ligulata* (71%) plants were found along road verges, most *A. carneorum* (95%) and

*A. loderi* (77%) stands were found on farm land, or on protected land such as National Parks (Figure 4.1 a).

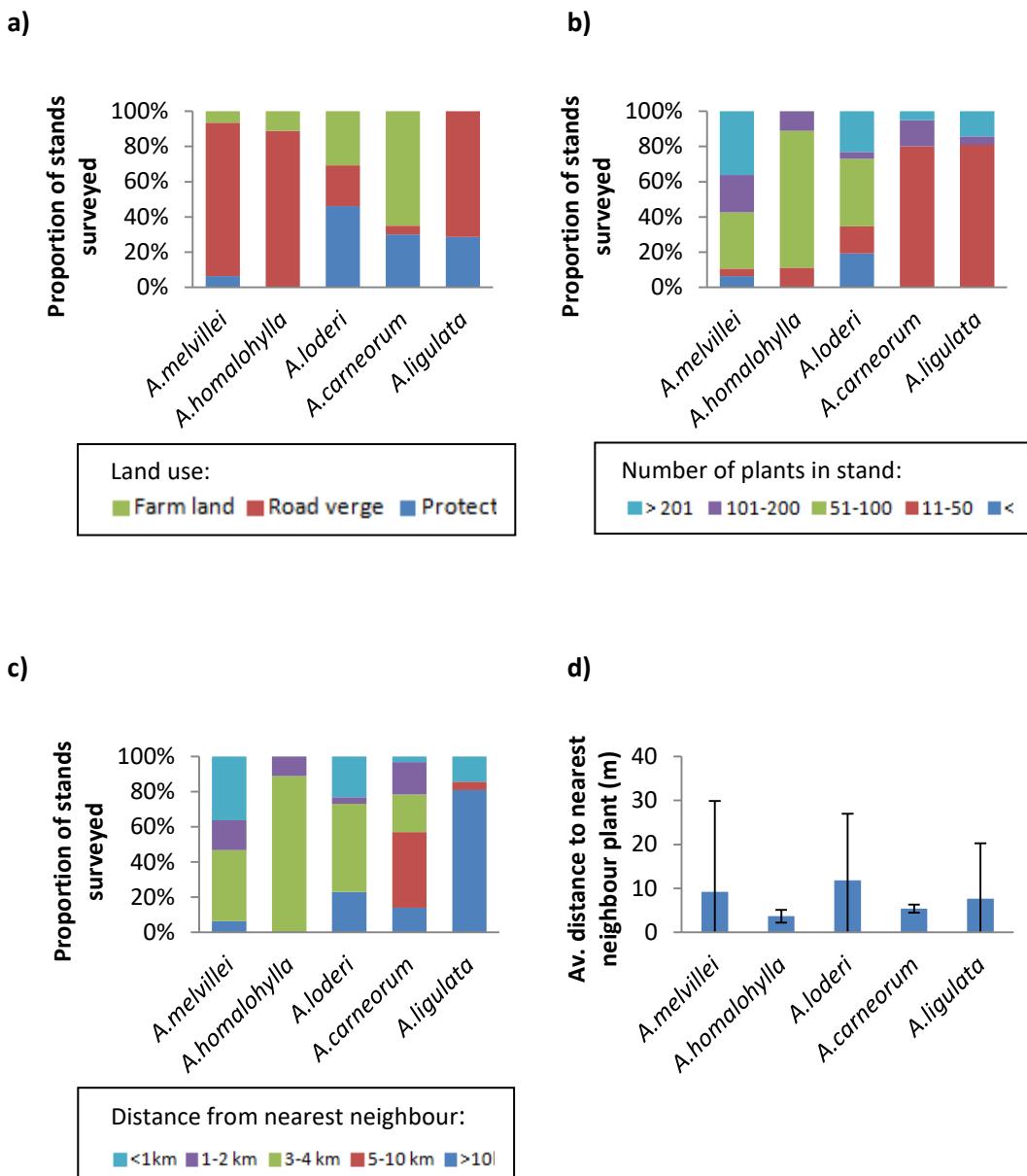
I found a higher level of variance in the size (# of plants) of *A. melvillei* and *A. loderi* stands compared to the other three species. Excluding suckers, which vastly increased the size of *A. homalophylla* stands and a minority of *A. carneorum* stands, a considerably higher percentage of *A. melvillei* stands (36%) and *A. loderi* stands (23%) contained more than 201 mature plants compared with the *A. homalophylla* stands (0%), *A. carneorum* stands (5%) and *A. ligulata* stands (14%) respectively. *A. melvillei* and *A. loderi* also had the highest percentages of stands with less than 10 plants with 6% and 19% respectively. Stands this small were not observed for the other three species. While 78% of *A. homalophylla* stands surveyed consisted of between 51 and 100 plants per stand, 80% and 81% of *A. carneorum* and *A. ligulata* stands respectively contained only between 11-50 plants per stand (Figure 4.1 b).

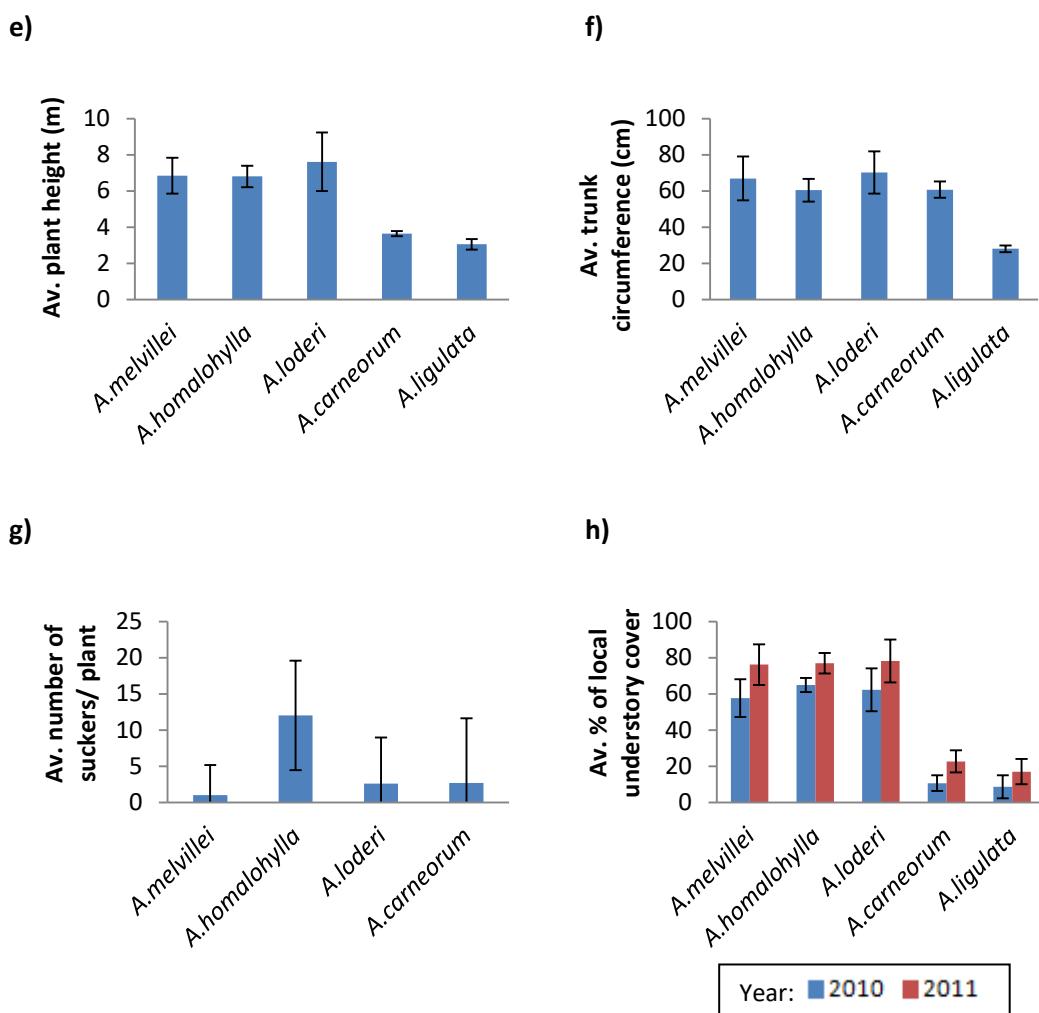
The degree of isolation between these stands followed a similar pattern as for stand size with a relatively high level of variance found between stands of *A. melvillei*, *A. loderi* and *A. carneorum* compared with *A. homalophylla* and *A. ligulata*. The majority of *A. homalophylla* stands (89%) were located 3-4 km from the nearest stand of the same species, while the majority of *A. ligulata* stands (81%) were located more than 10 km from their nearest neighbour (Figure 4.1 c).

While the average density of plants in stands was noticeably lower for *A. melvillei*, *A. loderi* and *A. ligulata* than for *A. homalophylla* and *A. carneorum*, indicated by nearest neighbour distances (Figure 4.1 d), of particular note was the considerably higher variance between stands found for *A. melvillei*, *A. loderi* and *A. ligulata* stands compared with *A. homalophylla* and *A. carneorum* stands.

Plant height and trunk width measures highlighted the similarity of the stature of plants between stands of each species as previously described in Chapter 2 of this thesis. While a low to moderate amount of variance in both measures between stands of *A. melvillei* and *A. loderi* was found, plant height and trunk width of *A. homalophylla*, *A. carneorum* and *A. ligulata* plants varied very little at all (Figure 4.1 e & f). The average number of suckers per plant was on average considerably higher in *A. carneorum* and *A. homalophylla* plants compared with *A. melvillei* and *A. loderi* plants (Figure 4.1 g), however, it must be noted that for *A. carneorum* this was driven solely by intense suckering at only two of the thirty sites surveyed. No other overstory plant species was found within sight of any of the stands of all five *Acacia* species.

The percentage of ground area around plants covered in understory vegetation was on average equivocally high for *A. melvillei*, *A. homalophylla* and *A. loderi*. This stood in stark contrast to levels of ground cover found in any *A. carneorum* or *A. ligulata* stands (Figure 4.1 h).



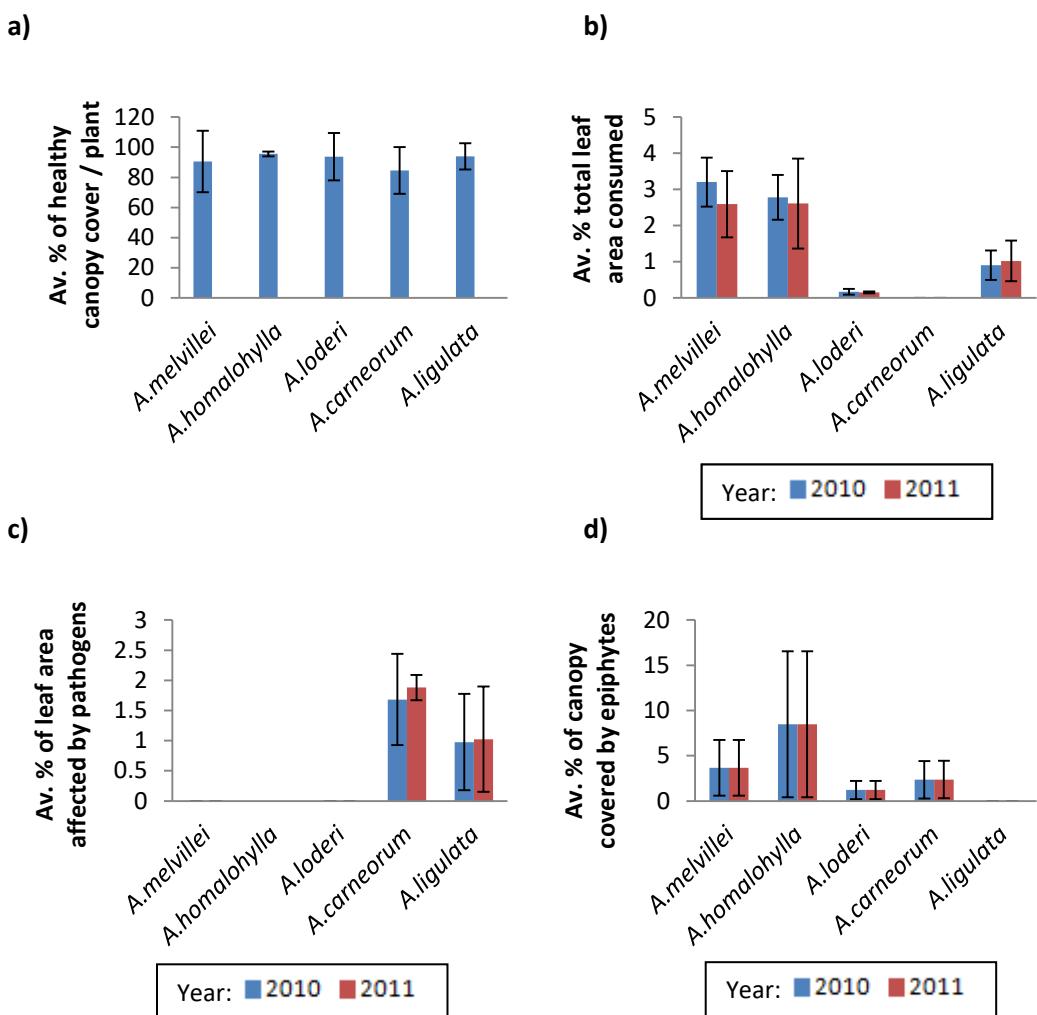


**Figure 4.1. Structure and condition of *A. melvillei*, *A. loderi*, *A. carneorum* and *A. homalophylla* stands.** Proportion of stands surveyed relative to a) land use, b) number of plants in a stand, c) distance to nearest neighbour, d) Average distance to nearest neighbor plant, e) Average plant height (m), f) Average trunk circumference (cm), g) Average number of suckers / plant, h) Average % of ground around plants covered in understory vegetation. \*Error bars (SE) represent variation between stands.

#### b) Condition of trees within stands

In general I found far less variance in the four measures of plant health in most stands of the five *Acacia* species compared with the structural characteristics of stands described above (Figure 4.2). The percentage of healthy canopy cover of trees did not vary much across all five *Acacia* species, or between stands of each species (Figure 4.2 a). Herbivory rates were negligible (<4.5% of leaves consumed on average), or undetected on plants of these five *Acacia*

species with little variation between stands of each species (Figure 4.2 b). Obvious signs of disease on leaves were only detected in *A. carneorum* and *A. ligulata* plants at very low levels (<3% of leaf surface affected on average) (Figure 4.2 c). Levels of epiphyte infection were very low (<5% of plants canopy surface covered), or nonexistent on *A. melvillei*, *A. loderi*, *A. carneorum* and *A. ligulata* plants with at least double the levels of epiphyte infection found on the more easterly distributed *A. homalophylla* plant canopies (Figure 4.2 d).



**Figure 4.2. Condition of *A. melvillei*, *A. loderi*, *A. carneorum* and *A. homalophylla* plants:** as measured by a) % of healthy canopy cover / plant, b) Average % total leaf area consumed, c) Average % of leaf area effected by pathogens, d) Average % of plant canopy covered by epiphytes, for 47, 10, 26, 30 and 20 stands of *A. melvillei*, *A. homalophylla*, *A. loderi*, *A. carneorum* and *A. ligulata* respectively. \*Error bars (SE) represent variation between stands.

c) Relationship between stand condition and plant health

Some significant positive linear relationships were found between several of the stand condition parameters and plant health measures used here for *A. melvillei*, *A. homalophylla*, *A. carneorum* and *A. ligulata* suggesting, as hypothesised, that some stand condition parameters have an effect on the condition of plants. A positive linear relationship between the density of healthy canopy cover of plants and the density of plants within stands (average distance to nearest neighbouring plant) was found for *A. melvillei* ( $r^2=0.762$ ,  $F=143.935$ ,  $p<0.001$ ), *A. carneorum* ( $r^2=0.267$ ,  $F=9.841$ ,  $p=0.004$ ) and *A. ligulata* ( $r^2=0.845$ ,  $F=98.312$ ,  $p<0.001$ ). A positive relationship was also found between the density of healthy canopy cover of plants and the proximity of stands to one another (level of isolation of stands) for *A. melvillei* ( $r^2=0.202$ ,  $F=11.418$ ,  $p=0.002$ ) and *A. ligulata* ( $r^2=0.245$ ,  $F=5.847$ ,  $p=0.026$ ). For *A. homalophylla* a positive linear relationship between the level of herbivory of plants and the size of stands ( $r^2=0.571$ ,  $F=9.320$ ,  $p=0.019$ ), as well as with the density of plants within stands ( $r^2=0.539$ ,  $F=8.182$ ,  $p=0.024$ ) was found. For *A. melvillei*, a positive relationship between the number of epiphytes infecting plants and the size of stands ( $r^2=0.133$ ,  $F=6.883$ ,  $p=0.012$ ) was found. For *A. carneorum*, a positive relationship between the number of epiphytes infecting plants and the level of understory ground cover in stands was also found ( $r^2=0.138$ ,  $F=4.333$ ,  $p=0.047$ ). For *A. ligulata*, positive linear relationships were found between the density of healthy canopy cover of plants and size of stands ( $r^2=0.594$ ,  $F=26.337$ ,  $p<0.001$ ), and between herbivory levels on plants and the level of understory ground cover in stands ( $r^2=0.254$ ,  $F=6.129$ ,  $p=0.023$ ). All other relationships were insignificant (Appendix 4.6.2).

#### 4.4.2 Assessing the role of stand condition and climate on sexual reproductive success

No significant correlations were found between the seven structural, four plant health and two climatic variables measured at each of the stands of the five *Acacia* species and the reproductive capacity of stands measured by the proportion of plants per stand that set any fruit. Hierarchical multiple regression analysis adding the seven structural measures (model 1) followed by the four measures of plant health (model 2) and lastly the two measures of local climatic conditions (model 3) also revealed no improvement in the capacity to predict reproductive response in combination for *A. melvillei*, *A. loderi* or *A. carneorum* (Appendix 4.6.1). While too few stands of *A. homalophylla* were surveyed to perform similar multiple regressions, it is doubtful that a significant correlation would be found given the lack of

relationship for any of the other three threatened species. As such, my hypothesis that reductions in stand or plant health will affect the reproductive capacity of these stands must be rejected for all five *Acacia* species.

No correlations were found between the seven structural, four plant health, and two climatic variables measured at each of the multiple stands of each of the five *Acacia* species and reproductive effort as measured by the average reproductive effort (fruiting intensity) of plants within stands. Regression analysis found no significant linear relationships between the capacity of plants to reproduce sexually and any of the variables measured within the five *Acacia* species (Appendix 4.6.3). Hierarchical multiple regression analysis adding the seven structural measures (model 1) followed by the four measures of plant health (model 2) and lastly the two measures of local climatic conditions (model 3) also revealed no improvement in predicting the fecundity of plants after the La Niña rains in combination for *A. melvillei*, *A. loderi* and *A. carneorum*, (Appendix 4.6.3). Again, while too few stands of *A. homalophylla* were surveyed to perform a similar multiple regressions, a similar lack of relationship as found for the other three threatened species is likely (Appendix 4.6.3).

#### **4.4.3 Comparison of the genetic make up of stands of *A. carneorum* and *A. loderi* with and without fruit set**

##### *(i) Acacia loderi*

I detected moderate to high levels of genetic diversity in 11 out of the 14 *A. loderi* stands that set fruit in response to the 2010-11 La Niña rain event, while the remaining three stands were genetically monoclonal (Table 4.2). The three non fruit setting *A. loderi* stands were also monoclonal, suggesting that while clonality seems associated with failure of these stands to set fruit, it cannot be used alone as a simple predictor of their capacity to sexually reproduce. Nevertheless, as all *A. loderi* stands that contained genetic diversity produced fruit, genetic diversity appears to be a consistent predictor of sexual capacity in stands of this species. None of the pair-wise tests for linkage equilibrium revealed significant associations between loci ( $P > 0.05$ ).

Of the 11 genetically diverse fruiting *A. loderi* stands surveyed, levels of genotypic diversity were typically high with many stands consisting of all genetically distinct individuals (Table 4.2). The average number of alleles per locus ( $Na$ ) was moderate to high for the 11

genetically diverse stands.  $N_a$  was equivalently low for all six clonal stands irrespective of their fruiting history, indicating that fruit set in clonal stands is unlikely determined by higher  $N_a$  within stands (Table 4.2).  $H_e$  was also on average much higher for the fruiting *A. ligulata* stands than the non fruiting stands, which were equivalently low, indicating that fruit set in clonal stands is also unlikely determined by higher  $H_e$  within stands (Table 4.2).

All *A. loderi* stands that contained diversity, but also contained multiple plants with the same multilocus, were likely a result of a combination of sexual and asexual recruitment.  $P_{sex}$  scores of  $<0.0001$  were reached for  $n=1$  copy of a multilocus genotype in all these stands suggesting it is improbable that any of the replicate genotypes within these genetically diverse stands were produced by distinct episodes of sexual reproduction. Given the capacity of these species to sucker, it is therefore highly probable that plants with the same genotype in these stands, of all three species, were produced by asexual reproduction.

For each of the clonal stands of all three *Acacia* species, it was highly improbable that plants in these stands were the product of sexual reproduction. In all cases, the number of repeat genotypes found in these stands (between 7 and 30) far exceeded the maximum number of replicates that might potentially be expected to occur from sexual reproduction ( $P_{sex}$  values of  $<0.05$  were reached with only between  $n=4$  replicates to  $n=7$  replicates among these stands, with all higher values of  $n$  resulting in  $P_{sex}$  values  $<0.05$  in every clonal stand). None of the multilocus genotypes found in the clonal stands were found in any of the stands that set fruit, whether they were genetically diverse or clonal.

While levels of genetic diversity and expected genotypic diversity were generally high for non clonal *A. loderi* stands characterized here, positive average  $F_{IS}$  scores across all loci indicated deficits of heterozygotes in all of these stands, suggesting inbreeding is a common phenomenon in these stands (Table 4.2).

#### (ii) *Acacia carneorum*

All 14 stands of *A. carneorum* that were genetically characterized, represented genetically distinct clones, irrespective of their capacity to set fruit. This suggests that sexual capacity in *A. carneorum* has little to do with genetic diversity. Comparative measures of average  $N_a$  and  $H_e$  between the six non fruiting and three fruiting stands revealed marginally higher average levels in the two fruiting *A. carneorum* stands compared with the non fruiting stands, suggesting that higher levels of either measure cannot account for the difference in the

capacity of some clonal plants to set seed (Table 2). For all 14 *clonal A. carneorum* stands, the probability that the plants populating them were produced by sexual reproduction was very low, irrespective of their capacity to set fruit. The number of repeat genotypes found in these stands (between 8 and 120) far exceeded the maximum number of replicates that might potentially be expected to occur from sexual reproduction ( $P_{sex}$  values of <0.05 were reached with only between n=4 to n=7 replicates among these stands, with all higher values of n resulting in  $P_{sex}$  values <0.05 in every clonal stand).

**Table 4.2: Comparison of genetic diversity in fruit setting and non fruit setting *A. loderi* and *A. carneorum* stands:** Number of samples ( $N$ ), average number of alleles per locus ( $Na$ ) per stand, Average number of alleles per locus per plant ( $Na/N$ ), # of private alleles per stand, average # private alleles per plant, average # of genotypes per stand, average # genotypes per plant, average expected heterozygosities ( $H_e$ ) per stand, Hardy-Weinberg equilibrium (HWeq) and fixation indices ( $F_{IS}$ ) per stand with standard errors ( $\pm$ ), in up to six stands which set fruit after the 2010 La Niña rain event, and up to six stands that did not set fruit of each species .

	History of fruit set	# stands	Plants/stand range ( $N$ )	Average # alleles per locus ( $Na$ )		Average # private alleles per stand		Average # multilocus genotypes per stand		# stands characterized that contained genetic diversity vs. clonal		Expected Heterozygosity ( $H_e$ )		Hardy-Weinberg Equilibrium (HWeq)		Fixation Indices ( $F$ )	
				Average	Range	Average	Range	Average	Range	# diverse stands	# clonal stands	Average	Range	Average	Range	Average	Range
<i>A. loderi</i>	F	14	8-30	6.420 ( $\pm 0.360$ )	1.44 ( $\pm 0.222$ )-91 ( $\pm 22$ )	1.5 ( $\pm 0.251$ )	0-3	20.786 ( $\pm 3.226$ )	1-30	11	3	0.613 ( $\pm 0.023$ )	0.2 ( $\pm 0.1$ )-0.763 ( $\pm 0.05$ )	p>0.05 (ns)	p<0.01-0.326	-0.140 ( $\pm 0.044$ )	-1 ( $\pm 0.00$ )-0.368 ( $\pm 0.074$ )
	NF	3	11-30	1.542 ( $\pm 0.104$ )	1.44 ( $\pm 0.2$ )-1.6 ( $\pm 0.22$ )	0 ( $\pm 0$ )	NA	1 (0)	clonal	0	3	0.271 ( $\pm 0.052$ )	0.188 ( $\pm 0.091$ )-0.313 ( $\pm 0.091$ )	p>0.05*	P<0.001-0.151	-0.992 ( $\pm 0.006$ )	-1(0.00)-1 (0.00)
<i>A. carneorum</i>	F	2	8-30	1.625 ( $\pm 0.067$ )	1.4 ( $\pm 0.2$ )-2.1 ( $\pm 0.3$ )	0.75 ( $\pm 0.218$ )	0-2	1(0)	clonal	0	12	0.254 ( $\pm 0.026$ )	0.250 ( $\pm 0.094$ )-0.250 ( $\pm 0.094$ )	P<0.001	P<0.001 for all	-0.843 ( $\pm 0.039$ )	-0.981 ( $\pm 0.014$ )-1.000 ( $\pm 0.000$ )
	NF	12	21-25	1.500 ( $\pm 0.129$ )	1.5 ( $\pm 0.2$ )-1.5 ( $\pm 0.2$ )	1.5 ( $\pm 1.5$ )	0-3	1(0)	clonal	0	2	0.250 ( $\pm 0.065$ )	0.187 ( $\pm 0.091$ )-0.395 ( $\pm 0.088$ )	P<0.001	P<0.001 for all	-0.990 ( $\pm 0.007$ )	-0.437 ( $\pm 0.279$ )-1.000 ( $\pm 0.000$ )

## 4.5 Discussion

### Evaluating the condition of Acacia stands and the affect of condition on reproductive health

While my findings here support the results of previous qualitative surveys that have reported that these overstory *Acacia* species existed in highly fragmented states, my finding of great variance in the physical structures of these stands is the first time such distinctions have been made. This gives a better indication of the true condition of stands in the region and should be used to prioritise conservation of smaller, most isolated stands over those that contain more individuals and are better connected, or vice versa. While many stands of these threatened *Acacia* species containing only a handful of senescent plants would undoubtedly represent some of the most extreme examples of unnaturally fragmented stands in any system, I did not find a strong link between their condition and reproductive health. Indeed, the finding of equivalently high numbers of fruit on plants in the smallest and most isolated stands, including lone plants isolated from any other plant by tens of kilometres, highlights the reproductive resilience of these species to their highly depleted and fragmented conditions.

The reproductive resilience displayed by highly fragmented plants has been noted in several overstory species both abroad (Severns et al., 2001; Nayak & Davidar, 2010; Aguilar et al., 2012; Córtes et al., 2013; Matesanz et al., 2015) and in Australia (Ottewell et al., 2010; Breed, et al., 2013; Bradbury & Krauss, 2013; Ashworth et al., 2015; Broadhurst, 2015). Such resilience must necessarily be conferred through a level of physiological tolerance to any altered local abiotic and biotic conditions. Also necessary is a resilient reproductive strategy, and for sexually reproducing plants a resilient mating system, as is obviously the case for many of these semi arid *Acacia* stands.

Mating systems can confer a level of resilience to sexually reproducing plants that have been fragmented in several ways; 1. Longevity of flowers/ length of flowering time requiring minimal pollen transfer to initiate fruit set (Fréville et al., 2007), 2. Possessing highly mobile pollinators able to bridge the vast gaps between remaining stands (Lowe et al., 2005; Petit et al., 2005; Bacchus and Jump, 2011; Kremer et al., 2012; Vranckx et al., 2011), 3. Having a high level of self compatibility meaning less chance of pollen limitation and inbreeding depression (Kalisz & Vogler & Hanley, 2004; Knight et al., 2005; Eckert, Samis & Dart, 2006; Brys et al., 2011; Rodger, et al., 2013), or 4. Possessing the ability to set fruit via parthenogenesis (Kearney, 2003), or a combination of these attributes. Given the flowers of these *Acacia* species are adapted for insect pollination (Gilpin et al., 2014), we might not expect large gaps

between isolated stands to be bridged to the degree or frequency capable by avian or mammal pollinators (Paton & Turner, 1985; Taylor & Whelan, 1988; Richardson et al., 2000; England et al., 2001). Whilst reductions in outcrossing rates can mean reproductive failure for obligately outcrossing species, fecundity can be maintained in species with higher levels of self compatibility despite reductions in outcrossing rates, so long as they are not pollen limited or affected by inbreeding depression. If we are to make better predictions about the reproductive histories and future reproductive success of these *Acacia* under worsening conditions, it is clear there is a need for further study to uncover more about their mating systems. It is possible that the lack of seed set in some stands represents a significant disturbance in the mating system of these particular stands, however it may simply reflect a natural difference between sites.

Stands of plants of the same species can differ in their maternal capacity, ranging from differences in degrees of maternal capacity between stands right up to segregation of male and female stands (Bierzychudek & Eckhart, 1988). This can be driven by phenotypic plasticity with some plant species capable of switching between the two modes of reproduction in response to changing environmental conditions. This switching has been noted as a response to increased density in some species (Abrahamson, 1975; Douglas, 1981; Cheplick, 1995; van Kleunen et al., 2002; Vallejo-Marín et al., 2010). Alternatively, differences in reproductive strategy between stands can represent a steady state, driven by genetic differences (Bierzychudek and Eckhart, 1988). The finding here that all *A. loderi* stands that did not set seed were always a single genet, whilst the majority of stands setting seed were genetically diverse, may suggest a genetic underpinning. It has been noted that clonality for this species increases west of the Darling River (Roberts et al., in press).

### **The mystery of *A. carneorum* reproduction**

The presence and lack of sexual reproduction in different *A. carneorum* stands could be explained theoretically in several ways; a lack of pollen movement between neighbouring stands, incompatible genotypes in neighbouring stands, or prevailing climatic conditions, however it may simply reflect natural differences in the sexual capacity between different clonal stands. Whilst it has been suspected that the lack of diversity within *A. carneorum* stands and corresponding lack of outcrossing coupled with unnaturally devastating levels of grazing is responsible for the historic lack of fruit set (Porteniers, 2001), recent carbon dating

results suggest this is unlikely. Carbon dating of recently deceased *A. carneorum* plants of equivalent stature from these same stands indicates a long history of genetic homogeneity in these stands, putting the majority of these largely senescent adult plants at over 200 years old (Auld & Denham, 2001; Chapter 9, thesis). As such the genetic homogeneity of these stands appears to predate local anthropogenic disturbance, lending support to a naturalistic explanation for a minimal role of sexual function in reproduction within these stands, rather than being a symptom of their highly fragmented conditions.

The finding here and previously that all *A. carneorum* stands separated by only a kilometre or two contained completely distinct genets (O'Brien et al., 2014; Roberts et al., *in review*) may suggest that while sexual recruitment has not been important within stands historically, seed may serve the purpose of colonizing and founding new stands. Given the bright arils on seed this would most likely be mediated by bird vectors (Whitney, 2005b). Sexual recruitment that is rare and therefore cryptic to us may yet be of long term importance for the health of these stands, particularly if rare seed are dispersed and found new stands, or if a rare sexual recruit adds some diversity into stands from time to time (Harper, 1977; Handel, 1985; Silvertown & Doust, 1993; Olivieri et al., 1995; Husband & Barrett, 1996; Tarasjev, 2005). Alternatively, separate stands once part of a single large population which have contracted, could have diverged genetically through a lack of connectivity and somatic mutation over time. A combination of both dynamics could also be at play. The lack of sexual reproduction and genetic diversity within *A. carneorum* stands could also reflect an ongoing evolutionary transition in this species' mating strategy.

Shifts between sexual and asexual function can come about through a relatively swift plastic response (Abrahamson, 1975; Douglas, 1981; Cheplick, 1995; van Kleunen et al., 2002; Vallejo-Marín et al., 2010), or as a result of a gradual evolutionary process (Silander, 1985; Caraco & Kelly 1991; Stuefer et al., 1996; Piquot et al. 1998; Honnay & Bossuyt 2005). For example, sexual function as a means to assist colonization or to maintain adaptive abilities can be jettisoned for more reliable asexual forms of reproduction better suited to harsh arid conditions (Olivieri & Gouyon, 1997; Piquot et al., 1998, Kearney, 2003; Vallejo-Marín et al., 2010). A shift to asexual reproduction has even been driven by fire frequency in some species (Millar et al., 2010; Gross et al., 2012) and has facilitated the persistence of relict species in marginal environments (Peakall et al., 2003). Under this model, the presence of stands of these *Acacia* that still contain plants capable of sexual reproduction, albeit at seemingly very low densities, might suggest they still retain an ever dwindling capability to set fruit, and that

this jettisoning has happened at different rates within each stand. Indeed a natural transition away from sexual to asexual reproduction would not be altogether surprising, especially in a climatically stable arid environment, where the benefits of sex are likely outweighed by the benefits of maintaining local adaptation and producing more drought resilient suckers (Silander, 1985; Caraco & Kelly 1991; Stuefer et al., 1996; Honnay & Bossuyt 2005). Moreover, this would explain the patchiness of remaining sexual function across the range of *A. carneorum* stands. Certainly the large numbers of young suckers found in a minority of *A. carneorum* stands (Chapter 2, thesis) shows that asexual reproduction can provide stands of this species with large enough numbers of recruits to persist in the absence of any sexual recruitment. The capacity for clonal growth has been linked to the persistence of species or stands with apparently dysfunctional mating systems (Sydes & Peakall, 1998; Bartolome et al., 2002; James & McDougall, 2007; Gross & Caddy, 2006). A switch to obligate asexual reproduction as a result of past and even current conditions however, may represent an evolutionary dead end. With predictions of climate change suggesting a further harshening of arid and semi arid environments in Australia (Hughes & Westoby, 1994; Hughes, 2003), greater genetic diversity afforded through sexual reproduction may be needed to cope with rapidly changing conditions.

### **Natural explanations for diversity of reproductive capacity between stands must be considered**

A major challenge for land managers is determining whether an observed lack of sexual reproduction in plant populations is truly worthy of concern. This should ultimately be based upon whether such deficits of seed set represent natural variance across stands, or whether they are a product of anthropogenically derived stresses. Whilst it appears that *A. carneorum* has at least heavily favoured asexual reproduction for some time, this is not the case for the other three threatened species. If clonal *A. melvillei*, *A. homalophylla* and *A. loderi* stands are a result of loss of genetic diversity in certain areas through reductions to stand size (fragmentation) and genetic drift, then active measures to restore genetic diversity to stands would be justified. If such stands have come about by natural founder events, then the lack of genetic diversity in these stands would be of relatively little concern to managers. As has been done for *A. carneorum*, carbon dating of recently deceased plants of these other *Acacia* species might help to clarify how these stands have established.

A steady reproductive strategy which contained stands with a range of different levels of sexual function, might come about and be maintained if local adaptations favour different reproductive strategies as a result of different pressures across the region (Richards, 1997; Sartor et al., 2011; Hardion et al., 2015). For instance, lower levels of sexual investment and higher levels of asexual reproduction might be selected for if an area was characterised by relatively harsh conditions that remained stable, such as higher temperatures, less water, or higher grazing pressures which served to disproportionately remove seedlings over hardier asexual recruits (Song et al., 2002; Honnay & Bossuyt, 2005; Silvertown, 2008). This is especially so where connectivity is relatively low and different micro-habitats and different micro-climates exist within the species distribution. *A. loderi* stands east and west of the Darling River may highlight this dynamic as they have been found to differ in their reproductive strategies. Stands west of the river tend to be more clonal than east of the river where they are generally genetically diverse (Roberts et al., 2016). In some cases, certain stands will differ in their level of ploidy with polyploidy favoured generally in harsher areas than diploids, especially in arid environments (Fawcett & van de Peer, 2010). In extreme cases, polyploid stands will no longer be able to mate with diploid stands leading to quicker divergences in the mating strategies of these groups (Richards, 1997; Sartor et al., 2011; Husband et al., 2012; Hardion et al., 2015). While some *A. carneorum* stands appear to be polyploid (Roberts et al., *in review*) we found only diploid plants amongst all the stands surveyed in this study, regardless of their capacity to set fruit.

Caution should be taken in suggesting that asexual reproduction (suckering) might be the natural more common in these *Acacia* stands that are not being observed to reproduce sexually, given that disturbance to the roots of plants by grazers and farming machinery can serve to promote mass suckering to grossly unnatural levels, regardless of sexual ability (Batty & Parsons, 1992; Fraser et al., 2004). The large numbers of suckers of the same age observed in most *A. homalophylla* stands (Chapter 2, thesis) should most likely be regarded as an unnatural consequence of relatively high disturbance given its relatively eastern distribution, especially considering that suckering is expected to be naturally very rare in closely related *A. melvillei* (Batty & Parsons, 1992).

### **Conservation concerns**

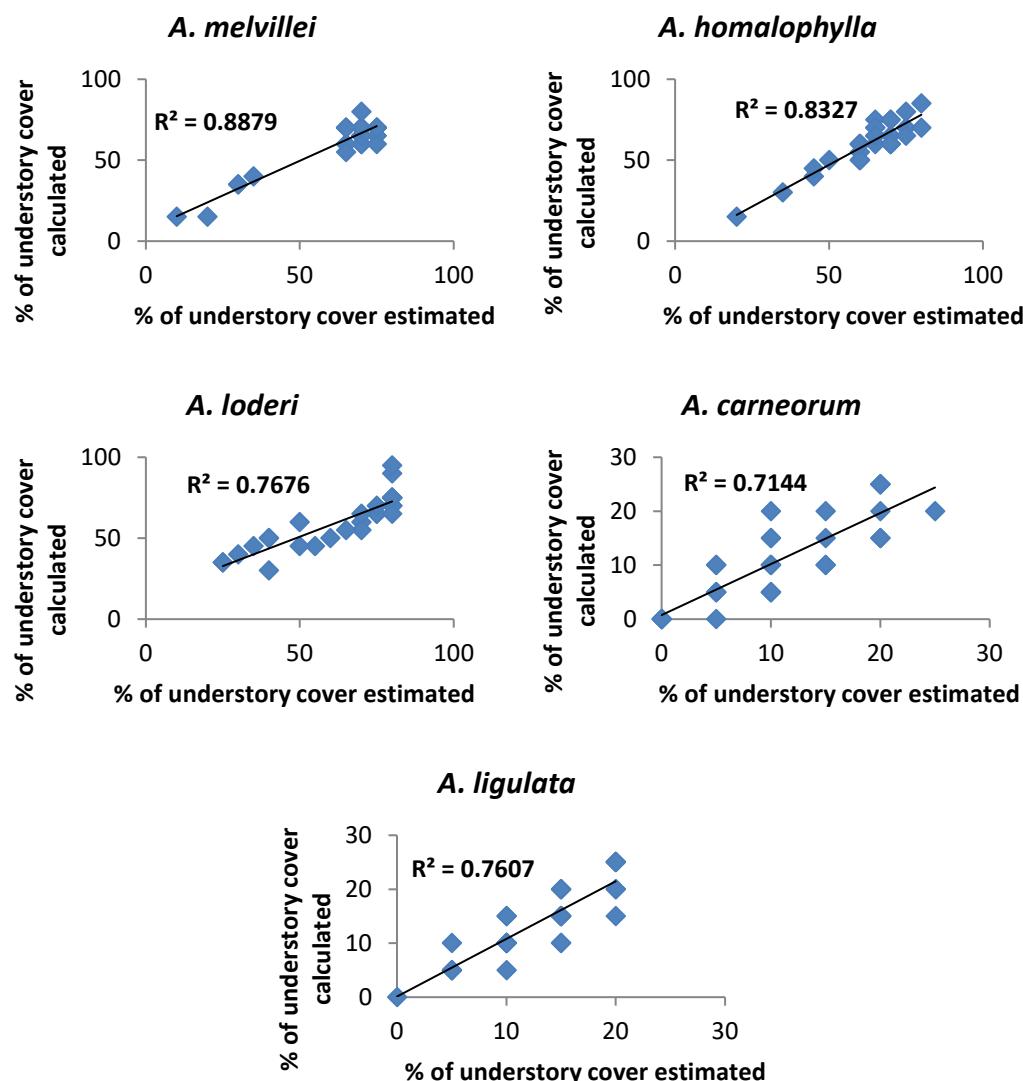
Whilst my findings here point to a potential genetic underpinning to the capacity to set seed for these semi arid *Acacia* species rather than differences in the condition of stands /plants, care must be taken not to overestimate their reproductive resilience either. The reproductive performance of the majority of the plants surveyed here may simply reflect the fact that they are mature, well established plants with large tap roots and largely impervious to now harsher ground conditions (Harris 1984; Kapos 1989; Parsons 1991; Newmark 2001; Pohlman et al., 2009). We might expect younger, yet reproductively mature plants, to be less physiologically resilient if they were present (Schuler & Orrock; 2012). Indeed, the similarity in the condition of trees between stands of all five species, despite the variance in the condition of stands they were found in, may simply reflect this. If larger numbers of younger plants were present in these remaining stands, it is possible that more obvious health consequences associated with severe isolation would be found, as we would expect them to be less resilient than their mature counterparts. No matter whether these species are capable of setting seed under the harsh conditions they currently exist in, their aging demographics combined with a historic lack of recruitment suggests that without active conservation measures, managers should not rely on natural recovery of these stands.

## 4.6 Appendix

**Appendix 4.6.1. Validating the visual method of estimating understory cover and plant health measurements:** To make consistent assessments of the a) density of understory cover within stands, b) percentage of healthy canopy cover, c) percentage of leaf surface area affected by disease, d) percentage of leaf surface area consumed by herbivory, and e) percentage of canopy vegetation surface area covered by epiphytes, the efficient visual estimation techniques used in this study were first verified for accuracy. The same ten plants from a subset of three of the 47, 10, 26, 30 and 20 stands of *A. melvillei*, *A. homalophylla*, *A. loderi*, *A. carneorum* and *A. ligulata* previously selected for this study (see Figure 1) were chosen randomly, to use for comparing data gathered via visual estimation of the five measures listed above against more accurate measurements:

### a) Percentage of understory cover within stands

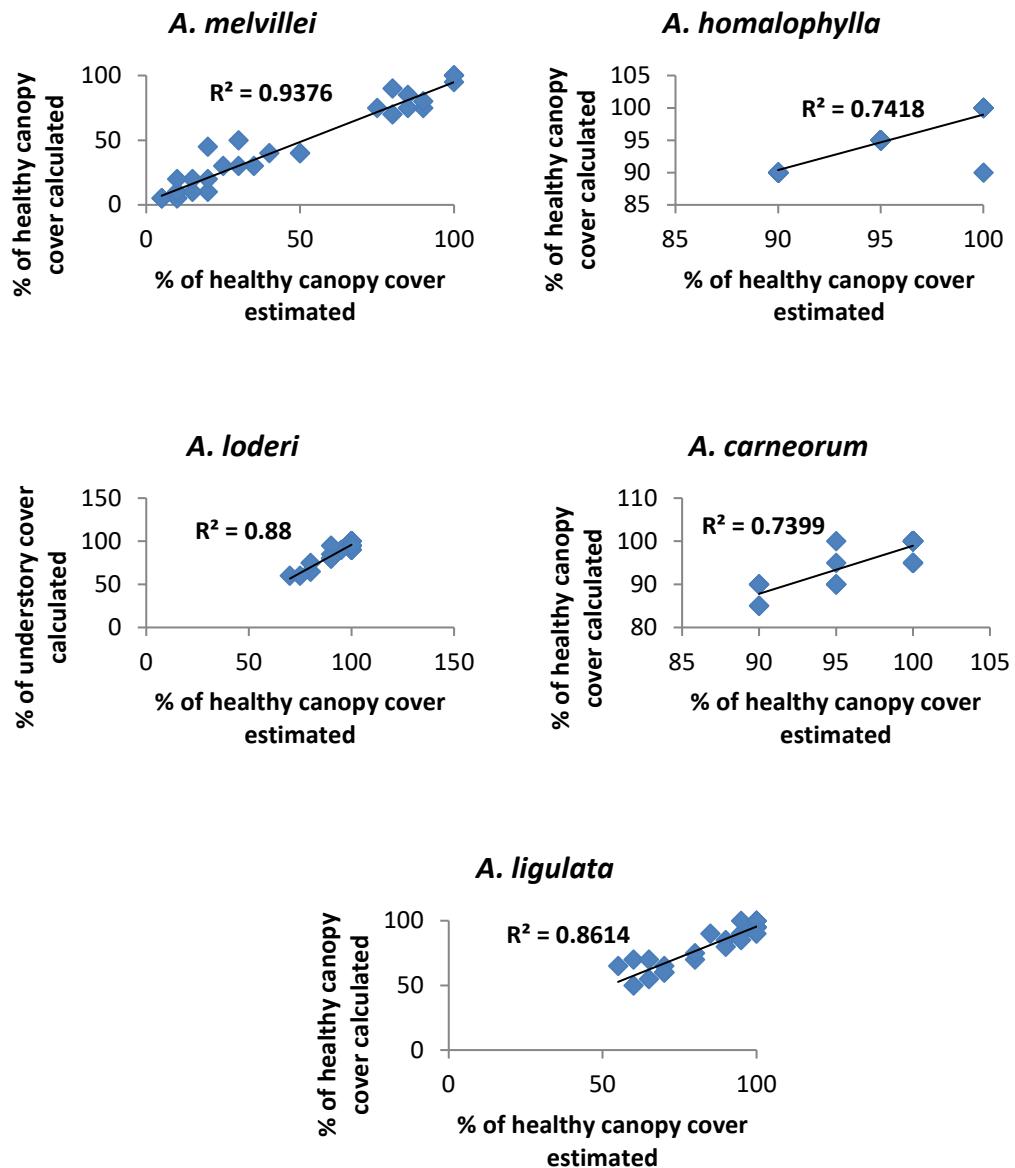
For each 2m<sup>2</sup> quadrat that the percentage of understory cover was visually estimated within, a 10cm<sup>2</sup> quadrat was placed over all areas of ground covered by the canopy of understory vegetation to precisely calculate the actual percentage of ground covered by understory vegetation versus bare ground. Linear regression analysis was performed to assess whether the visual estimates closely matched careful measurements. For all five species of plant my visual estimates of the percentage of ground covered in understory vegetation versus bare soil were very consistent between plants ( $r^2$  values ranging from 0.71 to 0.89 across the five *Acacia* species). As such, the visual technique was verified as a suitable means of estimating the percentage of understory cover within a wider range of quadrats, in a wider range of stands, to increase the efficiency of attaining these data.



b) Percentage of healthy canopy cover

To attempt to ensure my visual estimations of the amount of remaining healthy canopy cover on plants were consistent between plants, the surface area of one of the mature plants of each species deemed to have a full and healthy canopy was used as a standard by which all other canopy coverage was assessed. 1m<sup>2</sup> quadrats were used to measure the surface area of each of the 30 plants across three stands of each *Acacia* species to determine the surface area of their canopies. A linear regression analysis was performed to assess whether the visual estimates of canopy cover (surface area) closely matched these careful measurements. For all five species my visual estimates of the percentage of canopy cover were very consistent between plants ( $r^2$  ranging from 0.74 to 0.94 across the five *Acacia* species). As such, the visual

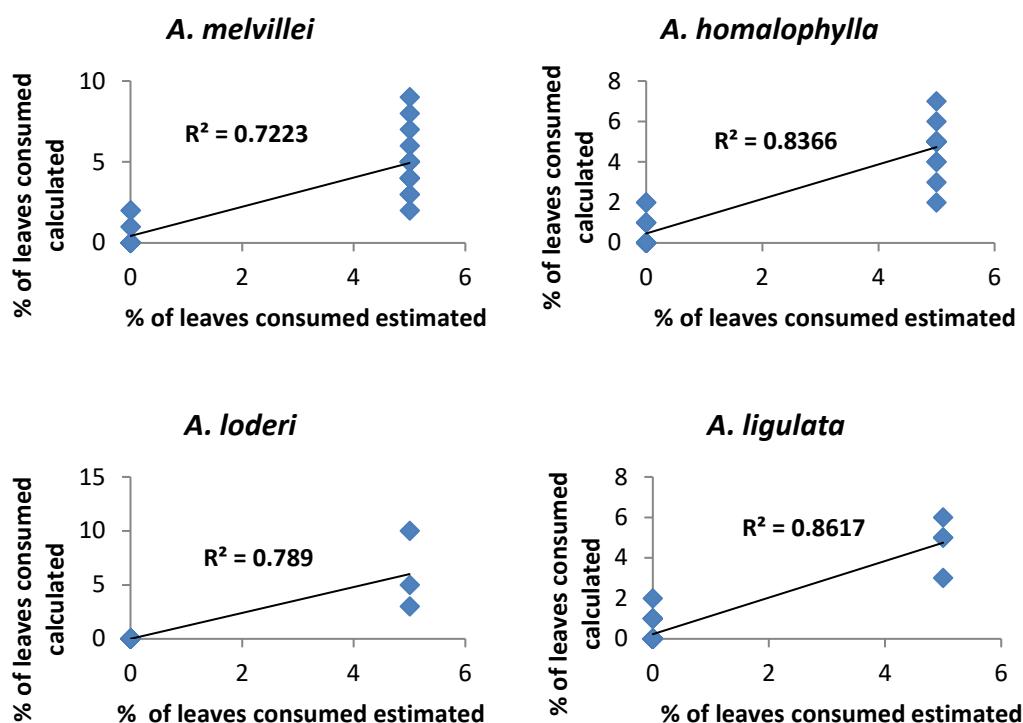
technique was verified as a suitable means of estimating the percentage of healthy cover remaining on plants within a wider range of quadrats in a wider range of stands to increase the efficiency of attaining these data.



c) Percentage of leaf surface area consumed by herbivory

1500 leaves were visually assessed for signs of herbivory. This was measured by tracing each leaf onto grid paper. The number of square centimetres their profiles encompassed on the grid paper was carefully estimated and this number was doubled for *A. melvillei*, *A. homalophylla*, *A. loderi* and *A. ligulata* leaves which are flat. For *A. carneorum* leaves which are cylindrical in

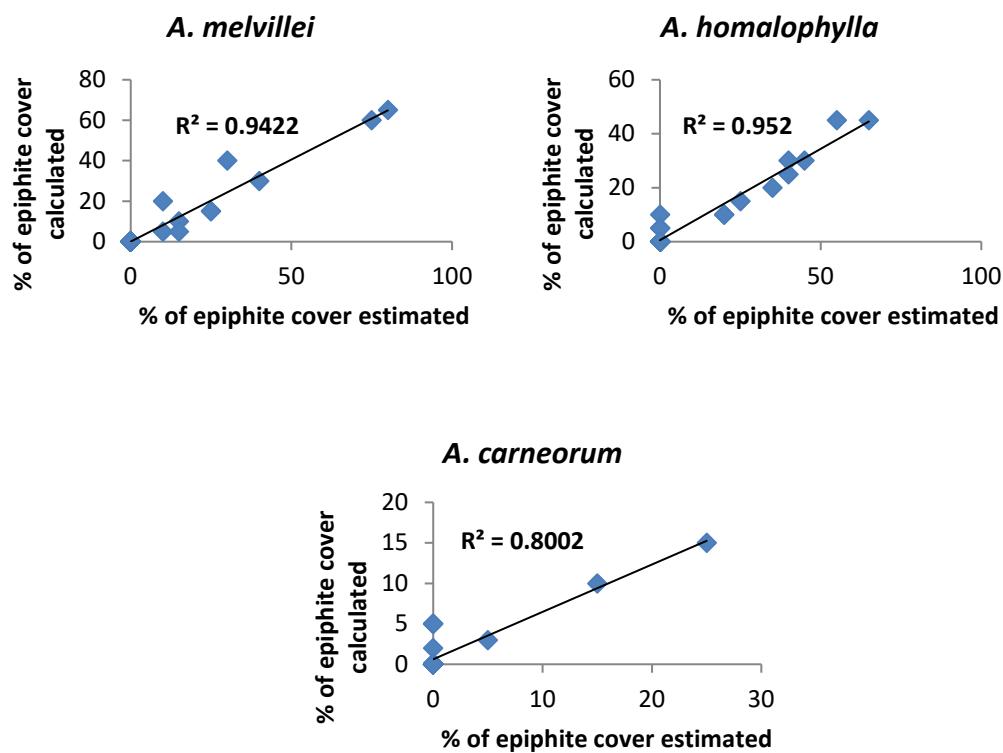
shape, the length and radius of each leaf was measured and used to calculate the surface area of the leaf. Any portion of each leaf that was obviously affected by herbivory (removed) was measured in surface area and added together to attain an accurate estimate of the total percentage of a leaf's surface area affected. An average proportion of leaf surface area affected by herbivory across each set of 50 leaves from each plant was then calculated and a linear regression analysis was performed to assess whether the visual estimates closely matched careful measurements. While no evidence of herbivory was found on *A. carneorum* leaves, my visual estimates of the proportion of the other four *Acacia* species leaves surface area consumed was very consistent between plants ( $r^2$  ranged from 0.81 to 0.88 across the four species). As such, the visual technique was verified as a suitable means of estimating the percentage of leaf surface area consumed within a wider range of quadrats in a wider range of stands to increase the efficiency of attaining these data.



#### d) Percentage of canopy vegetation surface area covered by epiphytes

The percentage of epiphyte foliage cover was estimated placing 1m<sup>2</sup> quadrats around the surface of remaining live canopy vegetation. Smaller 40 cm<sup>2</sup> quadrats were then used to measure the surface area of epiphyte vegetation found on plants in the same fashion to then determine the proportion of each canopy surface area infected by epiphytic vegetation. A

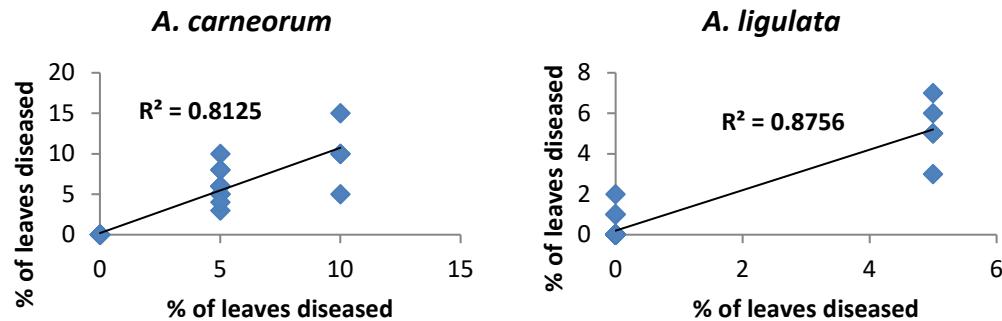
linear regression analysis was performed to assess whether the visual estimates of epiphyte cover closely matched my careful measurements of epiphyte cover. While no epiphytes were found on *A. loderi* and *A. ligulata* plants that were surveyed, my visual estimates of the proportion of the other three *Acacia* species were very consistent between plants ( $r^2$  values ranged from 0.80 to 0.95 across the four species). As such, the visual technique was verified as a suitable means of estimating the percentage of remaining canopy infected by epiphytes within a wider range of quadrats in a wider range of stands to increase the efficiency of attaining these data.



e) Percentage of leaf surface area affected by disease

The same method described above for estimating the surface area of leaves affected by herbivory was also used to accurately estimate the average proportion of leaves disease affected, by measuring the proportion of discoloured area on leaves. While no obvious evidence of pathogens were found on *A. melvillei*, *A. homalophylla* and *A. loderi* leaves, for *A. carneorum* and *A. ligulata* my visual estimates of the percentage of leaf surface area obviously affected by pathogens were very consistent between plants ( $r^2$  values of 0.81 and 0.88 for *A. carneorum* and *A. ligulata* respectively). As such, the visual technique was verified as a suitable

means of estimating the percentage of leaf surface area affected by pathogens within a wider range of quadrats in a wider range of stands to increase the efficiency of attaining these data.



**Appendix 4.6.2. Relationship between stand structure and plant health:** Results of regression and hierarchical multiple regression analysis between four key measures of stand structure (1. Size of stand, 2. Connectivity, 3. Density and 4. Understory cover) and three measures of plant health (a. Canopy condition (average % covered in healthy foliage), b. Leaf herbivory (average % of plants leaf surface area consumed), c. Epiphyte infection (average % of plants canopy parasitised by epiphytes)), tallied across two consecutive years following a La Niña rain event, using 10 (or as many in the stand) haphazardly chosen mature plants within each of 47, 10, 26, 30 and 20 stands of *A. melvillei*, *A. homalophylla*, *A. loderi*, *A. carneorum* and *A. ligulata* respectively.

\*denotes significant relationships.

Stand structure measures	Vs. Plant health measures	Relationship R <sup>2</sup> , F, P				
		<i>A. melvillei</i>	<i>A. homalophylla</i>	<i>A. loderi</i>	<i>A. carneorum</i>	<i>A. ligulata</i>
		<b>Regressions</b>				
<b>1. Size of stand</b> (# plants per stand)	a. Canopy condition	0.090, 4.474, <b>0.040*</b>	0.049, 0.359, 0.568	0.009, 0.217, 0.645	0.068, 1.963, 0.173	0.594, 26.337, <b>&lt;0.000*</b>
	b. Leaf herbivory	0.006, 0.276, 0.602	0.571, 9.320, <b>0.019*</b>	<0.000, 0.005, 0.945	n/a	0.002, 0.041, 0.842
	c. Epiphyte infection	0.133, 6.883, <b>0.012*</b>	<0.000, <0.000, 1.000	0.014, 0.347, 0.561	0.124, 3.822, 0.061	n/a
<b>2. Connectivity</b> (distance from nearest neighbour stand)	a. Canopy condition	0.202, 11.418, <b>0.002*</b>	0.154, 1.276, 0.296	0.001, 0.017, 0.898	0.026, 0.725, 0.402	0.245, 5.847, <b>0.026*</b>
	b. Leaf herbivory	0.013, 0.580, 0.450	0.099, 0.770, 0.409	0.012, 0.294, 0.593	n/a	0.028, 0.514, 0.483
	c. Epiphyte infection	0.157, 8.352, <b>0.006*</b>	0.114, 0.900, 0.374	0.027, 0.678, 0.418	0.045, 1.266, 0.270	n/a
<b>3. Density</b> (average distance of plants from the nearest neighbour plant)	a. Canopy condition	0.762, 143.935, <b>&lt;0.000*</b>	0.008, 0.059, 0.816	0.006, 0.141, 0.710	0.267, 9.841, <b>0.004*</b>	0.845, 98.312, <b>&lt;0.000*</b>
	b. Leaf herbivory	0.009, 0.428, 0.517	0.539, 8.182, <b>0.024*</b>	0.063, 1.537, 0.228	n/a	0.005, 0.088, 0.770
	c. Epiphyte infection	0.068, 3.292, 0.076	0.015, 0.106, 0.754	0.008, 0.183, 0.673	0.007, 0.191, 0.666	n/a
<b>4. Understory cover</b> (% of understory vegetation)	a. Canopy condition	0.083, 4.056, <b>0.050*</b>	0.002, 0.015, 0.905	0.107, 0.070, 0.103	0.045, 1.276, 0.269	0.020, 0.370, 0.550
	b. Leaf herbivory	0.031, 1.448, 0.235	0.135, 1.093, 0.331	0.002, 0.038, 0.846	n/a	0.254, 6.129, <b>0.023*</b>
	c. Epiphyte infection	0.010, 0.459, 0.502	0.001, 0.009, 0.926	0.004, 0.095, 0.761	0.138, 4.333, <b>0.047*</b>	n/a

Hierarchical multiple regressions							
Measures 1-4	a. Canopy condition	0.816, 46.716, <0.000*	0.312, 0.453, 0.769	0.285, 1.994, 0.134	0.352, 3.255, 0.029*	0.895, 31.826, <0.000*	
	b. Leaf herbivory	0.063, 0.708, 0.591	0.800, 4.001, 0.104	0.231, 1.499, 0.240	n/a	0.355, 2.066, 0.136	
	c. Epiphyte infection	0.225, 3.047, 0.027*	0.312, 0.453, 0.769	0.067, 0.358, 0.836	0.300, 2.573, 0.064	n/a	

**Appendix 4.6.3. Assessing the role of stand condition, plant health and local climate on sexual reproductive success:** Results of regression and Hierarchical multiple regression analysis between four key measures of stand structure (1. Size of stand, 2. Connectivity, 3. Density, 4. Height of plants, 5. Width of plants, 6. Number of suckers, 7. Understory cover) and three measures of plant health (8. Canopy condition, 9. Leaf herbivory, 10. Epiphyte infection) against a) the % plants where fruit was detected per stand, and b) the average % of plants canopies covered in fruit per stand, tallied over two consecutive years following a La Niña rain event, using 10 (or as many in the stand) haphazardly chosen mature plants within each of 47, 10, 26, 30 and 20 stands of *A. melvillei*, *A. homalophylla*, *A. loderi*, *A. carneorum* and *A. ligulata* respectively. \*denotes significant relationships.

a)

Independent variables	Vs. % of plants that set fruit			
	Relationship R2, F, P			
	<i>A. melvillei</i>	<i>A. homalophylla</i>	<i>A. loderi</i>	<i>A. carneorum</i>
<b>Regressions- structural condition of stand</b>				
1. Size of stand (# plants per stand)	<0.000, 0.002, 0.962	0.047, 0.630, 0.400	0.023, 0.574, 0.456	<0.000, 0.001, 0.971
2. Connectivity (distance from nearest neighbour stand)	0.001, 0.024, 0.876	0.056, 0.876, 0.210	0.065, 1.662, 0.210	0.033, 0.914, 0.348
3. Density (average distance of plants from the nearest neighbour plant)	0.021, 0.952, 0.335	0.236, 2.167, 0.184	0.018, 0.418, .0524	0.017, 0.454, 0.506
4. Height of plants (average height of independent plants)	0.045, 2.144, 0.150	0.014, 0.096, 0.766	0.121, 3.308, 0.081	0.066, 1.893, 0.180
5. Width of plants (average width of independent plants)	0.057, 2.639, 0.111	0.008, 0.059, 0.815	0.075, 1.939, 0.177	0.023, 0.625, 0.436
6. # of suckers (average number of suckers per independent plant)	0.052, 2.476, 0.123	0.283, 2.769, 0.140	0.120, 3.258, 0.084	0.011, 0.291, 0.594

7. Understory cover ( % of understory vegetation)	0.016, 0.721, 0.400	0.012, 0.086, 0.778	0.056, 1.423, 0.245	0.038, 1.077, 0.308
<b>Regressions- condition of plants</b>				
8. Canopy condition (average % covered in healthy foliage)	0.035, 1.653, 0.205	0.037, 0.266, 0.622	0.008, 0.205, 0.654	0.026, 0.719, 0.404
9. Leaf herbivory (average % of plants leaf surface area consumed)	<0.000, 0.007, 0.934	0.065, 0.484, 0.509	0.099, 2.633, 0.118	n/a
10. Epiphyte infection (average % of plants canopy parasitised by epiphytes)	0.005, 0.224, 0.639	0.333, 3.492, 0.104	0.017, 0.408, 0.529	0.001, 0.028, 0.869
<b>Regressions- local climatic conditions</b>				
11. Local rainfall (average annual local rainfall over 2 years (2010/2011))	0.002, 0.080, 0.778	0.232, 2.119, 0.189	<0.000, 0.002, 0.961	n/a
12. Local temperatures (annual average over 2 years (2010/2011))	<0.000, 0.022, 0.884	0.137, 1.113, 0.326	<0.000, 0.011, 0.919	0.012, 0.328, 0.571
<b>Hierarchical multiple regressions</b>				
Model 1: measures 1-7	0.238, 1.698, 0.139	n/a	0.241, 0.773, 0.618	0.253, 1.017, 0.448
Model 2: measures 1-10	0.105, 1.526, 0.171	n/a	0.336, 0.709, 0.704	0.293, 0.873, 0.564
Model 3: measures 1-12	0.090, 1.371, 0.228	n/a	0.338, 0.510, 0.871	0.361, 1.016, 0.467

\*\* No significant P values were found when Bonferroni adjustments were made to account for potential 'Type 1 errors' as a result of multiple testing

b)

Independent variables	Vs. fecundity (% fruit set)				
	Relationship R2, F, P				
	A. melvillei	A. homalophylla	A. loderi	A. carneorum	A. ligulata
<b>Regressions- structural condition of stand</b>					
1. Size of stand (# plants per stand)	0.003, 0.113, 0.738	0.278, 2.698, 0.144	0.036, 0.903, 0.351	0.001, 0.026, 0.873	0.058, 1.117, 0.305
2. Connectivity (distance from nearest neighbour stand)	0.020, 0.889, 0.351	0.151, 1.245, 0.301	0.075, 1.939, 0.177	0.033, 0.929, 0.344	0.015, 0.282, 0.602
3. Density (average distance of plants from the nearest neighbour plant)	<0.000, 0.016, 0.899	0.061, 1.088, 0.130	0.032, 0.772, 0.389	0.017, 0.467, 0.500	0.023, 0.432, 0.519

4. Height of plants (average height of independent plants)	0.025, 1.115, .297	0.017, 0.122, 0.737	0.096, 2.549, 0.123	0.057, 1.620, 0.214	0.134, 2.779, 0.113
5. Width of plants (average width of independent plants)	0.005, 0.211, 0.649	0.009, 0.061, 0.813	0.047, 1.187, 0.287	0.032, 0.901, 0.351	0.003, 0.054, 0.820
6. # of suckers (average number of suckers per independent plant)	0.047, 0.756, 0.190	0.046, 0.591, 0.450	0.089, 2.350, 0.138	0.011, 0.296, 0.591	n/a
7. Understory cover (% of understory vegetation)	0.011, 0.517, 0.280	0.048, 0.354, 0.571	0.061, 1.567, 0.223	0.041, 1.141, 0.295	0.065, 1.244, 0.279
<b>Regressions- condition of plants</b>					
8. Canopy condition (average % covered in healthy foliage)	0.007, 0.324, 0.572	0.028, 0.204, 0.665	0.028, 0.701, 0.411	0.028, 0.764, 0.390	0.105, 2.112, 0.163
9. Leaf herbivory (average % of plants leaf surface area consumed)	0.030, 1.360, 0.250	0.186, 1.600, 0.246	0.137, 3.808, 0.063	n/a	0.152, 3.219, 0.090
10. Epiphyte infection (average % of plants canopy parasitised by epiphytes)	0.006, 0.283, 0.597	0.191, 1.658, 0.239	0.042, 1.050, 0.316	0.001, 0.024, 0.878	n/a
<b>Regressions- local climatic conditions</b>					
11. Local rainfall (average annual local rainfall over 2 years (2010/2011)	0.053, 2.446, 0.125	0.432, 5.313, 0.055	<0.000, 0.001, 0.981	n/a	0.420, 0.782, 0.388
12. Local temperatures (annual average over 2 years (2010/2011)	0.001, 0.039, 0.845	0.376, 4.219, 0.079	0.004, 0.088, 0.769	0.012, 0.334, 0.568	0.001, 0.011, 0.918
<b>Hierarchical multiple regressions</b>					
Model 1: measures 1-7	0.259, 1.849, 0.107	n/a	0.249, 0.807, 0.593	0.249, 0.994, 0.462	0.306, 0.956, 0.491
Model 2: measures 1-10	0.383, 1.695, 0.051	n/a	0.409, 0.968, 0.509	0.289, 0.858, 0.576	0.456, 1.153, 0.403
Model 3: measures 1-12	0.389, 1.695, 0.115	n/a	0.410, 0.694, 0.731	0.356, 0.997, 0.481	0.486, 0.849, 0.601

\*\* No significant P values were found when Bonferroni adjustments were made to account for potential 'Type 1 errors' as a result of multiple testing

## **Chapter 5: Recruitment dynamics of long lived overstory *Acacia* in a degraded and heavily grazed arid landscape: effects of a rare La Niña rain event.**

### **5.1 Abstract**

It is estimated that approximately 40% of native vegetation around the world has been cleared for agricultural purposes and urbanisation, leaving many populations of plants drastically reduced in size and highly fragmented. The capacity of plants to persist in acutely fragmented populations does not guarantee recruitment or the long term survival of recruits under what are usually harshened local ground conditions. In particularly harsh environments such as arid and semi arid environments, where seedling mortality is already high, we might expect little chance of effective recruitment in acutely fragmented populations, even if plant fecundity is high. However little research has been done in such systems to quantify recruitment. Here I use four threatened semi arid *Acacia* species (*A. melvillei*, *A. homalophylla*, *A. loderi* and *A. carneorum*) existing now as a scattering of small and aging stands across 336,000 km<sup>2</sup> of western NSW and one thriving species (*A. ligulata*) as model systems. I employ a combination of field surveys (encompassing total of 133 stands), and manipulative seed burial and growth experiments in the laboratory and in the field, to investigate the capacity of acutely fragmented populations in a semi arid environment, to effectively recruit after a rare reproductive event. Whilst seed parasitism was high in all four threatened species across all surveyed stands, ranging from 37% (SE± 1.0) to 46% (SE± 3), I found high levels of seed viability in unparasitised seed ranging from 67.95% (SE± 0.1) to 77.3% (SE±0.0) which was comparable to viability in *A. ligulata* 45.6% (SE± 0.1). A substantial proportion of the seedlings grown from the four threatened species also survived to two years of age under coastal conditions ranging from 50% (SE± 5.0) to 54% (SE± 5.1), which was also comparable with *A. ligulata* 44% (SE± 1.8). Surveys of natural recruitment found highly variable numbers of new seedlings among stands and even among plants within the same stands of *A. melvillei*, *A. homalophylla*, *A. loderi* and *A. ligulata*, ranging from as high as 369 seedlings per plant to zero. In contrast, no sexual recruitment was found in any fruiting or non fruiting stand of *A. carneorum*. The presence of recruitment from soil stored seed banks especially within *A. melvillei* stands supported findings from seed burial experiments that these species are capable of maintaining a long lived soil stored seed bank with 25% and 32% of *A. melvillei* and *A. loderi* seed still viable three years after burial. Manually sown *A. melvillei* and *A. loderi* seed

recruited at far higher rates than that found naturally, with an estimated 60,000 times more *A. melvillei* seed and 6,443 times more *A. loderi* seed required to recruit one seedling naturally than if manually sown. I also found evidence that seedling mortality decreased over the long term if seedlings were protected from grazing by understory vegetation acting as ‘nurse plants’. Maintaining the quality of the understory within and around remaining stands is clearly of key importance for seedling recruitment and long term survival.

## 5.2 Introduction

Plants that reproduce sexually require suitable conditions for both the production of seed and the recruitment of seedlings. In anthropogenically disturbed environments, factors such as urbanization, agricultural activity, and increased grazing may reduce the probability of successful seed set or recruitment (Jennersten, 1988; Menges, 1991; Ouborg et al., 1991). Whilst long lived iteroparous plants may persist in the landscape for long periods of time without reproducing, if conditions for reproduction remain hostile, local extinction is ultimately inevitable. Estimates of reproductive success in trees are often based on seed production (Fuchs et al., 2003, Schoen & Stewart, 1986; Herrera & Jovani, 2010), however, from an evolutionary or stand-dynamics perspective, a plant has not successfully reproduced until its offspring are themselves of reproductive age (Howard, 1979; Primack & Kang, 1989). Observations of successful fruit / seed set does not guarantee that recruitment will follow (Baskin & Baskin, 1998; Fenner, 2000). This is especially true within harsh environments, such as alpine, arid and semi arid environments (Boyd & Brum, 1982; De La Cruz et al., 2008). Irrespective of the climatic conditions of a region, recruitment rates and survival of recruits are generally reduced in fragmented landscapes (Vergeer et al., 2003; Winter et al., 2008; Matezans et al., 2009; Tsaliki & Diekmann, 2010), and are drastically reduced in areas of high grazing pressure (Sinclair, 1995). Isolation between plants in fragmented landscapes can lead to lowered chances of mating if pollinators cannot locate stands, often termed ‘pollen limitation’ (Jennersten, 1988; Andrieu et al., 2009; Nayak, et al. 2010), however there can also be an increased chance of inbreeding depression in offspring when reproduction does occur, making successful long term recruitment less likely (Kolreuter, 1761; Crow & Kimura, 1970; Lande & Schemske, 1985; Charlesworth & Charlesworth, 1990). Offspring can also display reduced fitness if seed development is suboptimal as a result of stressed mature plants restricting resource allocation to seeds (Roach & Wulff, 1987; Obeso, 1993).

Regardless of the fitness of the seed produced by these plants or their adaptive capacity, if local ground conditions have become so harsh that even the fittest seed cannot effectively recruit, speculation about the quality of seed may be of little consequence. In environments that have been highly disturbed and which are subject to continuous and prolonged intense grazing pressure, harshening of local abiotic conditions, and hardening of soils, are all likely to reduce the chances of both mature plants and their offspring’s survival. Edge effects become more pronounced with increasing levels of fragmentation as more plants at these edges are exposed to harsher climatic conditions, such as higher temperatures and loss of soil moisture

(Kapos, 1989; Matlack, 1993, 1994; Malcom, 1998; Jules & Rathcke, 1999; Meiners & Pickett, 1999) and loss of nutrients through run off (Dorrough & Moxham, 2005; Gibbons et al., 2008; Dardel et al., 2014) and increased grazing pressure from invertebrate and vertebrate herbivores (MacGarvin et al. 1986; Warren, 1987; Terborgh & Wright 1994; Asquith et al., 1997; Harrington et al., 1997). As such, scattered tree stands often experience elevated mortality at all stages of life as a result of physiological stress, with recruitment often totally absent (Dorrough & Moxham, 2005; Gibbons et al., 2008). While large mature plants with long tap roots can be resilient to harshened ground conditions in fragmented populations, new seedling recruits are especially vulnerable (Baskin & Baskin, 1998; Fenner, 2000). Even the chances of seed being able to lodge and recruit in the first place, are generally reduced in denuded landscapes (Dardel et al., 2014).

The importance of a healthy understory for recruitment and seedling survival is well known (Padilla & Pugnaire, 2006; Gul et al., 2007; Jankju, 2013). Whilst understory vegetation can trap seed that may get washed away in run off as well as maintain softer soils for seed to penetrate (Dardel et al., 2014), it can also offer protection to seedlings by buffering microclimatic extremes often referred to as the ‘nurse effect’, a form of ecological facilitation (Callaway, 1995; Rousset & Lepart, 1999; García et al., 2003; Padilla & Pugnaire, 2006). Often the nurse effect cannot be attributed solely to a single factor, rather to multiple factors (Ren et al., 2008) divided into canopy effects and soil effects (Gomez-Aparicio et al., 2005). Canopy effects include light reduction (increased shade) (Valladares et al., 2005; Kos & Poschlod, 2007) and temperature buffering (Fulbright et al., 1995; Arroyo et al., 2003; Drezner, 2004, 2007). A thorny or unpalatable canopy can protect target species from herbivores (Garcia & Obeso, 2003; Baraza et al., 2006) and flowering species can increase pollinator visits to target species (Feldman et al., 2004). Understory vegetation affects the quality of local soils by increasing hydraulic lift (Callaway et al., 199; Castro et al., 200; Armas & Pugnaire, 2005), altering the physical and chemical traits of soil (Carrillo-Garcia et al., 2000; Suzan-Azpiri & Sosa, 2006), affecting mycorrhizae and other soil microorganisms (Carrillo-Garcia et al., 1999; Ouahmane et al., 2006) and encouraging animal activity, which can also increase local soil nutrients (Dean et al., 1999). Indeed, a growing body of experimental studies are reporting a facilitative effect of shrubs and grasses for the early establishment of reforested woody species (Maestre et al., 2001; Gómez et al., 2001; Castro et al., 2002; Jankju et al., 2013; Perea & Gil, 2014).

Predicting levels of recruitment within fragmented plant populations based on their fecundity is often very difficult given the challenges modified landscapes pose for the

establishment and survival of new recruits. However in order for managers to plan conservation strategies for these threatened stands, it is important to determine whether they can still recruit naturally. Further complicating predictions of how much recruitment to expect following the setting of seed, is the presence or absence of a soil stored seed bank in some species.

Persistent seed banks are often favoured in environments that are subject to high rates of recruitment failure, and / or where opportunities for recruitment and establishment are rare in either time or space (Venable & Lawlor, 1980; Cohen, 1966; Thompson, 2000; Carta, et al., 2015). As such it is expected that persistent seed banks should be common-place in arid and semi arid vegetation, given that frequent drought increases the probability of recruitment failure (Kemp, 1989). Whilst a long-lived soil stored seed bank has been suggested in some arid species, including Australian species such as *A. loderi* (Auld, 1995), long drought periods may exhaust seed banks leaving few or no soil stored seed for recruitment when rains return (Ooi, 2012). If a substantial soil stored seed bank survives a period of drought however, arid and semi arid zone seed banks are known to produce an ephemeral “flush” of seedlings after rainfall, thus greatly adding to recruitment that would otherwise result from post-rainfall seed production (Guttermann, 2000).

The majority of research into understanding the effects of fragmentation on the capacity of plant populations to effectively recruit has been carried out on short lived annual and perennial species in the northern hemisphere. Due to practical difficulties, little research has been carried out on the capacity for long-lived iteroparous plants that reproduce infrequently such as after rare large scale rain events in arid and semi arid environments (Letnic & Dickman, 2006; Wardle et al, 2013). Threatened overstory *Acacia* plants in the semi arid region of far western NSW in Australia, considered to be ecological engineers (Morton & Davis, 1983; Recher & Davis, 1997; Sharp, 1997; Facelli & Brock, 2000; Singh & Rathod, 2002) provide model systems to study how such species persist in fragmented landscapes within an already harsh semi arid environment.

Here I take advantage of the breaking of a two decade long drought in the semi arid western region of NSW, to estimate recruitment and seedling survival of four threatened and one thriving *Acacia* species. Stands of these plants set considerable quantities of seed during a period of high water availability despite not being observed to set fruit for an extended period.

I employ both field surveys and manipulative experiments to discover; a) what proportion of seed produced post La Niña rains are viable; b) whether there is evidence of a functional soil stored seed bank; c) whether any seedlings recruit as a result of the recent seed produced; d) the longer term prognosis for any seedlings that recruit in the field and e) what local macro-and microhabitat factors are important in predicting seed fitness and recruitment success in these species.

Specifically for these five *Acacia* species, I test these hypotheses:

1. Many seed produced in these fragmented stands are likely to be inviable.
2. Soil stored seed banks have perished in these stands over the last two decades of drought.
3. Any seedlings produced are likely to show evidence of being unfit at different stages of growth and /or survival.
4. Poorer stand and maternal plant condition will result in lower levels of recruitment and higher mortality rates of seedlings that do emerge.
5. Seedlings that recruit under the canopy of overstory plants survive longer and grow slower than those located outside the canopy of overstory plants.
6. Understory ‘nurse’ plants protect new seedlings from grazing and provide seedlings with a better chance of long term survival than those that grow outside of their canopies

## **5.3 Methods**

### **5.3.1 Study species and study area**

The same plants from within the same 47, 10, 26, 30 and 20 *A. melvillei*, *A. homalophylla*, *A. loderi*, *A. carneorum* and *A. ligulata* stands spread across a region of approximately 336,000 km<sup>2</sup> across the semi arid regions of NSW in southeastern Australia that were used in previous studies (Chapter 2, 3 & 4, thesis), were again the focus of this study (see Figure 2.1, in Chapter 2 of this thesis).

### **5.3.2 Assessing the viability and fitness of seed produced after the 2010-2011 La Niña rainfall events**

Various health proxies including a) seed weight, b) seed viability, c) seedling growth rates, and d) seedling survival rates, were utilized to assess the viability and performance of the seed and seedlings produced after a large scale La Niña rain event beginning in January of 2010. I collected fruit from the same 10 mature plants in each of the same *A. melvillei*, *A. homalophylla*, *A. loderi*, *A. ligulata* stands set after the rain event (Figure 2.1 of Chapter 2, thesis). Identical collections were also made at the two fruiting stands of *A. carneorum* that produced enough mature seed. Collections were made when fruits were dehiscing (*A. carneorum* in May of 2010 and 2011 and *A. melvillei*, *A. homalophylla*, *A. loderi* and *A. ligulata* in January of 2011 and 2012). This ensured that all seed were collected when mature. In order to representatively sample the whole plant, in case there were developmental or genetic differences between fruit / seed on different parts of the plant, I sampled 1200 fruit from each plant, which were taken from 12 positions on each plant (from four sides of the plant (representing the compass points north, south, east and west), as well as lower, mid and upper parts of the canopy). The seed from each plant was mixed to get a representative sample for each plant and 500 randomly selected seed were assessed for viability. For *A. carneorum*, where fruit set was limited to a minority of stands, a total of 600 seeds were collected to be grown from a total of 13 plants in two neighbouring stands separated by only one kilometre just south of Kinchega National Park.

### **5.3.3 Measuring seed health under lab conditions**

#### **(i) Seed parasitism level**

Seed was assessed for damage by seed parasites by visual inspection and by putting physical pressure on the seeds to check their integrity. If seeds were found to have an obvious entry or exit hole on their surface, or they crushed and were soft inside when pressure was applied it was deemed parasitized. The percentage of seed that was parasitized was calculated for each cohort of seed from each of the mature plants.

#### **(ii) Seed viability**

Of the remaining non parasitised seed, 100 *A. melvillei*, *A. homalophylla*, *A. loderi* and *A. ligulata* seed per stand and 125 seed from each of the two *A. carneorum* stands were selected for germination. Physical dormancy was broken by scarifying seed (with sandpaper), which

were then placed in Petri dishes, 20 per dish, on top of moistened filter paper. The dishes were placed in an incubator with a day / night regime of 28 to 14 °C. Seed was checked each day for 3 weeks for signs of swelling, germination and death (rotting). This was recorded. The percentage of seed that successfully germinated was calculated for the seed cohort of each plant.

(iii) Seedling growth rates

From those seeds that germinated, 20 were randomly selected per *A. melvillei*, *A. homalophylla*, *A. loderi* and *A. ligulata* stand, and a total of 96 from the two *A. carneorum* stands were selected to assess their early stage growth rates. These seeds were kept on filter paper in the Petri dishes and in the incubator, and were measured from root tip to shoot tip at 2, 4, 5 and 6 weeks after germination. The filter paper was kept moist throughout the period of incubation. After six weeks these seedlings were planted into square plastic punnets (of 10cm length and width by 20 cm deep) into coastal soils inoculated with soil taken from where these seed were collected (20% of total soil in punnets), watered daily and grown under coastal conditions (outdoors) for two years. The height of stems was measured at 3, 6, 12, 18 and 24 months.

(iv) Survival to two years of age

Within the two years, the time period when any potted seedlings died was recorded.

### **5.3.4 Assessing the capacity for seed to recruit and contribute to a long lived soil stored seed bank**

Seeds of *A. melvillei* and *A. loderi* collected after the 2010 La Niña rain event were manually sown in their natural environment, as well as buried in bags to be unearthed periodically, allowing tests of viability and recruitment potential, and to examine changes in dormancy characteristics over time under natural field conditions.

a) **Seed plantings:** Assessing the fitness of seed produced by the 2010-11 La Niña rain event under natural field conditions

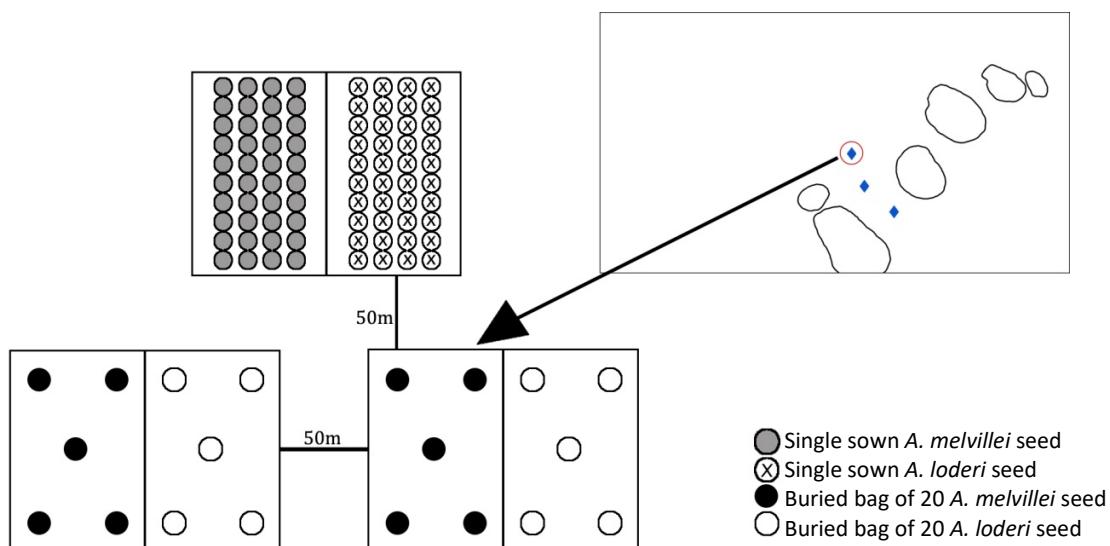
The recruitment rate, growth rate and mortality rate of *A. melvillei* and *A. loderi* seed was determined under natural field conditions. Seed were pooled from multiple plants across multiple stands to overcome any provincial diversity in seed viability and dormancy. Three

locations where *A. loderi* naturally occurs were chosen on Kinchega National Park for seed of both species to be buried (Figure 5.1). Soon after their collection and while La Niña rains were still heavy, 40 randomly selected unparasitised seed from the pooled seed were planted in the field at each site side by side for both *Acacia* species. The plots were approximately 1 m<sup>2</sup> and were surrounded by caging to prevent disturbance by animals and vertebrate herbivory. Seed were buried 1-2 cm below the soil surface to avoid loss through erosion, while exposing them to near-surface temperatures and water. The i) germination rate and ii) longer term survival of any seedlings emerging were assessed on five occasions over a three year period (5, 9, 13, 23 and 36 months after sowing).

b) **Seed burials:** Searching for evidence of a long lived soil stored seed bank

A total of 600 unparasitised *A. melvillei* and *A. loderi* seed, collected from the same trees used above were split into five lots of 200 and buried to uncover the capacity of these species to maintain a viable but dormant long lived soil stored seed bank, in order to estimate the proportion of canopy seed that remain dormant after burial. At each of the same three locations across Kinchega National Park where seed was sown (Figure 5.1), the 200 seed were divided into 10 lots of 20 seeds which were placed into mesh bags, filled with local soil and stapled closed. These bags were buried in two plots at least 50 metres apart at each site, such that each plot contained five bags full of 20 seeds each. Plots were approximately 1 m<sup>2</sup> and the bags were buried under approximately 2-5 cm of soil, so as to remain buried but close to the soil surface, under the conditions we would expect dormant seed to experience (Figure 5.1). Above the bags, wire mesh was pegged down over the surface of the soil to prevent animals digging them up.

Single bags from each plot across all five sites were unearthed periodically over a three year period at 146, 266, 384, 690 and 1080 days after burial. At each check, the retrieved bag was sieved to assess how many seed remained intact, germinated, or were missing. Any remaining un-germinated seed were assessed for viability. The seed coat of these seeds was scratched to break dormancy, they were placed on wet filter paper in Petri dishes, incubated at 28 / 14° C and monitored for germination for three weeks. Seeds that germinated were classed as dormant but viable, while those that did not germinate were considered inviable.



**Figure 5.1. Design of seed sowing and seed burial experiments:** Position of 40 and 200 *A. melvillei* and *A. loderi* seed sown and buried respectively, at each of three sites within Kinchega National Park.

### 5.3.5 Quantifying the scale of natural recruitment following the 2010-2011 La Niña rainfall event

I aimed to determine; a) whether the seed produced after the 2010 rain event is healthy enough to successfully recruit in the field; b) the approximate number of recruits that they produce per plant; and c) the ratio of seed to seedling recruits and the variance between stands for all three measures.

a) Surveying and quantifying relative success and failure of stands to recruit across the landscape

I conducted a region wide survey of sexual recruitment in each of the same stands of all five species previously surveyed for demographic structure, fruit / seed set and to determine the physical condition of stands in Chapters 2, 3 & 4 of this thesis. At each of these stands, I conducted timed searches for ten minutes per plant, under and around the canopy of the same ten mature plants used in previous chapters, to count all seedlings in a circular search area of equal size surrounding each plant.

b) Quantification of seedlings emerging from the canopy cohort initiated by La Niña rains versus from the soil stored seed bank.

From the same stands surveyed for recruitment above and in previous chapters, a subset of five stands each of *A. melvillei*, *A. loderi* and *A. ligulata* were chosen haphazardly (Figure 5.2). Six mature plants were selected haphazardly from these stands in the same manner as how plants were chosen in Chapter 2 of this thesis (see methods of Chapter 2, thesis). In April of 2011, at each of these six trees, four people rigorously searched every section of ground under the canopy and around the tree as far as mid way to the next neighbouring plant. Every seedling detected was measured to determine whether it was likely to have originated from canopy seed produced after the 2010 La Niña rain or from seed set in previous years that had been dormant in the soil stored seed bank.

Seedlings likely to have originated from canopy seed set in response to the 2010 La Niña rains were defined as those being from 0.5 to 2 cm high, while old seedlings were classed as being greater than 2 cm in height, based on plausible growth rates since seeds were released onto the ground. These ranges in seedling heights were also supported in retrospect, when after one year of monitoring, seedlings originating from seed set after the 2010 La Niña rain event were measured to be approximately the same height as the tallest seedlings categorized as originating from the soil stored seed bank. Moreover, given that the La Niña rains began approximately one year prior to when most fruit set on these *Acacia* began to dehisce and potentially recruit, the seedlings of a size larger than 2 cm on my initial visit were the right size to have begun growing from the soil stored seed bank from any time after January of 2010 up until the new fruits began to dehisce. I acknowledge that a portion of the smaller seedlings may have originated from soil stored seed rather than from 2010 canopy seed. Nevertheless, given the uniformity in height of the seedlings in each of the two classes used here, it was deemed likely that the smaller seedlings were most likely to be a product of the same 2010 canopy seed whilst the larger seedlings were almost certainly too big to have grown from the 2010 canopy seed. Suckers were not counted, identified as not possessing pinnate leaves and where possible their surface / subterranean lateral roots were traced back to a mature parent plant to confirm their status.

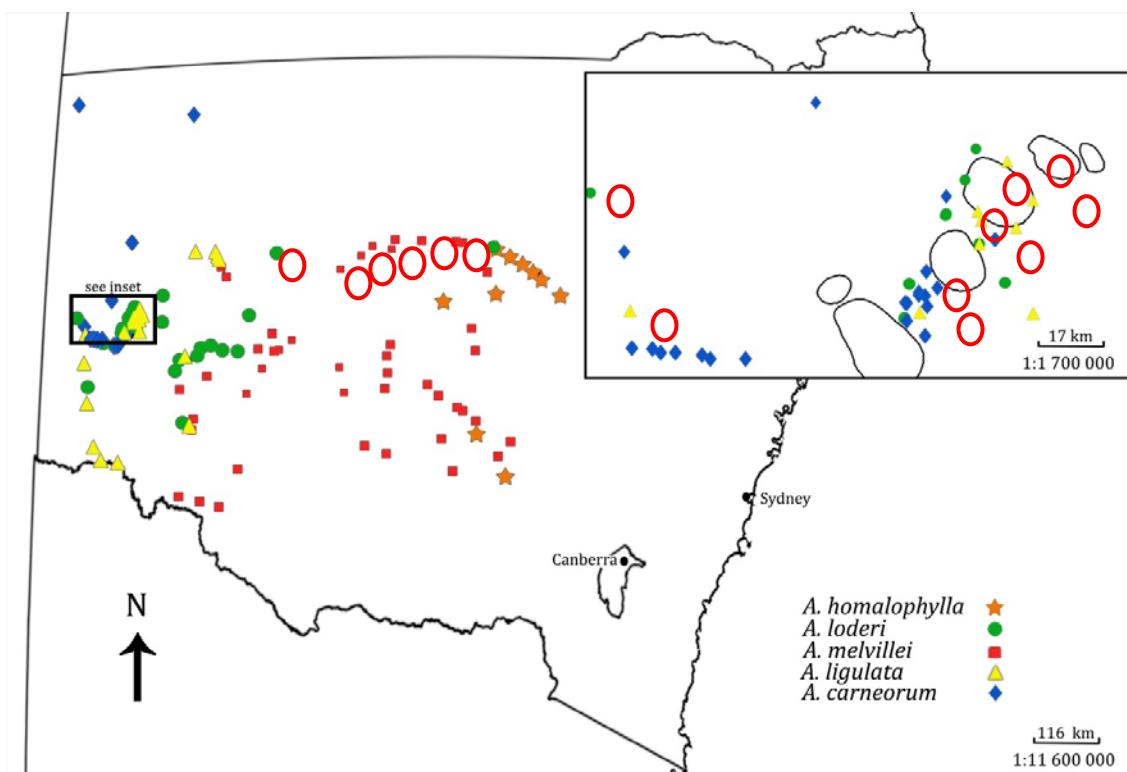


Figure 5.2. Subset of five stands each of *A. melvillei*, *A. loderi* and *A. ligulata* used to determine the contribution of seedlings from the canopy seed and the soil stored seed bank after a La Niña rain event and for monitoring their long term persistence and condition.

### c) Estimates of the ratio of seed to seedlings

Utilizing previous estimates of the numbers of seed set on plants (Chapter 3, thesis) and the counts of seedlings obtained here, the ratio of seed set post La Niña rains to new seedlings recruited was estimated for each maternal plant. I acknowledge that some seed found around any given plant have potentially been moved there by wind, water or seed dispersers, however local seed are likely to be lost in a similar way at a similar rate, making my estimate a reasonable one.

### **5.3.6 Assessment of the potential driving factors of initial recruitment success and failure within stands**

With the aim of finding reliable predictors for initial recruitment success, failure and effort in these species, I looked for evidence that recruitment success in the stands of all five *Acacia* species shown in Figure 2.1 (Chapter 2, thesis) was either determined by differences in; i) the numbers of seed produced by plants (quantified in Chapter 3, thesis); or ii) the level of anthropogenic disturbance in these stands (quantified in Chapter 4, thesis); or iii) differences in local climates (quantified in Chapter 4, thesis). Specifically, for each stand of *A. melvillei*, *A. horalophylla*, *A. loderi*, *A. carneorum* and *A. ligulata* surveyed for recruitment, I looked for any significant relationship between; a) the average number of seed produced by the ten plants surveyed in each stand and the average number of seedlings recruited per plant, as well as any relationships between; b) local conditions within stands including demographic integrity, environmental integrity, health of plants and climatic variables (listed in Table 4.1 of Chapter 4, thesis); c) the presence of seedlings (percentage of these ten plants that were found with newly recruited seedlings under or around their canopy); and d) the average number of seedlings recruited under or around the canopy of plants.

### **5.3.7 Assessing the performance and survival of recruits**

The fate of seedlings in the same subset of *A. melvillei*, *A. loderi* and *A. ligulata* stands used in method **5.3.4 b**, were followed for three years under natural field conditions to assess their fitness and mortality rates, while also gaining information about the grazing pressures they experience and their growth rates. A metal stake with an identifying tag was placed next to each seedling so I could track their progress over the long term. All seedlings, of any size, found in the search were assigned to the tree they were found closest to or under. I revisited each site a total of five times over three years (at approximately 3, 6, 12, 24 and 36 months after seed were released from the canopy of trees) up until the number of new recruits became proportionally very small. Any new recruits previously untagged that emerged within the same original search area of each plant were tagged, measured and added to the tally for the tree. At each check I recorded the presence and absence of seedlings as well as their height and whether there was any evidence of new grazer damage. Seedlings with any obvious signs of grazing such as chewed leaves and cut stems, were assigned a value of one while a seedling that was found to have no signs of being grazed was assigned the value zero. After the

five checks the total of these values was summed up to assign a score of 0-5, with 0 representing no grazing pressure and five representing maximal grazing pressure. Descriptions and /or photos were taken of seedlings at each check to guard against recounting the same grazer damage twice. The height of seedlings at the final check (three years) was recorded and reported.

### **5.3.8 Driving factors for long term survival of recruits**

The effect of two microhabitat features, thought to be important for seedling health and survival within a hot and dry semi arid environment with high grazing rates (canopy shade and protection by ‘nurse’ plants), were assessed utilizing the same tagged seedlings used above. The proximity to canopy shade, and protection by understory vegetation (‘nurse plants’) were assessed as predictors of seedling condition and persistence in the field. This was achieved by measuring and comparing the average, height, levels of grazing pressure and three year survivorship rates between seedlings located in different positions with respect to the canopy cover of mature plants and understory ‘nurse’ plants.

The position of each seedling was also recorded to gain information about the microhabitats / microclimates in which seedlings exist within, as the difference in daily temperatures experienced by seedlings in shade versus fully exposed on bare ground, as well as the protective cover other understory plants may provide, are likely to affect growth and mortality rates.

When these seedlings were originally tagged they were each assigned to one of three distinct zones where seedlings could recruit and where levels of shade vary; 1) under, 2) edge and 3) outside the canopy of mature independent plants. Recruits classed as ‘under’ the canopy were any seedlings that were located anywhere from the trunk of the parent plant out to the edge of the overlying canopy. Those that were classed as at the ‘edge’ of the canopy were those that were found anywhere from the edge of the canopy to one metre outside the edge of the canopy, such that they would receive partial shade at certain times. Those classed as “outside” the canopy were those located from the boundary of the ‘edge’ to half way between the parent plant and the closest neighbouring plant, in all directions up to ten metres from the trunk of the parent plant. The position of each seedling in relation to understory vegetation was also recorded. Seedlings were either classed as existing under the canopy of an understory plant (‘nurse plant’), or outside a ‘nurse plant’ where they were not concealed in

any way. I then looked for significant differences between the measures of survivorship, herbivory and height obtained above between groups of seedlings after three years of observation based on their proximity to the canopy cover of mature plants and nurse plants.

### **5.3.9 Statistical analysis of data**

I used two way analysis of variance (2 way ANOVA) to compare the viability of seed (parasitism and germination rates) and fitness of seedlings produced (seedling growth rates, survival to three years) in response to the 2010-2011 La Niña rainfall event, between species and between stands of the same species, as well as estimates of the ratio of seed to seedlings found to have recruited. These data passed the strict assumptions of the Shapiro-Wilks and Levene's tests for normality and equality of variance. Tukey's post hoc tests were used to test for differences between each of the *Acacia* species and each of the stands.

I used Kruskal-Wallis tests to compare the number of seedlings recruited in stands between the four *Acacia* species that recruited them, and between multiple stands of each species. Mann-Whitney U tests were used to test for differences between each of the *Acacia* species and each of the stands against each of the other species and stands.

Linear regression analysis was used to look for linear relationships between the fecundity of plants and the number of seedlings that recruited under and around *A. melvillei*, *A. loderi* and *A. ligulata* plants. Linear regression analysis was also used to look for relationships between the five structural, three environmental, four plant condition and two climatic measures and a) the percentage of plants within stands that were found to have recruits around them and b) the average number of seedlings recruited under and around plants in each stand. Hierarchical multiple regression analysis was used to look for any relationship between combinations of the demographic, environmental, plant condition and local climatic conditions. All percentage data to be analysed with linear regression was transformed using an arcsine-root transformation, and data involving counts was square root transformed, and both were found to be normally distributed.

I used Kaplan-Meier (*Log rank test*) survival analysis to compare the long term survival of recruits between *Acacia* species, as well as between seedlings originating from canopy seed and soil stored seed. This was also performed to compare the long term survival of these

seedlings with respect to their position to the canopy of mature plants and to understory ‘nurse’ plants.

I used Kruskal-Wallis tests to compare the long term performance (grazing damage and growth) and survival of recruits, as well as comparing these same measures between seedlings located in different areas with respect to the canopy of mature plants and to understory ‘nurse’ plants. Mann-Whitney U tests were used to test for differences between each of the *Acacia* species and each of the stands against each of the other species and stands.

## 5.4 Results

### 5.4.1 Assessment of the viability and fitness of seed produced after the 2010-2011 La Niña rainfall event

(a) Measuring seed and seedling health under lab conditions

(i) Seed parasitism level

Over 35% of seed produced on *A. melvillei*, *A. homalophylla*, *A. loderi* and *A. carneorum* plants were parasitized, whilst in stark contrast less than 20% of the *A. ligulata* seeds collected were parasitized (Figure 5.3 a). Two way analysis of variance comparing the proportion of seed parasitized on *A. melvillei*, *A. homalophylla*, *A. loderi* and *A. ligulata* plants revealed a significant overall effect of species on the average level of seed parasitism ( $F(4, 95) = 200.55$ ,  $p < 0.001$ ). Tukey’s post hoc tests revealed that the levels of seed parasitism were significantly higher on average for *A. melvillei* and *A. homalophylla* plants 43.2% ( $SE \pm 1.1$ ) and 42.6% ( $SE \pm 1.4$ ) than for *A. loderi* plants 36.9% ( $SE \pm 1.2$ ), which was in turn significantly higher than *A. ligulata* plants 16.8% ( $SE \pm 0.09$ ). Seeds collected from *A. carneorum* plants were noticeably more affected by seed parasites than the other four species with 46% ( $SE \pm 3$ ) parasitized. No significant effect of stand on parasitism levels was found for *A. melvillei* ( $F(38, 351) = 0.920$ ,  $p = 0.601$ ), *A. homalophylla* ( $F(7, 77) = 0.822$ ,  $p = 0.732$ ), *A. loderi* ( $F(20, 189) = 0.923$ ,  $p = 0.598$ ) and *A. ligulata* ( $F(19, 180) = 0.801$ ,  $p = 0.702$ ).

(ii) Seed viability

The majority of seed produced on plants of the four threatened species was viable, whilst a little less than half of the *A. ligulata* seed collected was viable (Figure 5.3 b). Two way analysis of variance revealed a significant overall effect of species on the levels of viability in

the non parasitised seed cohorts collected from stands of all five *Acacia* species ( $F(4,95) = 183.936$ ,  $p < 0.001$ ). Tukey's post hoc analysis revealed that the percentage of seed that germinated after scarification and hydration for *A. melvillei* 77.3% ( $SE \pm 0.01$ ), *A. homalophylla* 76.1% ( $SE \pm 0.02$ ) and *A. loderi* 76.1% ( $SE \pm 0.01$ ) was equivalent, whilst being significantly higher than for *A. carneorum* 67.9% ( $SE \pm 0.70$ ) which was, in turn, significantly higher than *A. ligulata* 45.6% ( $SE \pm 0.05$ ). No significant effect of stand on seed viability was found for *A. melvillei* ( $F(38, 351) = 0.671$ ,  $p = 0.798$ ), *A. homalophylla* ( $F(7, 77) = 0.910$ ,  $p = 0.576$ ), *A. loderi* ( $F(20, 189) = 0.739$ ,  $p = 0.655$ ) and *A. ligulata* ( $F(19, 180) = 0.900$ ,  $p = 0.628$ ).

### (iii) Seedling growth

#### a) Early stage (shoot tip to root tip lengths at six weeks after germination)

Early stage growth of *A. melvillei*, *A. homalophylla* and *A. loderi* seedlings was noticeably higher than for *A. ligulata* seedlings, but noticeably lower than for *A. carneorum* seedlings (Figure 5.3 c). Two way analysis of variance revealed a significant effect of species on the early stage growth of seedlings in situ ( $F(4, 95) = 403.930$ ,  $p < 0.001$ ). Tukey's post hoc test revealed that while height at six weeks was equivalent for closely related *A. melvillei* 3.8cm ( $SE \pm 0.02$ ) and *A. homalophylla* 3.7cm ( $SE \pm 0.1$ ), they were significantly lower for *A. loderi* 3.1cm ( $SE \pm 0.01$ ) and significantly higher for both *A. carneorum* 4.6cm ( $SE \pm 0.01$ ) and *A. ligulata* 4.5cm ( $SE \pm 0.01$ ), which were found to be statistically equivalent. The effect of stand on early stage seedling growth was not significant for *A. melvillei* ( $F(38, 741) = 0.840$ ,  $p = 0.701$ ), *A. homalophylla* ( $F(7, 152) = 0.890$ ,  $p = 0.689$ ), *A. loderi* ( $F(20, 399) = 0.907$ ,  $p = 0.598$ ) and *A. ligulata* ( $F(19, 380) = 0.992$ ,  $p = 0.703$ ).

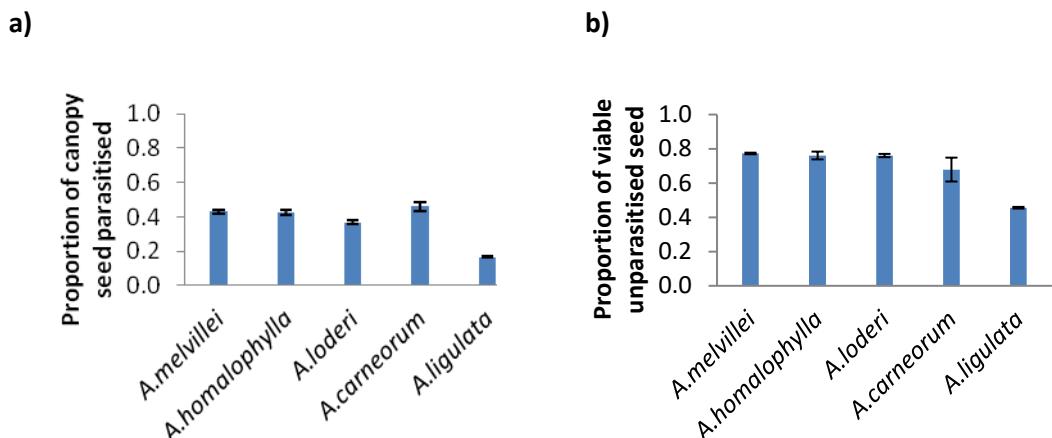
#### b) Seedling growth to two years of age

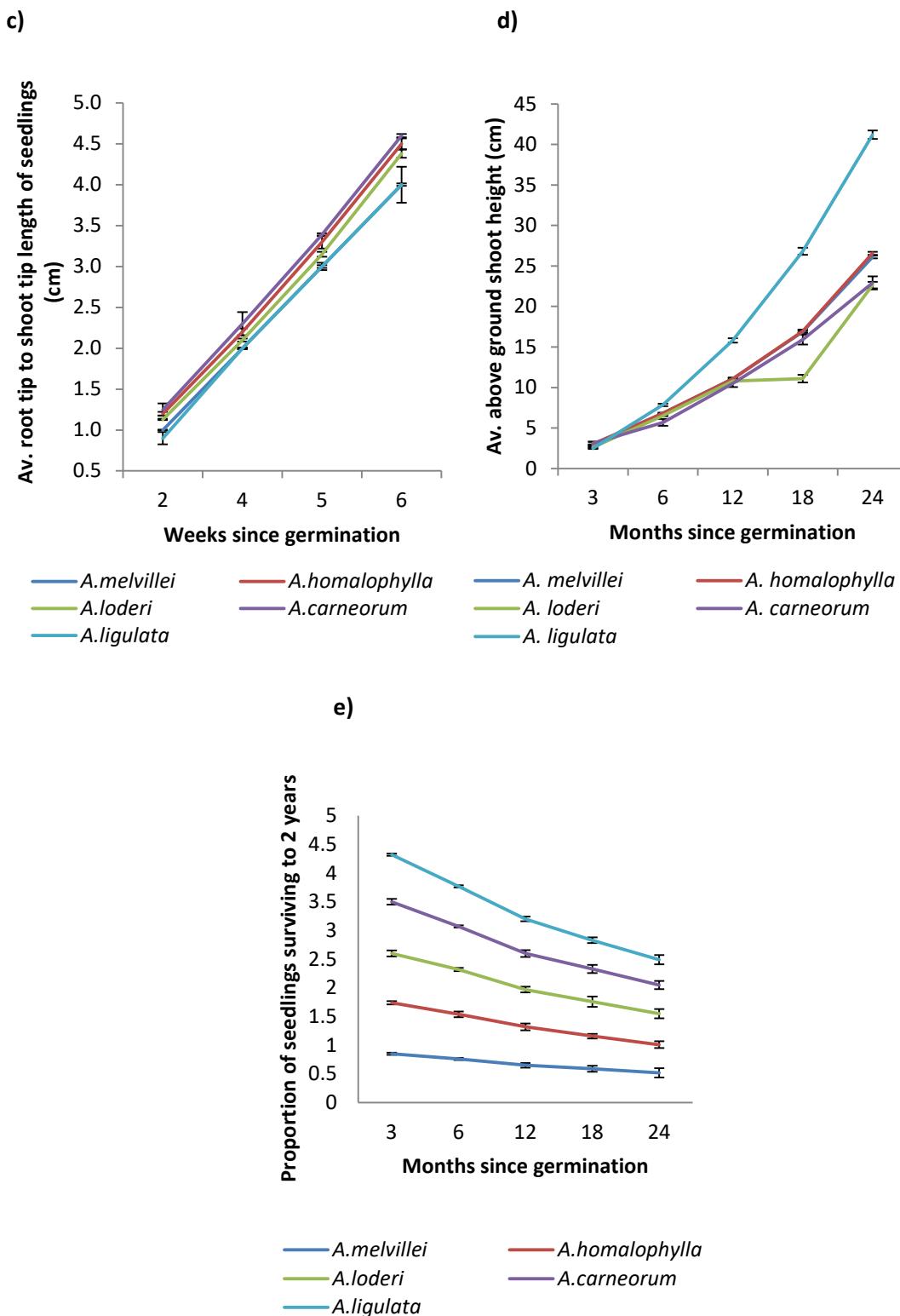
With the exception of *A. carneorum* seedlings, whose growth slowed relative to their early growth (Figure 5.3 d), growth of seedlings after being potted showed similar trends to early growth with little variation among sites. Two way analysis of variance revealed a significant effect of species on growth of potted seedlings ( $F(4, 99) = 403.930$ ,  $p < 0.001$ ). Tukey's post hoc test revealed that while two year heights were equivalent for closely related *A. melvillei* 26.1 cm ( $SE \pm 0.2$ ) and *A. homalophylla* 26.6 cm ( $SE \pm 0.2$ ), they were significantly lower for both *A. loderi* 22.6 cm ( $SE \pm 0.1$ ) and *A. carneorum* 22.9cm ( $SE \pm 0.1$ ) which were equivalent. *A. ligulata* seedlings were significantly taller than the other species at the final check 41.2 cm ( $SE \pm 0.2$ ) (Figure 2 c). The effect of stand on seedling growth was not significant

for *A. melvillei* ( $F(38, 741) = 0.597, p=0.899$ ), *A. homalophylla* ( $F (7, 152) = 0.654, p=0.812$ ), *A. loderi* ( $F (20, 399) = 0.961, p=0.560$ ) or *A. ligulata* ( $F (19, 380) = 0.941, p=0.521$ ).

(iv) Survival to two years

Two year survival of potted *A. melvillei*, *A. homalophylla*, *A. loderi* and *A. carneorum* was noticeably higher than for *A. ligulata* (Figure 5.3 e). Two way analysis of variance revealed a significant effect of species on two year survival of potted seedlings ( $F (4, 99) = 334.222, p=0.001$ ). Tukey's pot hoc tests revealed that while the two year survival of potted seedlings of *A. melvillei* 52% ( $SE \pm 8.7$ ), *A. homalophylla* 49% ( $SE \pm 2.000$ ), *A. loderi* 54% ( $SE \pm 5.1$ ), and *A. carneorum* 50% ( $SE \pm 5.0$ ) were not significantly different, the overall difference between *Acacia* species was solely driven by the lower survival rates of potted *A. ligulata* seedlings 44% ( $SE \pm 1.800$ ). The effect of stand on seedling survival was not significant for *A. melvillei* ( $F (38, 741) = 0.722, p=0.721$ ), *A. homalophylla* ( $F (7, 152) = 0.979, p=0.551$ ), *A. loderi* ( $F (20, 399) = 0.892, p=0.755$ ) and *A. ligulata* ( $F (19, 380) = 0.598, p=0.871$ ).





**Figure 5.3. Viability and performance of seed tested under lab and coastal conditions:** a) Proportion of canopy seed parasitized by insects ( $n= 15000, 3500, 8000, 600$  and  $10000$  seed for *A. melvillei*, *A. homalophylla*, *A. loderi*, *A. carneorum* and *A. ligulata* respectively) b) Proportion of unparasitised seed that were found to be viable after scarification and addition

of water in an incubator (n=3000, 700, 1600, 200 and 2000 seed for *A. melvillei*, *A. homalophylla*, *A. loderi*, *A. carneorum* and *A. ligulata* respectively) c) Average root tip to shoot tip length of seedlings (cm) (n= 600, 140, 320, 96 and 400 seed for *A. melvillei*, *A. homalophylla*, *A. loderi*, *A. carneorum* and *A. ligulata* respectively) d) Average above ground shoot height (cm) e) Proportion of seedlings surviving to two years (n= 600, 140, 320, 96 and 400 seed for *A. melvillei*, *A. homalophylla*, *A. loderi*, *A. carneorum* and *A. ligulata* respectively).

#### 5.4.2 Assessing the capacity for seed to recruit and contribute to a long lived soil stored seed bank

a) **Seed plantings:** Assessing the fitness of seed produced by the 2010-2011 La Niña rain event under natural field conditions

On average 4% and 1.7% of the manually sown *A. melvillei* and *A. loderi* seed at three different sites emerged during a three year period of observation (Figure 5.4 a). Recruitment levels of manually sown seed varied from 0-10% for the *A. melvillei* seed between burial sites and from 0-2.5% between different *A. loderi* burial sites (Figure 5.4 b). Greater than 150, 000 and almost 10,000 times as many seedlings per seed emerged naturally in the *A. melvillei* and *A. loderi* stands surveyed above. None of the seedlings of either species that did emerge survived to the next check after they were discovered.

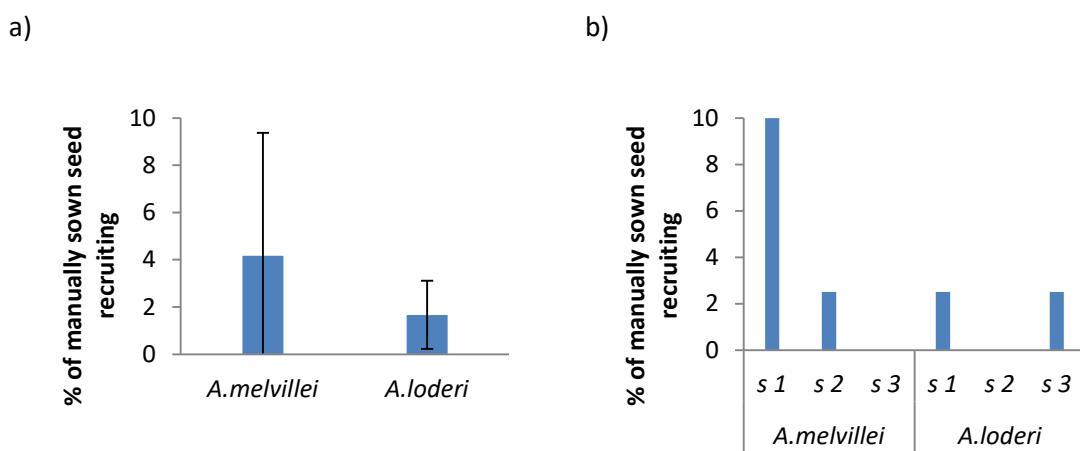
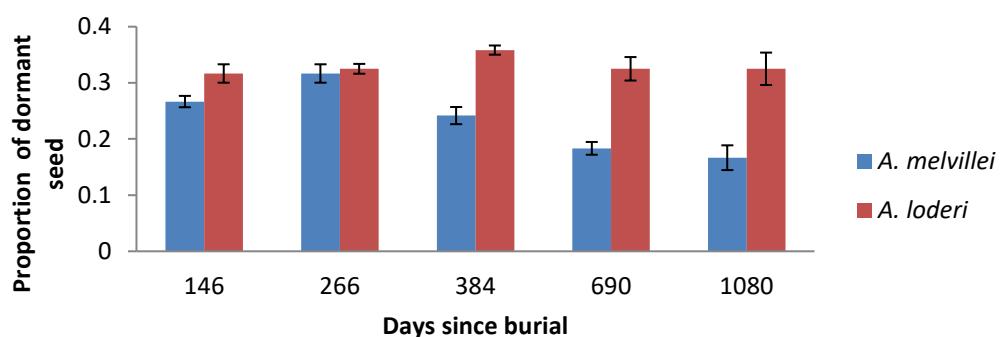


Figure 5.4: Recruitment rates of manually sown *A. melvillei* and *A. loderi* seed in Kinchega National Park during the time of natural recruitment: a) % of all 120 manually sown *A. melvillei* and *A. loderi* seed within three sites within Kinchega National Park that recruited, b) % of 40 manually sown *A. melvillei* and *A. loderi* seed within each of three sites within Kinchega National Park.

b) **Seed burials:** Searching for evidence of a long lived soil stored seed bank.

A significant proportion of the seed cohort of both *A. melvillei* and *A. loderi* were found to be dormant, with the potential to become part of a long lived soil stored seed bank remaining dormant for many years. I found 25.2% and 31.7% of the total number of buried *A. melvillei* and *A. loderi* seed remained dormant and viable under the ground for 1080 days, with dormancy rates dropping very little over that time period (Figure 5.5 a). Dormancy rates were consistent across the three sites seed were buried, suggesting these rates are true indicators of the number of dormant seed we might expect (Figure 5.5 b).

a)



b)

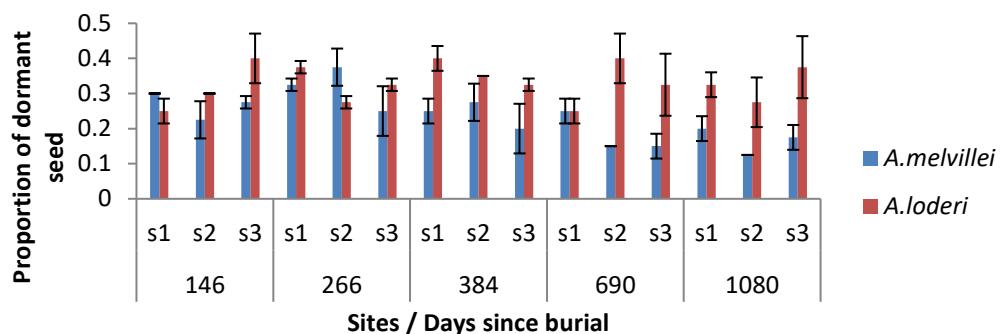


Figure 5.5. Assessments of the dormancy characteristics of *A. melvillei* and *A. loderi* seeds after burial at three sites (s1, s2, s3) within Kinchega National Park: a) Proportion of 60 unparasitised *A. melvillei* and *A. loderi* seed found to be dormant and viable after burial in the soil across three sites within Kinchega National Park, b) Proportion of 20 unparasitised *A. melvillei* and *A. loderi* seed that were found to be dormant and viable after 146, 266, 384, 690 and 1080 days buried in the soil at each of three sites within Kinchega National Park. \* Error bars (SE) in a) represents variation in a) between three sites and in b) between five treatments (buried bags).

### **5.4.3 Quantifying the scale of natural recruitment following the 2010-2011 La Niña rainfall event**

#### *a) Surveying and quantifying relative success and failure of stands to recruit*

I observed patchy recruitment of *A. melvillei*, *A. homalophylla*, *A. loderi* and *A. ligulata* seedlings across the region, with some stands recruiting seedlings and others not (Figure 5.6). No obvious patterns across the landscape were seen with respect to stands that recruited seedlings and those that failed to recruit seedlings for any of the five *Acacia* species surveyed, suggesting more local determinants of recruitment success and failure (Figure 5.7). Whilst recruitment of seedlings was not observed in any of 30 *A. carneorum* stands surveyed, and only observed in a minority of the *A. homalophylla* (30%) and *A. loderi* (38%) stands surveyed, more than half the *A. melvillei* (66%) stands surveyed showed recruitment of at least some seedlings after the La Niña rains, as did every one of the 20 *A. ligulata* stands surveyed (Figure 5.8 a). The percentage of stands where more than 8 out of the 10 plants surveyed was observed to recruit was only 13%, 20%, 15% and 20% of *A. melvillei*, *A. homalophylla*, *A. loderi* and *A. ligulata* respectively (Figure 5.8 b). Kruskal-Wallis tests revealed that a significant difference existed in the proportion of plants per stand with recruits under and around their canopies among the four *Acacia* species surveyed in 2012 ( $\chi^2$  (3, n=133)=31.961, p<0.001). Mann-Whitney U tests revealed that the average proportion of plants per stand that recruited seedlings for *A. melvillei* (39.8% SE± 5.3), *A. homalophylla* (23.9% SE 14.7), *A. loderi* (3.4% SE± 1.3) and *A. ligulata* (86.5% SE± 2.4) were all significantly different from one another (p<0.001 for all comparisons). Kruskal-Wallis tests also revealed a significant difference in the number of seedlings recruited in stands among the four *Acacia* species that recruited them ( $\chi^2$  (3, n=133) = 21.997, p<0.001). Mann-Whitney U tests revealed that the average number of seedlings recruited per plant per stand (detected in the ten minute timed search) for *A. melvillei* (3.14 SE± 1.19), *A. homalophylla* (1.54 SE± 0.87), *A. loderi* (11.7 SE± 9.74), and *A. ligulata* (4.72 SE± 0.18) were all significantly different from each other (p<0.001 for all comparisons) (Figure 5.8 c). Kruskal-Wallis tests also revealed that the average number of seedlings recruited per plant was significantly different on among the multiple stands of *A. melvillei* ( $\chi^2$  (46, n=460) =235.1, p<0.001), *A. homalophylla* ( $\chi^2$  (9, n=139) = 69.9, p<0.001) and *A. loderi* ( $\chi^2$  (25, n=887) =144.2 df=25, p<0.001) on average, but in contrast no significant difference was found between the *A. ligulata* stands surveyed ( $\chi^2$  (19, n=946)= 10.68 df=19, p=0.934).



**Figure 5.6. Seedling recruitment around overstory *Acacia* in western NSW after a region wide La Niña driven rain event in 2011:** (clockwise from top left): A newly recruited *A. loderi* seedling; Digging down to the roots of a newly recruited *A. loderi* seedling; An *A. loderi* seedling after 1 year of growth; Tagged *A. melvillei* seedlings after 1 year of growth.

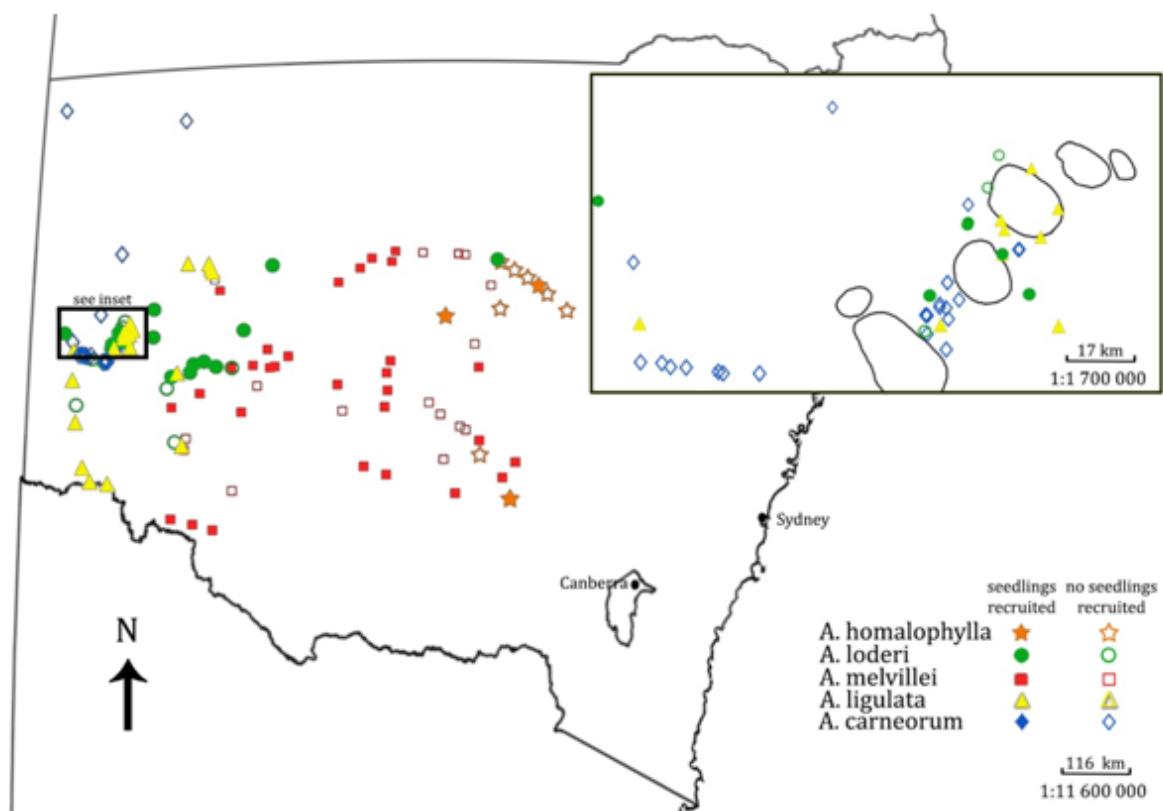
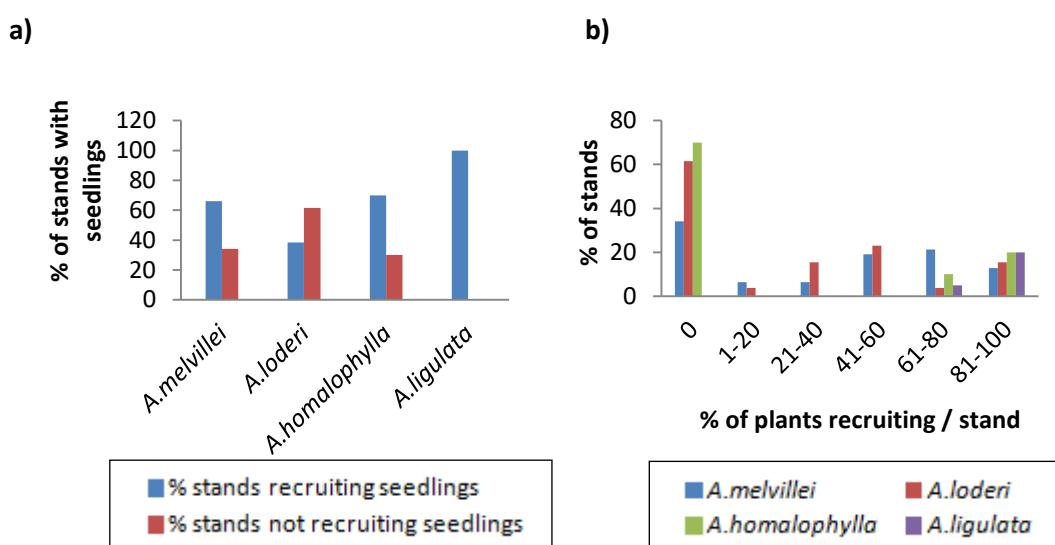
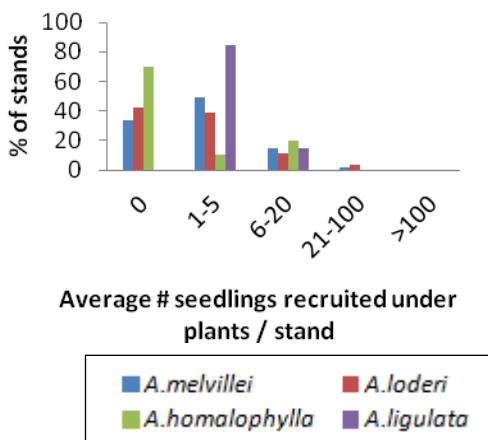


Figure 5.7. Sexual recruitment success or failure of surveyed *Acacia* stands in western NSW: The presence and absence of seedlings recruited in 47, 10, 26, 30 and 20 stands of *A. melvillei* *A. homalophylla*, *A. loderi* and *A. carneorum* in at least one of two consecutive years following the beginning of the La Niña rains in January of 2010. Inset displays Kinchega National Park and the region around the Menindee Lakes.



c)



**Figure 5.8. Surveys of sexual recruitment from 20 to 47 stands of *A. melvillei*, *A. homalophylla*, *A. loderi* and *A. ligulata* spread across western NSW utilizing timed searches of ten minutes each of ten mature plants within each stand:** a) Percentage of stands that recruited seedlings, b) Percentage of stands with respect to the percentage of plants recruiting seedlings per stand, c) Percentage of stands with respect to the average number of seedlings recruited per plant per stand.

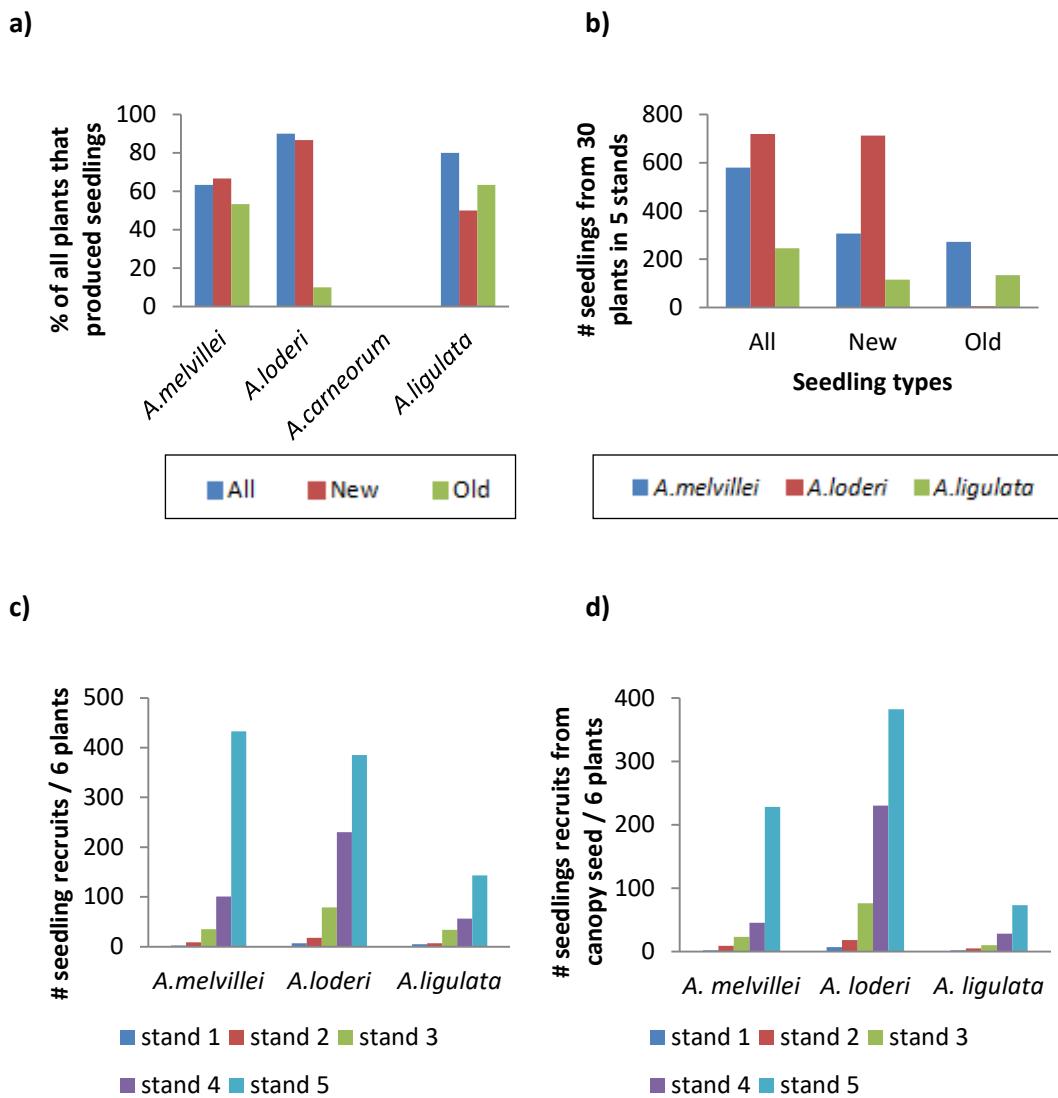
b) Quantification of seedlings emerging from the canopy cohort initiated by La Niña rains versus from the soil stored seed bank.

Seedlings recruited around the majority of plants (Figure 5.9 a), with numbers varying greatly between plants and stands (Figure 5.9 f). There were comparable numbers of *A. melvillei* and *A. ligulata* seedlings originating from the canopy seed cohort produced after the La Niña rainfall, and those larger seedlings that were likely to have originated from within the soil stored seed bank (Figure 5.9 b). In stark contrast, while many seedlings originated from the canopy seed cohort, only three seedlings that originated from a soil stored seed bank were detected across the five *A. loderi* stands (Figure 5.9 b & c). Again, no seedlings were found after searching a subset of five *A. carneorum* stands more thoroughly.

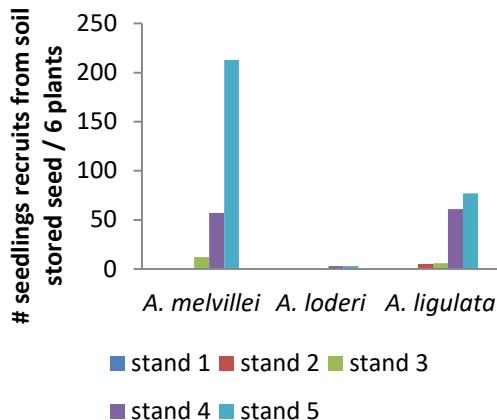
Great variation in the level of seedling recruitment was found between the five stands of each species surveyed (Figure 5.9 c, d, e), as well as between the 30 plants across these sites of each species, regardless of the origin of seedlings (Figure 5.9 f, g, h).

A general trend in the timing of recruitment was observed for all three species surveyed following the dehiscing of seed from fruit pods, with 77%, 74% and 79% of *A. melvillei*, *A. loderi*

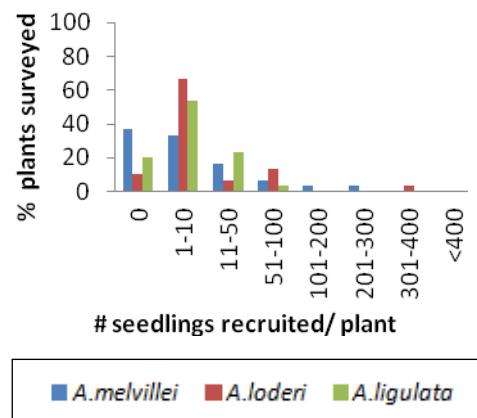
and *A. ligulata* seedlings, respectively recruiting within approximately six months of seed pods opening. While seedling recruitment tapers off considerably after approximately one year, seedlings were still emerging in small numbers at least three years after initial recruitment was observed (Figure 5.9 i).



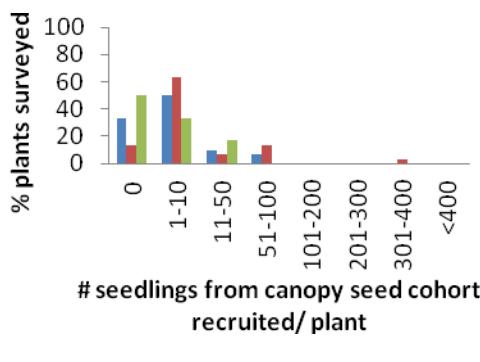
e)



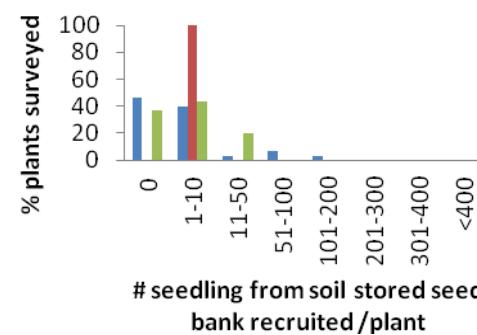
f)



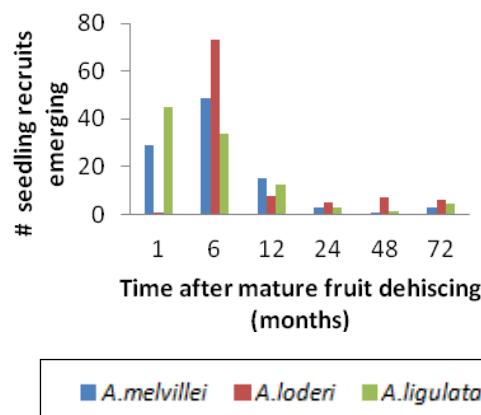
g)



h)



i)

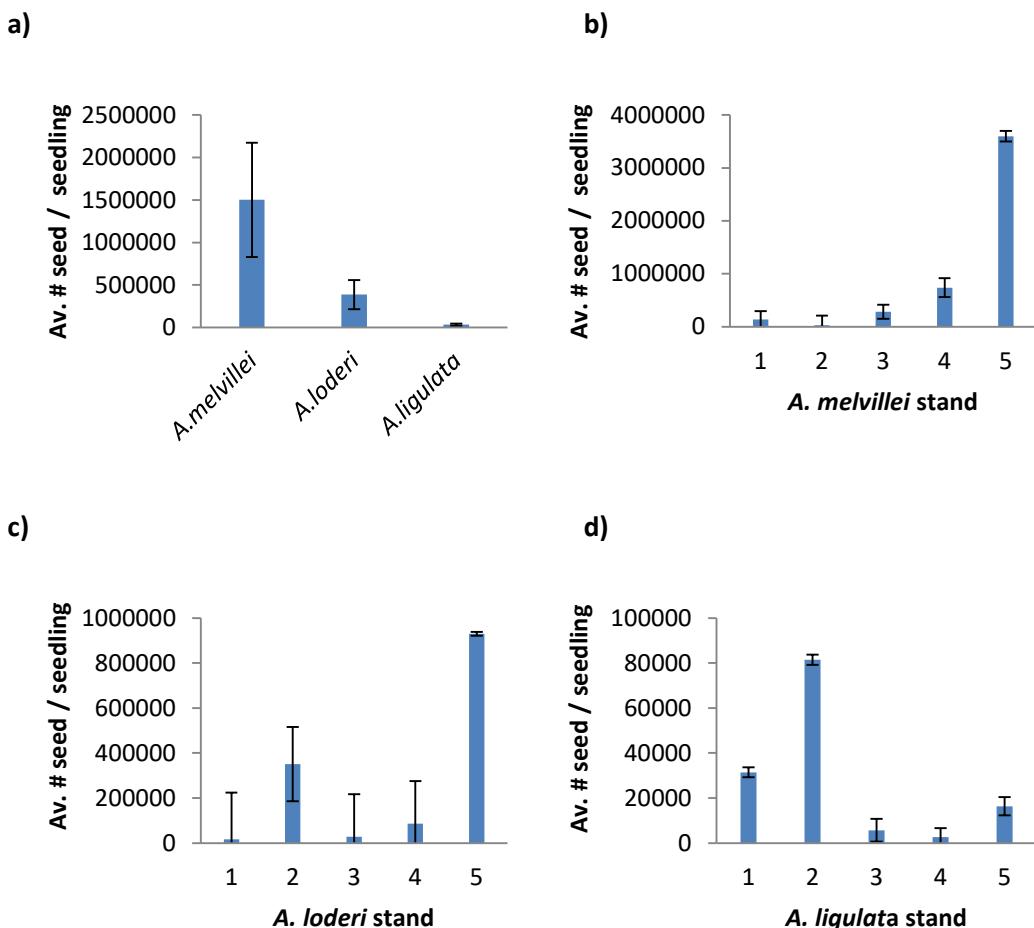


**Figure 5.9. Surveys of sexual recruitment under 30 *A. melvillei*, *A. loderi* and *A. ligulata* plants spread across five stands for each species to determine actual numbers of recruits:** a) % plants that produced seedlings post La Niña rainfall beginning in January of 2010 (separated into all seedlings regardless of origin (All), seedlings originating from the canopy seed cohort of 2011 (New), and those originating from the soil stored seed bank (Old)), b) # seedlings from 30 plants across five stands (separated into All, New and Old), c) # seedling recruits / six plants, d) # seedling recruits from canopy seed cohort / six plants, e) # seedling recruits from soil stored seed bank / six plants f) % of plants with respect to the # seedlings recruited / plant , g) % of plants with respect to the # seedlings from canopy seed cohort recruited / plant, h) % of plants with respect to the # seedlings from soil stored seed bank recruited /plant, i) # seedling recruits emerging with respect to the time after mature fruit dehiscing (months).

c) Estimates of the ratio of seed to seedlings

The average number of seed expected to produce one seedling differed noticeably among the three species surveyed with an average of 1.5 million ( $SE \pm 671,665$ ), 386,583 ( $SE \pm 172,885$ ) and 32,191 ( $SE \pm 14,396$ ) seed required to set a single seedling under *A. melvillei*, *A. loderi* and *A. ligulata* plants respectively (Figure 5.10 a). There was great variation between plants in the number of seed set per seedlings recruited ranging from 12,055 to <1,200,000, 2,744.66 to <1,109,091 and 1,397.13 to <27,272.72 across all five stands of *A. melvillei*, *A. loderi* and *A. ligulata* respectively. For *A. melvillei* and *A. ligulata* variance among plants within stands was not great (Figure 5.10 b, c, d). In contrast for four of the five *A. loderi* stands surveyed, far more variance was seen among plants in the number of seed required to recruit a single seedling (Figure 5.10 c). One way analysis of variance revealed that the estimated ratio of seed set on plants to seedlings recruited, varied significantly among the three *Acacia* species for which this was estimated (*A. melvillei*, *A. loderi* and *A. ligulata*) ( $F(2, 88) = 130.276$ ,  $p < 0.001$ ). Tukey's post hoc tests revealed that significantly more seed on average was required to produce each *A. melvillei* seedling (107580) than an *A. loderi* seedling (44551) and far fewer seeds were required, again on average, to produce an *A. ligulata* seedling (7574.614). For all three species however, there was great variation between the five different stands in average number of seed required to recruit a seedling with averages ranging from 28,577 ( $SD \pm 178,317$ ) to 36 million ( $SE \pm 1$  million), 15,981 ( $SE \pm 207,635$ ) to 929,416 ( $SE \pm 8,355$ ) and 2,623 ( $SE \pm 3,966$ ) to 81,455 ( $SE \pm 2,333$ ) for *A. melvillei*, *A. loderi* and *A. ligulata* stands respectively, suggesting an effect of stands on the recruitment rate of plants within them (Figure 5.10 b, c, d). While no seedlings were found in the proximity of *A. melvillei*, *A. loderi* or *A. ligulata* plants that did not set any fruit in response to the La Niña rainfall, no relationship was found between the number of seeds set by plants that did set at least some seed and the number of seedlings

that recruited in their vicinity for *A. melvillei* ( $r^2=0.49$ ,  $F=2.316$ ,  $p=0.135$ ), *A. loderi* ( $r^2<0.001$ ,  $F=0.007$ ,  $p=0.932$ ), or *A. ligulata* ( $r^2=0.015$ ,  $F=0.292$ ,  $p=0.595$ ).



**Figure 5.10. Estimates of the average ratio of seed produced to seedlings recruited on ten plants in each of five *A. melvillei*, *A. loderi* and *A. ligulata* stands respectively:** a) average # of seed per seedling recruited under *A. melvillei*, *A. loderi* and *A. ligulata* plants with standard error bars representing the average variance between five stands, b), c), d) average # of seed per seedling recruited per *A. melvillei*, *A. loderi* and *A. ligulata* plants respectively in each of the five stands surveyed. \* Standard error bars represent the variance between plants within stands.

#### **5.4.4 Assessment of the potential driving factors of initial recruitment success and failure within stands.**

##### **a) Effect of fecundity on seedling recruitment**

No seedlings were found in stands where no seed were seen to be set post La Niña rain. Nevertheless, no relationship was found between the number of seed set and the number of seedlings recruited for any of the five *Acacia* species with  $r^2$  ranging from 0.008 to 0.094 (Appendix 5.6.2).

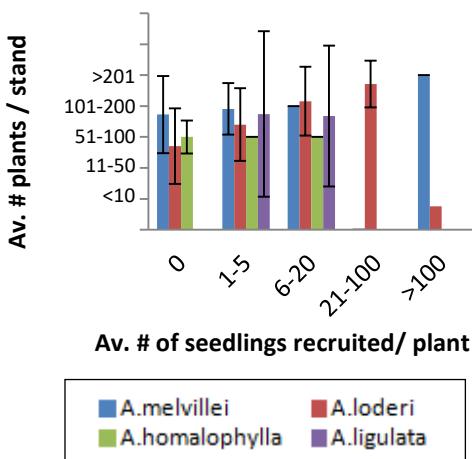
##### **b) Effect of stand, plant and climatic conditions on seedling recruitment**

###### **(i) Numbers of seedlings recruited**

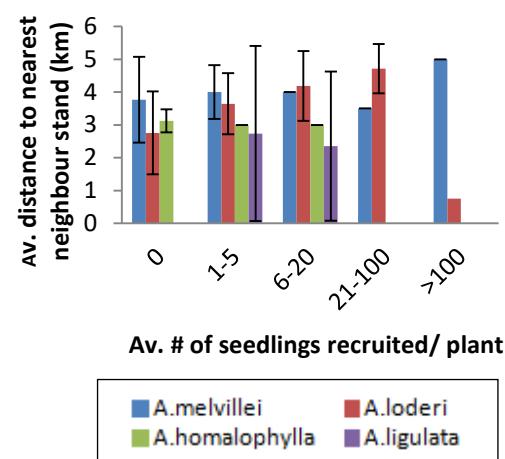
The 14 stand, plant and local climatic variables assessed here were generally not noticeably different between stands that recruited different numbers of seedlings (0, 1-5, 6-20, 21-100, >100 seedlings / plant) for any of the *Acacia* species surveyed (Figure 5.11). Other than a significant positive linear relationship between the average number of suckers per *A. homalophylla* plant and the average number of seedlings recruited ( $r^2=0.825$ ,  $F=32.999$ ,  $p=0.001$ ), regression analysis found no significant linear relationships between the number of recruits and any of the 14 variables measured in stands that recruited seedlings, with  $r^2$  values ranging from <0.000 to 0.411 ( $p>0.05$ ) for all relationships (Appendix 5.4). Moreover, no other non linear relationships were apparent between any of the stands and plant condition measures and their reproductive capacity.

Hierarchical multiple regression analysis, adding the seven structural measures in combination (model 1) followed by the four measures of plant condition (model 2) and lastly the two measures of local climatic conditions (model 3) also revealed no improvement in the capacity of any of these measures of stand structure, plant condition and climatic conditions to predict the number of seedlings recruited by plants after the La Niña rains in combination for *A. melvillei*, *A. loderi* and *A. carneorum*, with  $r^2$  values ranging from 0.102 to 0.451 ( $p >0.5$  for all) (Appendix 5.6.4). Again, while too few stands of *A. homalophylla* were surveyed to perform similar multiple regressions, a similar lack of relationship as found for the other three threatened species might be expected (Appendix 5.6.4).

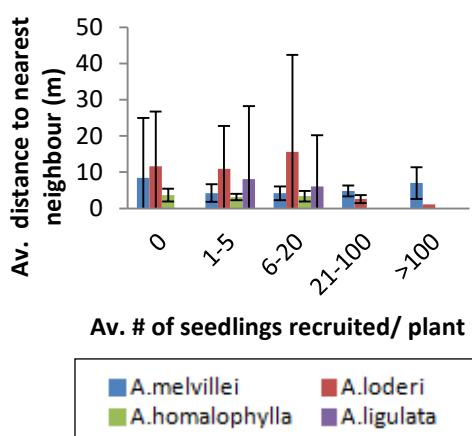
a)



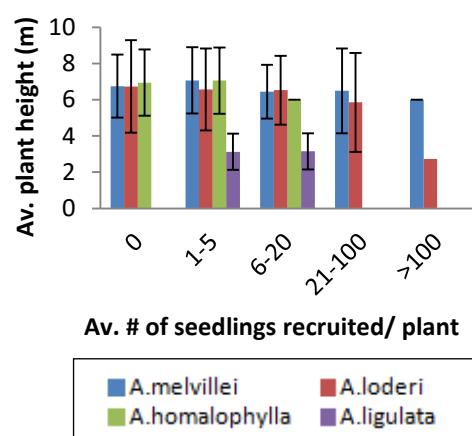
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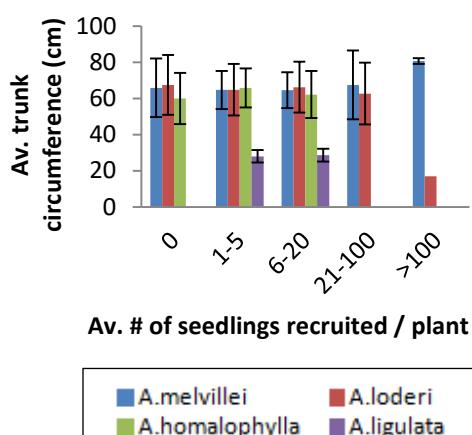
c)



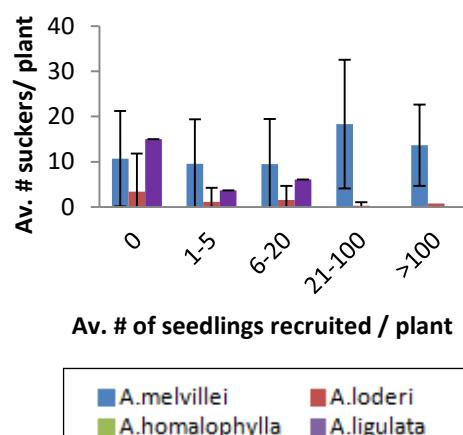
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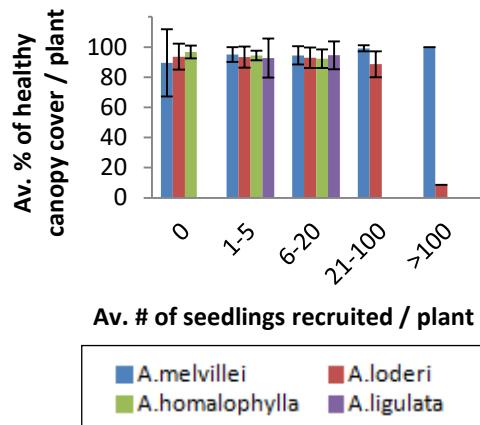
e)



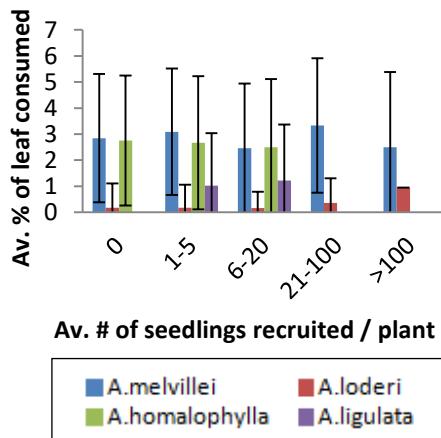
f)



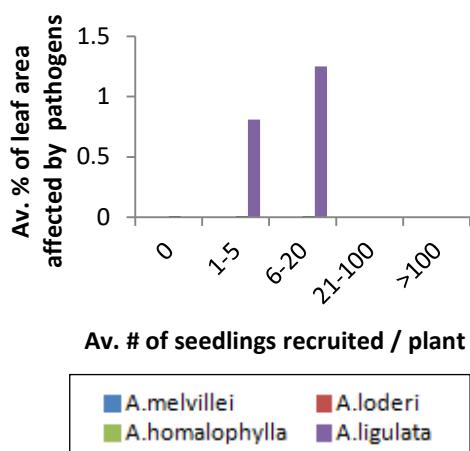
g)



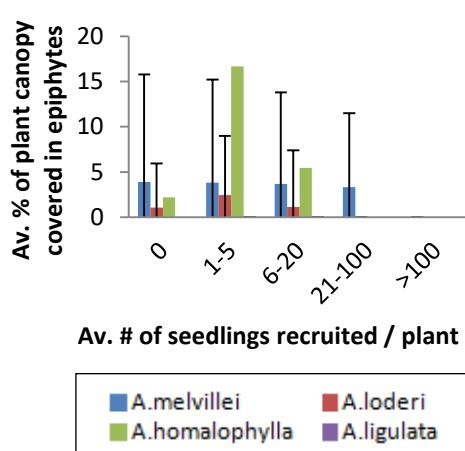
h)



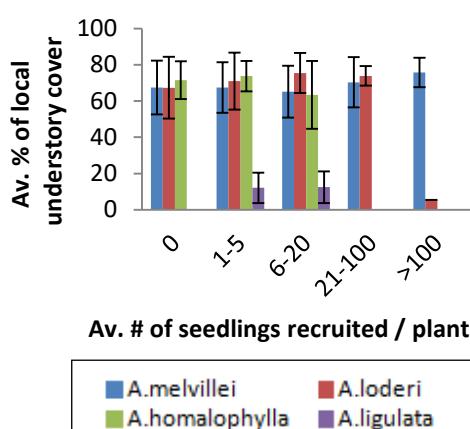
i)



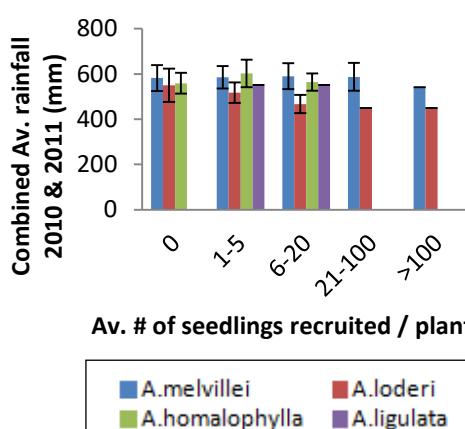
j)

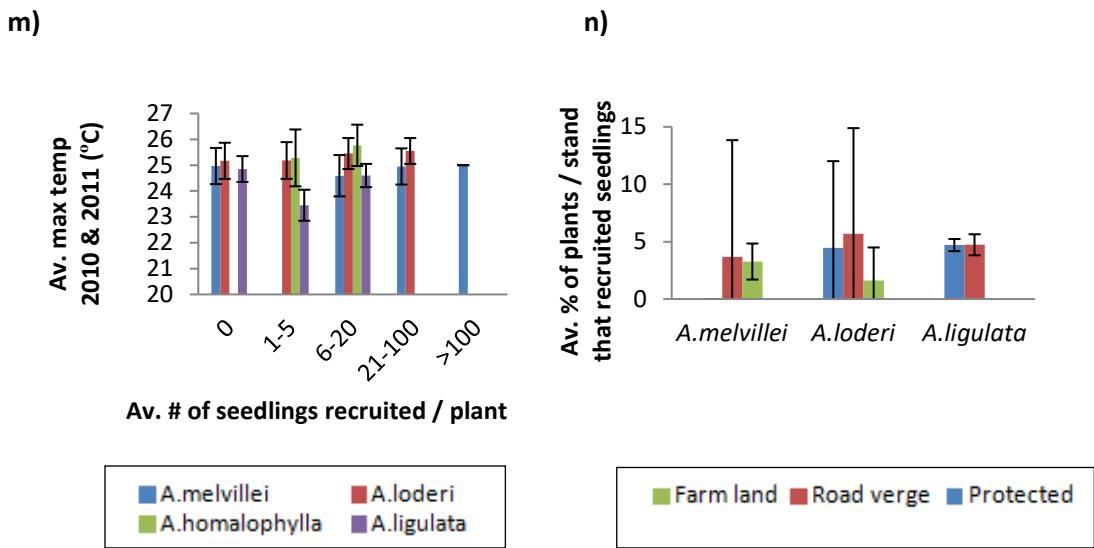


k)



l)





**Figure 5.11. Comparison of local structural and climatic conditions between *Acacia* stands that recruited on average 0, 1-5, 6-20, 21-100 and <100 seedlings per plant:** For n=16, 23, 7, 1 & 0 stands of *A. melvillei*, n= 11, 10, 3, 1 & 0 *A. loderi*, 7, 1, 2, 0 & 0 *A. homalophylla* and 0, 17, 3, 0 & 0 *A. ligulata* stands producing 0, 1-5, 6-20, 21-100 and < 100 seedlings per plant respectively. Condition of stands and local climatic conditions were measured as: a) Size of stands (average # of plants per stand categorized as <10, 11-50, 51-100, 101-200, >201 plants per stand), b) Connectivity of stands (average distance (km) to nearest neighbouring stand categorized as <1, 1-2, 3-4, 5-10, >10 km), c) Average distance to nearest neighbour plant within stands (m), d) Average plant height (m), e) Average trunk circumference (cm), f) Average # suckers / plant, g) Average % of healthy canopy cover / plant, h) Average % of leaf surface area consumed, i) Average % of leaf surface area affected by observable pathogens, j) Average % of plant canopy covered in epiphytes, k) Average % of ground around plants covered in understory vegetation, l) Annual rainfall averaged over 2010 & 2011 (mm), m) Maximum temperatures averaged over 2010 & 2011 (°C), n) Average % of plants / stand that recruited seedlings with respect to the type of land stands were located within; farmland, road verge or protected land.

#### 5.4.5 Assessing the long term performance and survival of recruits.

##### (a) Survival rates (to three years)

Survival rates of seedlings recruiting after the La Niña rain differed greatly between the species with the highest rates found for *A. melvillei*, followed by *A. loderi* and considerably lower survival rates found for *A. ligulata* (Figure 5.12 a). Kaplan-Meier (*Log rank test*) survival analysis confirmed a significant difference among species ( $\chi^2$  (2, n=1541) =356.661, p<0.000). A large amount of variation was observed between the three year survival of seedlings originating in and around the different mature plants surveyed for all three *Acacia* species (Figure 5.12 e), notably more (81-90%) seedlings survived to three years under and around *A.*

*melvillei* plants than the other two species (Appendix 5.6.3). Whilst moderate to low levels of variance in three year survival was found across all seedlings of each species (see SD bars in Figure 5.12 b), the average three year survival of seedlings varied greatly among some stands (Figure 5.12 b), suggesting a stronger effect of stands on seedling mortality than individual plants. Average three year survival ranged from 22.2% ( $SD \pm 0.14$ ) to 94.3% ( $SD \pm 0.07$ ), 14.3% ( $SD \pm 0.01$ ) to 59.49% ( $SD \pm 0.14$ ) and 50.4% ( $SD \pm 0.11$ ) to 100% ( $SD \pm 0.00$ ) among the five *A. melvillei*, *A. loderi* and *A. ligulata* stands respectively. A significant difference between the three year survival of *A. loderi* ( $\chi^2 (4, n=718) = 73.904, p < 0.000$ ) and *A. ligulata* ( $\chi^2 (4, n=244) = 29.357, p < 0.000$ ) seedlings in the five different stands was found, however this was not found for *A. melvillei* ( $\chi^2 (4, n=579) = 4.349, p = 0.361$ ).

When seedlings from the current seed cohort and the older seedlings from the soil stored seed bank were considered separately, a higher proportion of the younger seedlings died over the three year period they were observed, compared to older ones that were more established at the point of tagging (Figure 5.12 a).

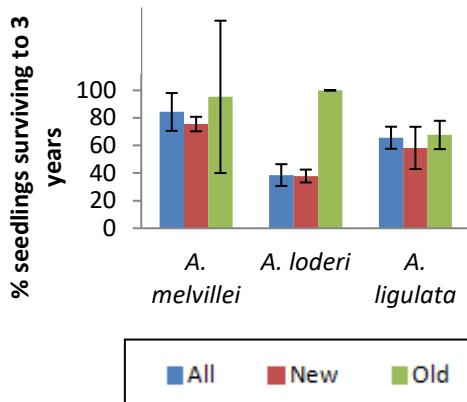
Kaplan-Meier (*Log rank test*) survival analysis revealed that for *A. melvillei*, the survival rates of seedlings originating from seed banks were significantly higher than those originating from the 2011 canopy seed cohort ( $\chi^2 (1, n=579) = 21.829, p < 0.001$ ). Whilst too few *A. loderi* seedlings were found to have originated from the soil stored seed bank to perform a similarly robust statistical comparison, all seven *A. loderi* seedlings originating from the soil stored seed bank survived until the final check.

The variance in three year survival rates of the average seedlings originating from the canopy cohort mirrored the high levels found for all seedlings combined (Figure 5.12 f & c). Unsurprisingly far less variance between individual plants in the same stand or different stands was seen for the older seedlings, given their very low mortality rates over the period of observation (Figure 5.12 g & d). Kaplan-Meier (*Log rank test*) survival analysis revealed that there was a significant difference in the three year survival rates between seedlings originating from the canopy cohort of *A. melvillei*, *A. loderi* and *A. ligulata* ( $\chi^2 (2, n=1135) = 145.264, p < 0.000$ ) as well as between *A. melvillei* and *A. ligulata* seedlings originating from the soil stored seed bank ( $\chi^2 (1, n=406) = 64.067, p < 0.001$ ).

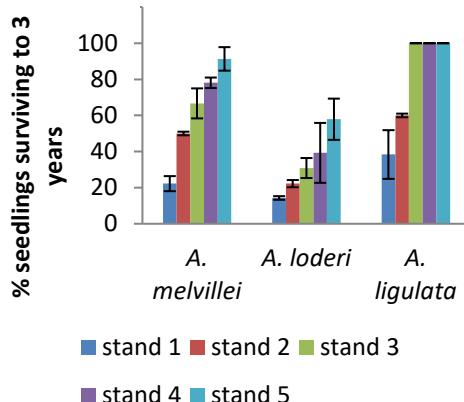
While a significant difference in the three year survival rates of seedlings originating from the canopy cohort of 2011 was found between the five stands of *A. loderi* ( $\chi^2 (4, n=713) = 18.488, p < 0.001$ ) and *A. ligulata* ( $\chi^2 (4, n=115) = 13.064, p < 0.011$ ), the survival rates of

seedlings was equivalent between the five *A. melvillei* stands. No significant difference was found in the three year survival rates of seedlings originating from the soil stored seed bank, between the five stands of *A. melvillei* ( $\chi^2$  (4, n=272) =0.101, p<0.951) or the five stands of *A. ligulata* ( $\chi^2$  (4, n=134) =6.458, p=0.167) surveyed.

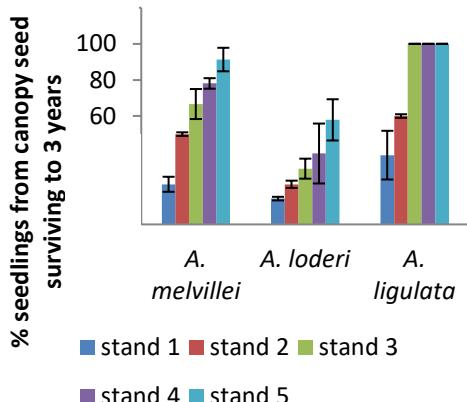
a)



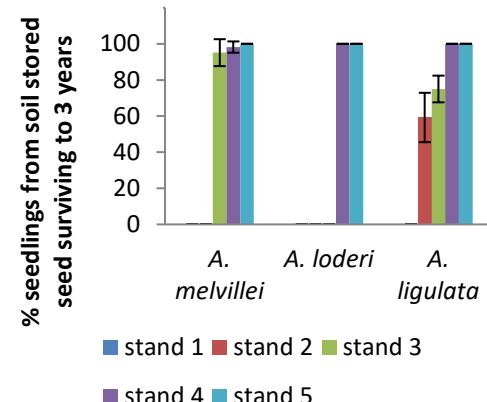
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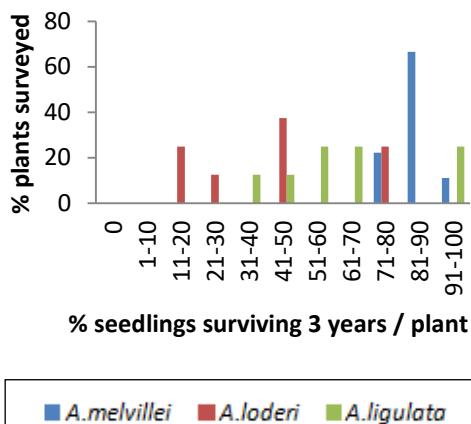
c)



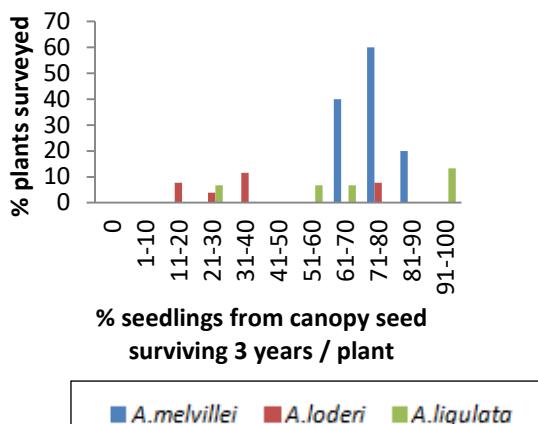
d)



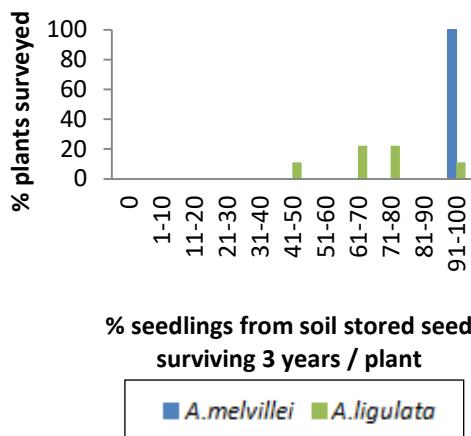
e)



f)



g)



**Figure 5.12. Assessments of the long term performance and survival of naturally occurring sexual recruits under 30 *A. melvillei*, *A. loderi* and *A. ligulata* plants spread across five separate stands:** a) % of seedlings originating from the 2011 canopy seed cohort ( $n=307, 713, 115$ ), seed from the soil stored seed bank ( $n= 272, 6, 134$ ) and all seedlings irrespective of origin ( $n=580, 719, 245$ ), for *A. melvillei*, *A. loderi* and *A. ligulata* respectively, surviving to a minimum of three years (standard deviation bars represent variance between five stands), b) % of *A. melvillei* ( $n= 101, 433, 35, 9, 2$ ), *A. loderi* ( $n= 385, 18, 230, 79, 7$ ) and *A. ligulata* ( $n=5, 7, 34, 143, 53$ ) seedlings in each of five stands respectively surviving 3 years of age (standard deviation bars represent variance between six plants per stand), c) % of *A. melvillei* ( $n= 45, 228, 23, 9, 2$ ), *A. loderi* ( $n= 302, 18, 230, 76, 10$ ) and *A. ligulata* ( $n=5, 2, 28, 73, 10$ ) seedlings originating from canopy seed cohorts in each of five stands respectively surviving to three years of age (standard deviation bars represent variance between six plants per stand), d) % of *A. melvillei* ( $n= 57, 213, 12, 0, 0$ ), *A. loderi* ( $n= 3, 3, 0, 0, 0$ ) and *A. ligulata* ( $n=0, 5, 6, 77, 61$ ) seedlings originating from soil stored seed cohorts in each of five stands respectively surviving to three years of age (standard deviation bars represent variance between six plants per stand).

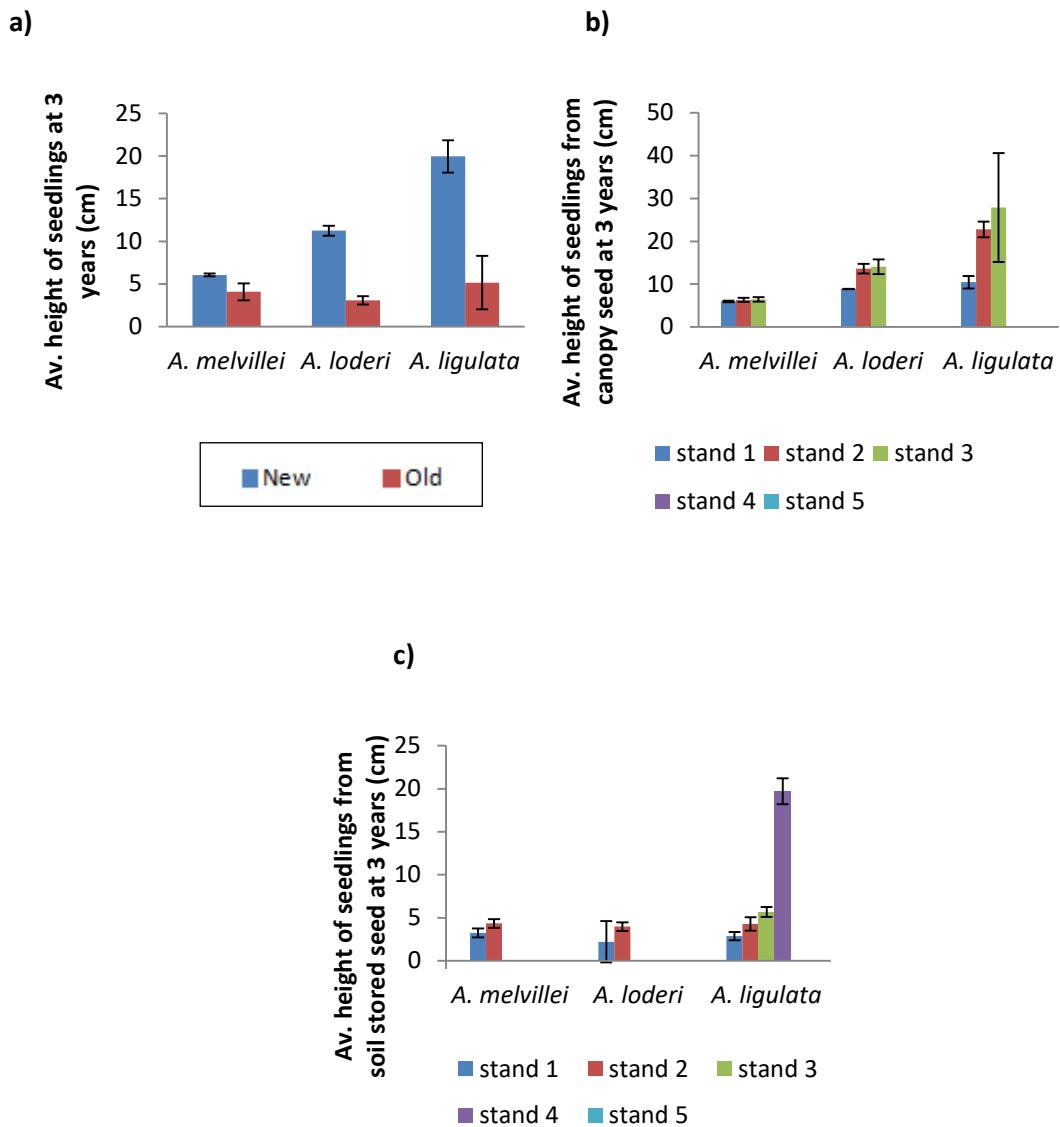
stand), e), f), g) % of plants with respect to the % (0%, 1-10%, 11-20%, 21-30%, 31-40%, 41-50%, 51-60%, 61-70%, 71-80%, 81-90%, 91-100%) of all seedlings, seedlings originating from canopy seed cohort and seedlings originating from soil stored seed cohort respectively, recruited under them surviving to three years of age.

b) Growth

Natural growth rates of seedlings in the absence of herbivory were unable to be conclusively determined given the exposure of all seedlings to grazers, which in most instances reduced their height. Nevertheless, as a likely consequence of lesser grazing pressures on seedlings originating from canopy seed emerging later than those from the seed bank, Kruskal-Wallis tests revealed that seedlings originating from seed banks were significantly shorter on average than those originating from the 2011 canopy seed cohort at the final check for *A. melvillei* ( $\chi^2$  (1, n=579) =98.715, p<0.000), and *A. ligulata* seedlings ( $\chi^2$  (1, n=244) =57.343, p<0.000). The few *A. loderi* seedlings originating from the soil stored seed bank were also shorter compared to the many originating from the 2011 canopy seed cohort (Figure 5.13 a). Whilst little variance was generally found between the heights of seedlings recruited under the different plants surveyed for all three species (see SD bars in Figure 5.13 b & c), moderate levels of variance for *A. melvillei* and *A. loderi* and noticeably higher levels of variance for *A. ligulata* were found between the five different stands surveyed (Figure 5.13 b & c).

While Kruskal-Wallis tests revealed that there was a significant difference in the height of seedlings originating from the canopy seed cohort at three years of age among species ( $\chi^2$  (2, n=1135) =139.064, df=2, p<0.001), no such difference was found for seedlings originating from the soil stored seed bank. Mann-Whitney U tests revealed that on average new *A. ligulata* seedlings were significantly taller than new *A. loderi* seedlings, which were in turn significantly taller than new *A. melvillei* seedlings at the final check (Figure 5.13 a, Appendix 5.6.3).

Significant differences were found in the height of seedlings originating from the canopy cohort within the five stands of *A. loderi* ( $\chi^2$  (4, n=713) =18.488, p<0.000) and *A. ligulata* ( $\chi^2$  (4, 115) =13.064, p=0.011), however the height of *A. melvillei* seedlings originating from the canopy cohort were not significantly different among the five stands (Figure 5.13 b, Appendix 5.6.3). The height of seedlings originating from the soil stored seed bank were at final check significantly different in the five *A. melvillei* stands ( $\chi^2$  (4, 272) =20.086, df=4, p<0.000) and five *A. ligulata* stands ( $\chi^2$  (4, 134) =25.154, p<0.000) surveyed (Figure 5.13 c)

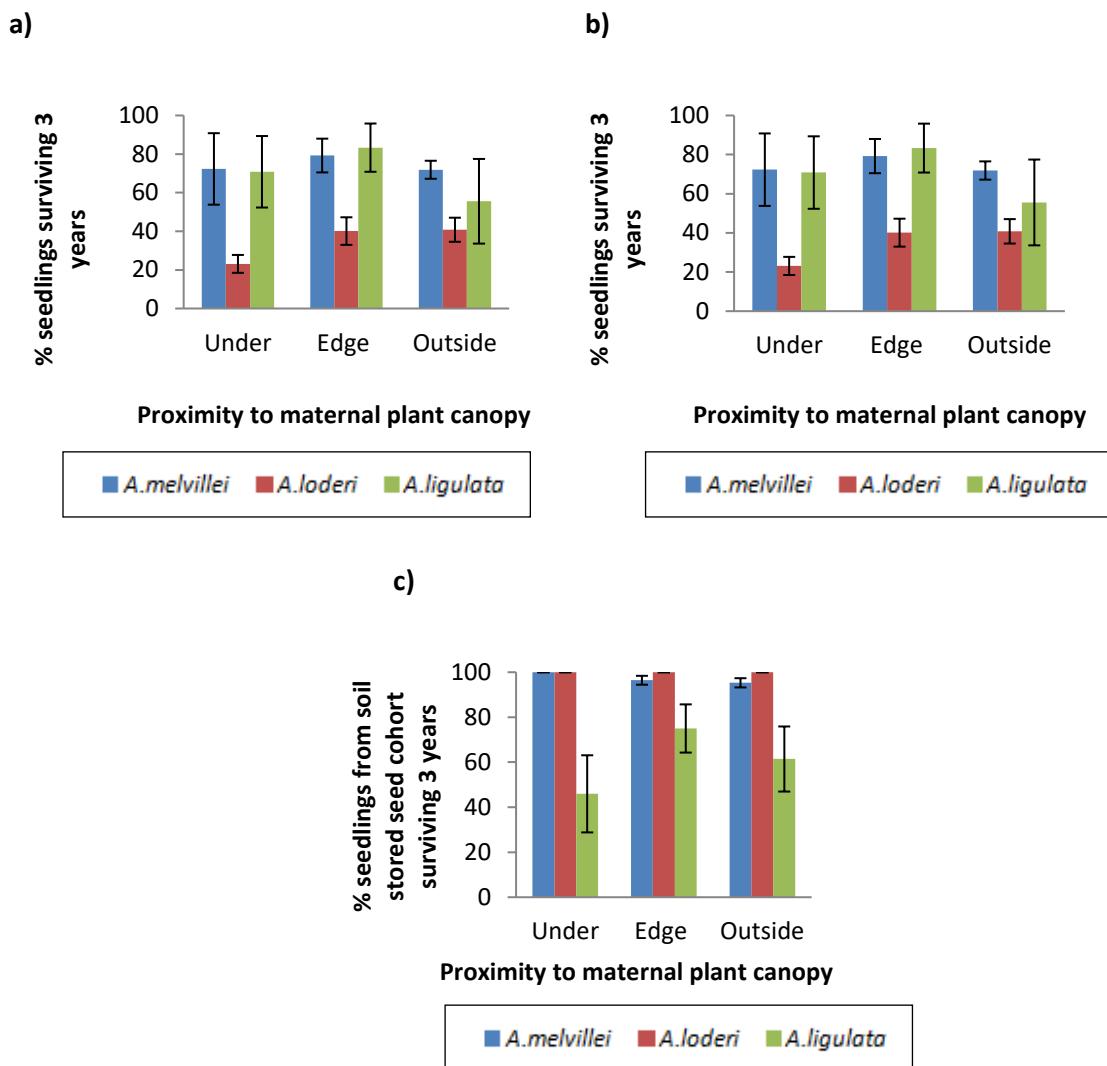


**Figure 5.13. Average height of seedling recruits located under 30 *A. melvillei*, *A. loderi* and *A. ligulata* plants spread across five separate stands:** a) Average height of seedlings at final check originating from the 2011 canopy seed cohort ( $n=307, 713, 115$ ), seed from the soil stored seed bank ( $n= 272, 6, 134$ ) and all seedlings irrespective of origin ( $n=580, 719, 245$ ), for *A. melvillei*, *A. loderi* and *A. ligulata* respectively, surviving to a minimum of three years (\*Standard errors represent variance between 5 stands), b) Average height of *A. melvillei* ( $n=45, 228, 23, 9, 2$ ), *A. loderi* ( $n= 302, 18, 230, 76, 10$ ) and *A. ligulata* ( $n=5, 2, 28, 73, 10$ ) seedlings originating from canopy seed cohorts in each of five stands respectively (\*Standard errors represent variance between six plants per stand), c) Average height of *A. melvillei* ( $n= 57, 213, 12, 0, 0$ ), *A. loderi* ( $n= 3, 3, 0, 0, 0$ ) and *A. ligulata* ( $n=0, 5, 6, 77, 61$ ) seedlings originating from soil stored seed cohorts in each of five stands respectively (\*Standard errors represent variance between six plants per stand).

#### **5.4.6 Driving factors for long term survival of recruits**

##### **a) Proximity to canopy shade**

Kaplan-Meier (*Log rank test*) survival analysis revealed a significant effect of proximity to the canopy of maternal plants on seedling survival for *A. loderi* seedlings ( $\chi^2$  (2, n=718) = 37.394,  $p<0.001$ ), with lower rates of survival of seedlings located under the canopy of mature trees than either at the edge or outside their canopies (Figure 5.14 a, Appendix 5.6.4 a). Whilst no significant differences were found between the survival rates of all *A. melvillei* or *A. ligulata* seedlings located in the three different zones with respect to mature plant's canopies (Figure 15.14 a, Appendix 5.6.4 a), for *A. ligulata* seedlings originating from the soil stored seed bank, a significant effect of proximity to mature plants on their three year survival rates was found ( $\chi^2$  (2, n=134) = 9.886,  $p=0.007$ ). *A. ligulata* seedlings located under the canopy were less likely to survive on average compared to those located at the edge or outside a canopy (Figure 5.14 c, Appendix 5.6.4 a). Differences in the survival rates of *A. loderi* seedlings with respect to their proximity to mature plants were driven by differences in survival rates of the younger seedlings originating from canopy seed alone given that all of the seven older seedlings from the soil stored seed bank survived to three years irrespective of their proximity to the canopy of plants (Figure 5.14 b, Appendix 5.6.4 a). The effect of proximity to mature plants on the survival of *A. ligulata* seedlings originating from the soil stored seed bank was driven by significantly lower survival rates of seedlings located under the canopy of mature plants than at the edge or outside the canopy (Figure 5.14 c, Appendix 5.6.4 a).



**Figure 5.14. Assessments of the long term performance and survival of naturally occurring sexual recruits under 30 *A. melvillei*, *A. loderi* and *A. ligulata* plants spread across five separate stands with respect to their proximity to the canopy of mature plants:** a) % of *A. melvillei* (n= 155, 191, 234), *A. loderi* (n= 218, 230, 271) and *A. ligulata* (n=62, 44, 160) seedlings located under, at the edge and outside the canopy cover of mature plants respectively surviving three years of age from five stands (\*Standard errors represent variance between stands), b) % of *A. melvillei* (n= 134, 108, 65), *A. loderi* (n= 214, 231, 268) and *A. ligulata* (n=26, 21, 68) seedlings originating from canopy seed cohorts located under, at the edge and outside the canopy cover of mature plants respectively surviving three years of age, from five stands (\*Standard errors represent variance between stands), c) % of *A. melvillei* (n= 21, 85, 169), *A. loderi* (n= 2, 1, 4) and *A. ligulata* (n=49, 24, 70) seedlings from originating from soil stored seed cohorts located under, at the edge and outside the canopy cover of mature plants respectively surviving three years of age, from five stands (\*Standard errors represent variance between stands).

Kruskal-Wallis tests revealed a significant effect of the proximity to mature plants on the heights of *A. melvillei* ( $\chi^2 (2, 307) = 33.687$ ,  $p < 0.001$ ) and *A. ligulata* seedlings ( $\chi^2 (2, n=115) = 10.644$ ,  $p=0.005$ ) originating from the canopy cohort of 2011, as well as for *A. melvillei* ( $\chi^2 (2, 272) = 10.297$ ,  $p=0.006$ ) and *A. ligulata* ( $\chi^2 (2, 134) = 10.309$ ,  $p=0.006$ ) seedlings originating from the soil stored seed bank. No such difference was found for the *A. loderi* seedlings originating from the 2011 canopy seed cohort (Figure 5.15 a, Appendix 5.6.4 a). Mann-Whitney U tests revealed the heights after three years of *A. melvillei* seedlings originating from the 2011 canopy seed were on average significantly smaller when located under the canopy of plants than those located at the edge or outside the canopy (Figure 5.15 a, Appendix 5.6.4 a). Whilst for *A. melvillei*, the height of these seedlings increased steadily with distance away from the canopy, for *A. loderi*, seedlings at the edge were marginally taller than those outside the canopy, which were in turn significantly taller than those located under the canopy. In contrast the younger *A. ligulata* seedlings originating from the 2011 canopy cohort which were located outside the canopy were significantly smaller than those located under or at the edge of the mature plant canopies (Figure 5.15 a, Appendix 5.6.4 a). The average heights of the older *A. ligulata* seedlings originating from the soil stored seed bank followed a similar trend to the younger seedlings (Figure 5.15 b, Appendix 5.6.4 a). The older *A. melvillei* seedlings located outside the canopy were on average proportionally significantly smaller than those under and at the edge of canopies contrasting with the younger seedlings originating from the canopy seed (Figure 5.15 b).

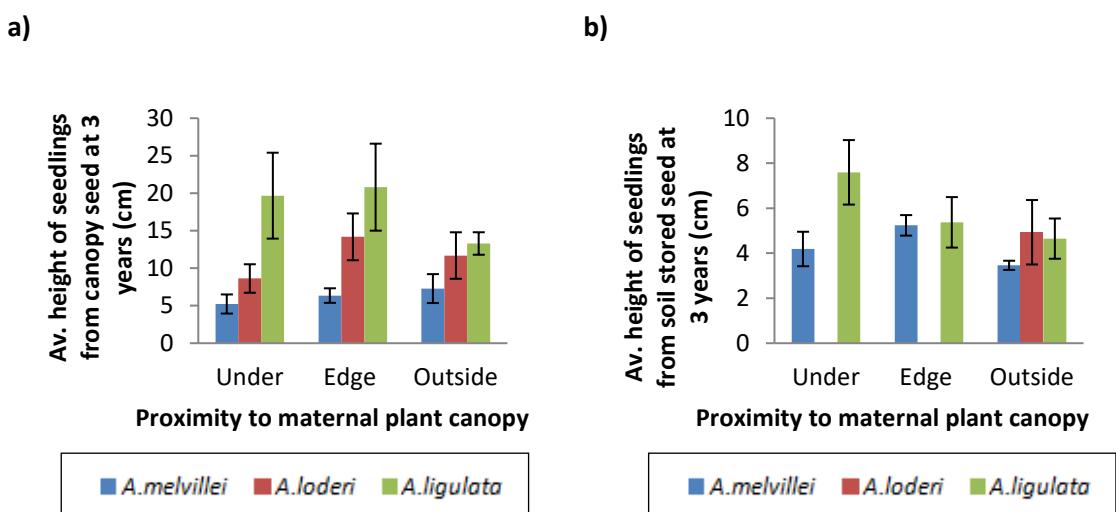


Figure 5.15. Quantifying the growth rates of naturally occurring sexual recruits under 30 *A. melvillei*, *A. loderi* and *A. ligulata* plants spread across five separate stands with respect to

**their proximity to the canopy of mature plants:** a) Average height *A. melvillei* (n= 134, 108, 65), *A. loderi* (n= 214, 231, 268) and *A. ligulata* (n=26, 21, 68) seedlings originating from the canopy seed cohort located under, at the edge and outside the canopy cover of mature plants respectively across five stands at final check, b) Average height of *A. melvillei* (n= 21, 85, 169), *A. loderi* (n= 2, 1, 4) and *A. ligulata* (n= 49, 24, 70) seedlings originating from the soil stored seed cohort located under, at the edge and outside the canopy cover of mature plants respectively across five stands at final check. \*Standard errors represent variation between the heights of the five different stands.

b) Association with understory nurse plants

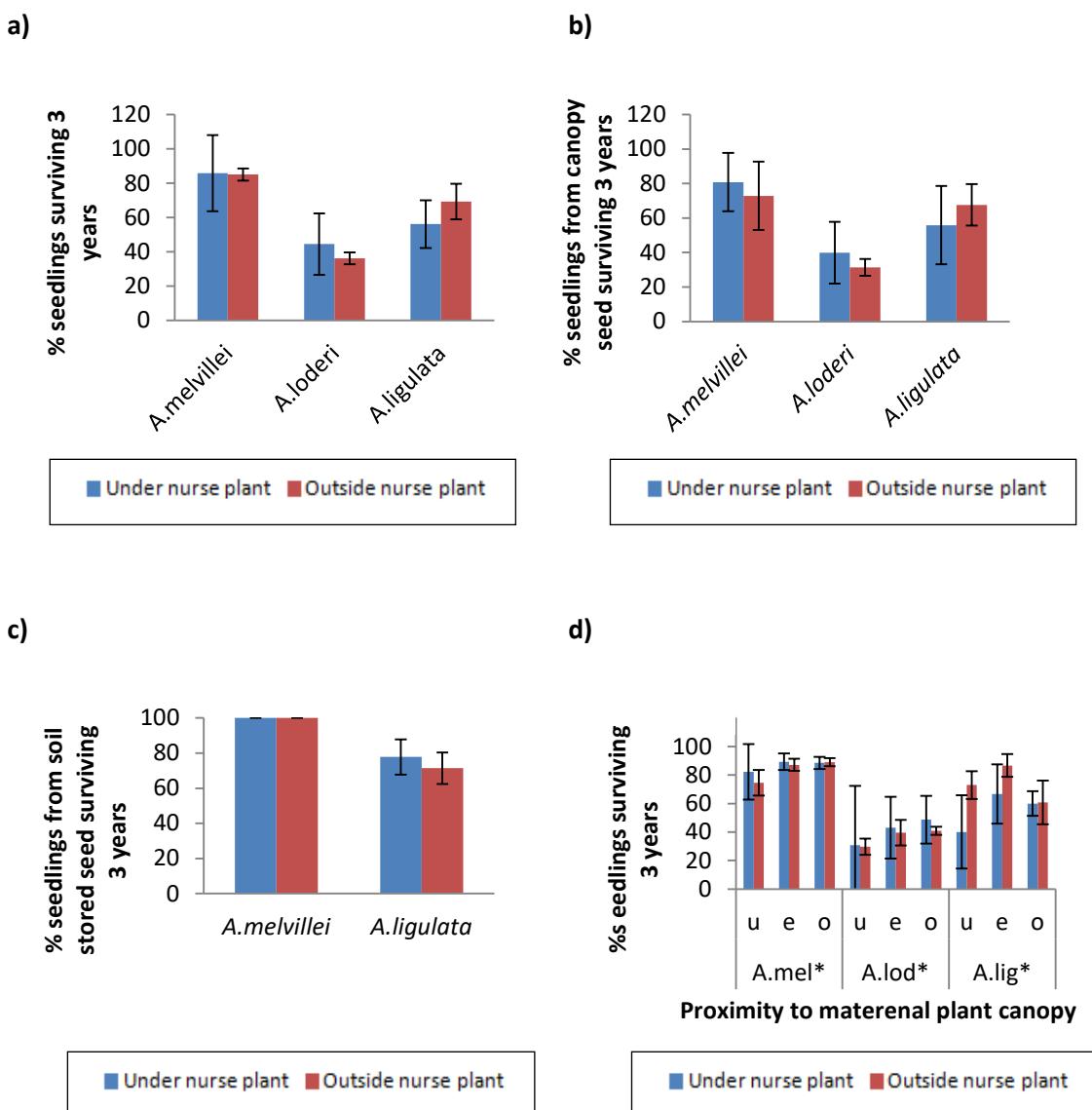
Kaplan-Meier (*Log rank test*) survival analysis revealed a significant effect of proximity to nurse plants on three year survival rates of *A. loderi* seedlings ( $\chi^2$  (1, n=718) = 34.398, p<0.001) with lower rates of survival for seedlings located outside the cover of nurse plants (Figure 5.16, Appendix 5.6.4 b). This same pattern was found when seedlings originating from the canopy cohort of 2011 and those from the soil seed bank were analysed separately. However, too few *A. loderi* seedlings were determined to have originated from the soil stored seed bank to run statistical tests (Figure 5.16 b & c, Appendix 5.6.4 b). No significant effects of nurse plants on survival were found for the other two species (Appendix 5.6.4 b).

A significant survival advantage for seedlings under nurse plants, irrespective of the proximity of seedlings to mature plants, was found for *A. loderi* seedlings ( $\chi^2$  (5, n= 718) = 58.095, p<0.001) (Figure 5.16 d), with lower rates of survival for seedlings located outside the cover of nurse plants and also outside the canopy cover of mature plants (Figure 5.16 a, Appendix 5.6.4 b). There was no significant survival advantage associated with proximity to nurse plants, irrespective of the proximity of seedlings to the canopy of mature plants, for *A. melvillei* or *A. ligulata* seedlings.

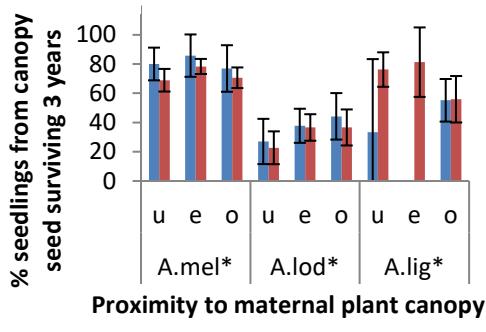
When seedlings classed as being under or outside a nurse plants protection were further partitioned into those that were located under, at the edge and outside the canopy of mature plants, I found that the significantly higher survival rates of *A. loderi* seedlings under the protection of nurse plants, was driven solely by those located also outside the canopy of mature plants (Figure 5.16 d). In contrast, survival rates of *A. melvillei* seedlings remained similar irrespective of their location (Figure 5.16 a). *A. ligulata* seedlings located outside the protection of nurse plants had marginally higher survival rates than those under the protection of a nurse plant if located at the edge of a mature plants canopy cover. Those located outside the protection of a nurse plant but under the canopy of a mature plant had noticeably higher

survival rates than those located under the canopy of mature plants and also under a nurse plant (Figure 5.16 d).

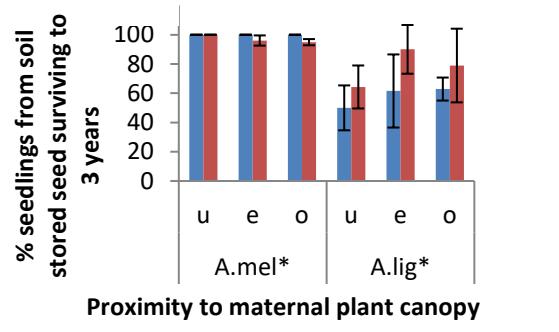
When seedlings were further partitioned into those that originated from the 2011 canopy seed cohort, and those from the soil stored seed bank, noticeably higher rates of survival were found for *A. melvillei* seedlings originating from the 2011 canopy seed cohort that were located under the protection of nurse plants irrespective of their position in relation to mature plants (Figure 5.16 e, Appendix 5.6.4 b). This was not evident for those *A. melvillei* seedlings originating from the soil stored seed bank (Figure 5.16 f).



e)



f)



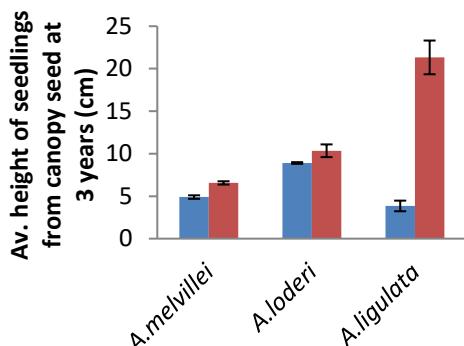
**Figure 5.16. Assessments of the long term performance and survival of naturally occurring sexual recruits under 30 *A. melvillei*, *A. loderi* and *A. ligulata* plants spread across five separate stands positioned in relation to understory vegetation:** a) % of *A. melvillei* (n=99, 481), *A. loderi* (n=245, 472) and *A. ligulata* (n= 106, 140) seedlings located under and outside ‘nurse’ plants respectively surviving three years of age from five stands (\*Standard errors represent variance between stands), b) % of *A. melvillei* (n= 76, 307), *A. loderi* (n=243, 470) and *A. ligulata* (n=35, n=115) seedlings originating from canopy seed cohorts located under and outside ‘nurse’ plants respectively surviving 3 years of age from five stands (\*Standard errors represent variance between stands), c) % of *A. melvillei* (n=22, 252), *A. loderi* (n=5, 2) and *A. ligulata* (n= 70, 59) seedlings from originating from soil stored seed cohorts located under and outside ‘nurse’ plants respectively surviving three years of age, from five stands (\*Standard errors represent variance between stands), d) % of *A. melvillei* (n=45 & 110, 28 & 163, 25 & 209 ), *A. loderi* (n=39 & 179, 58 & 172, 150 & 121) and *A. ligulata* (n= 25 & 37, 15 & 30, 65 & 74) seedlings located under and outside ‘nurse’ plants in turn located under, at the edge and outside the canopy cover of mature plants respectively, surviving to three years of age from five stands (\*Standard errors represent variance between stands), e) % of *A. melvillei* (n=93 & 91, 21 & 87, 13 & 52), *A. loderi* (n= 39 & 177, 57 & 172, 147 & 121) and *A. ligulata* (n= 3 & 23, 2 & 19, 30 & 38) seedlings originating from canopy seed cohorts, located under and outside ‘nurse’ plants in turn located under, at the edge and outside the canopy cover of mature plants respectively, surviving to three years of age from five stands (\*Standard errors represent variance between stands), f) % of *A. melvillei* (n= 2 & 19, 7 & 76, 12 & 157) and *A. ligulata* (n=22 & 14, 13 & 11, 35 & 35) seedlings originating from soil stored seed cohorts, located under and outside ‘nurse’ plants in turn located under, at the edge and outside the canopy cover of mature plants respectively, surviving to three years of age from five stands (\*Standard errors represent variance between stands.)

Kruskal-Wallis tests revealed a significant effect of proximity to nurse plants on the height of *A. melvillei* ( $\chi^2$  (1, n=307)= 13.537, p<0.001) and *A. ligulata* seedlings ( $\chi^2$  (1, n=115)= 22.419, p<0.001) originating from the 2011 canopy seed cohort, as well as *A. melvillei* ( $\chi^2$  (1, n=272) = 18.520, p<0.001) and *A. ligulata* seedlings ( $\chi^2$  (1, 134) = 10.731, p=0.001) originating

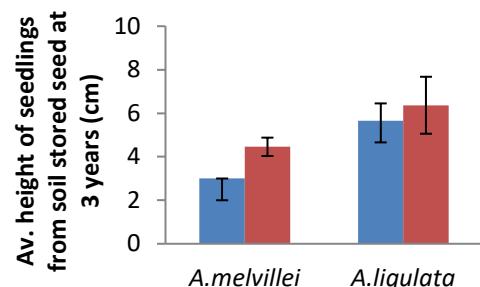
from the soil stored seed bank, at the final check. In contrast *A. loderi* seedlings were no different in height with respect to their position in relation to nurse plants (Figure 5.17 a, Appendix 5.6.4 b). Mann-Whitney U tests revealed that seedlings of all three species originating from the 2011 canopy seed cohort were significantly taller when located outside the protection of a nurse plant, than when under the protection of a nurse plant (Figure 5.17 a, Appendix 5.6.4 b). Similar significantly taller *A. melvillei* and *A. ligulata* seedlings originating from the soil stored seed cohort were found located outside the protection of a nurse plant compared with *A. melvillei* and *A. ligulata* seedlings located under the protection of a nurse plant (Figure 5.17 b, Appendix 5.6.4 b).

When the effect of nurse plants and mature canopy on the height of seedlings was considered, being located under a nurse plant reduced the average height of seedlings regardless of their proximity to mature plants and regardless of the original of the seedlings for *A. melvillei* and *A. ligulata* (Figure 5.17 c & d). Whilst for *A. melvillei* this effect was strongest for seedlings located outside the canopy of mature plants when they originated from the 2011 canopy seed, for seedlings originating from the soil stored seed bank, the effect was strongest for those located at the edge of a mature plant's canopy. *A. ligulata* seedlings outside the protection of nurse plants grew equivalently tall irrespective of their position to mature plants. I also found *A. loderi* seedlings originating from the 2011 canopy seed cohort to be taller after three years if located outside the protection of a nurse plant, which was located under the canopy of a mature plant, but this was reversed if found outside the canopy (as a likely result of higher grazing rates) (Figure 5.17 c).

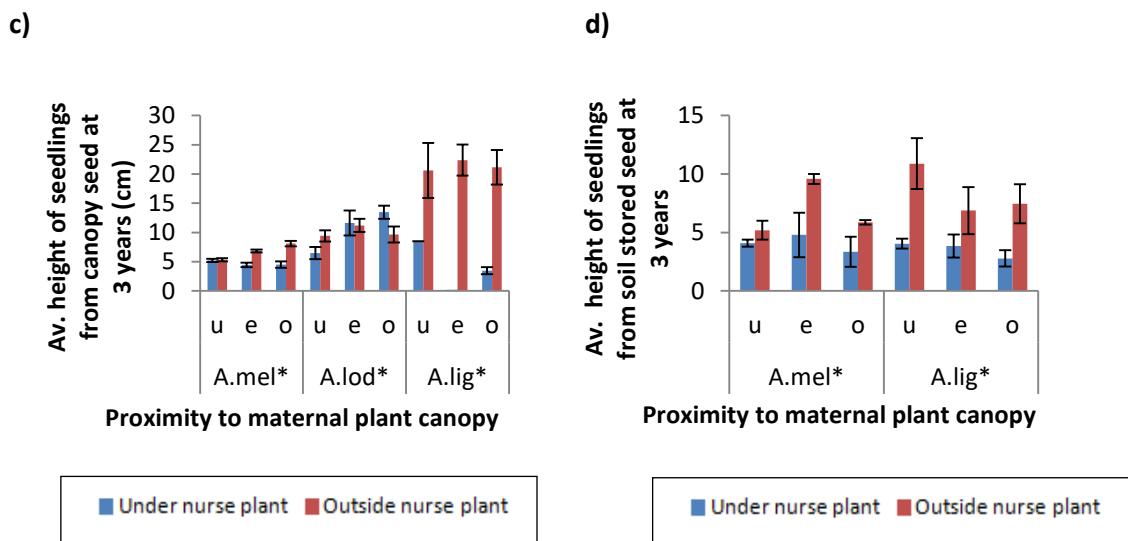
a)



b)



■ Under nurse plant ■ Outside nurse plant



**Figure 5.17. Assessments of the of the height of naturally occurring sexual recruits under 30 *A. melvillei*, *A. loderi* and *A. ligulata* plants spread across five separate stands positioned in relation to understory vegetation:** a) Average height *A. melvillei* (n= 76, 307), *A. loderi* (n=243, 470) and n= *A. ligulata* (n=35, n=115) seedlings originating from canopy seed cohorts located under and outside ‘nurse’ plants respectively across five stands at final check (\*Standard errors represent variation between the average grazing levels of the five different stands), b) Average height of *A. melvillei* (n=22, 252 ), *A. loderi* (n=5, 2) and *A. ligulata* (n= 70, 59) seedlings originating from soil stored seed cohorts located under and outside ‘nurse’ plants respectively across five stands at final check (\*Standard errors represent variation between the average grazing levels of the five different stands), c) Average height *A. melvillei* (n=93 & 91, 21 & 87, 13 & 52), *A. loderi* (n= 39 & 177, 57 & 172, 147 & 121) and *A. ligulata* (n= 3 & 23, 2 & 19, 30 & 38) seedlings originating from canopy seed cohorts located under and outside ‘nurse’ plants in turn located under, at the edge and outside the canopy cover of mature plants respectively across five stands at final check (\*Standard errors represent variation between the average grazing levels of the five different stands), d) Average height of *A. melvillei* (n= 2 & 19, 7 & 76, 12 & 157), *A. loderi* (n=0 & 2, 1& 0, 3 & 0) and *A. ligulata* (n=22 & 14, 13 & 11, 35 & 35) seedlings originating from soil stored seed cohorts located under and outside ‘nurse’ plants in turn located under, at the edge and the canopy cover of mature plants respectively across five stands at final check (\*Standard errors represent variation between the average grazing levels of the five different stands).

## 5.5 Discussion

### Evaluating the recruitment potential of stands

My finding of sexual recruitment in the majority of these threatened semi arid *Acacia* species emphasises the importance of either conducting very long term studies, or seizing the opportunity to survey populations under apparently optimal conditions, when estimating recruitment in populations of long lived arid plants. Along with my finding that most seeds

produced by these plants were viable, these data highlight the potential of many remaining stands of most of these long lived overstory species to generate sexual recruits, despite their highly fragmented conditions, which is encouraging from a conservation viewpoint. Nevertheless, given my finding that seed were unlikely to all be dormant, the total lack or minimal recruitment in many stands that produced large amounts of fruit, suggests local conditions are affecting the capacity of stands to recruit.

The total lack of recruitment in any of the *A. carneorum* stands surveyed was not altogether surprising, given the very low levels of seed set (Chapter 3, thesis), and this supports genetic and carbon dating data that suggests sexual recruitment is at best likely to be a very rare event (Auld & Denham, 2001; O'Brian et al., 2014; Roberts et al, *in review*; Chapter 9, thesis). Whilst I could not find any evidence that the structure and condition of stands and plants could wholly predict which stands would and would not recruit, or which would recruit the most, positive correlations between the number of recruits and stand size for *A. melvillei* and *A. loderi* and number of suckers for *A. homalophylla*, as well as a negative correlation with the distance between *A. ligulata* stands may provide some predictive power.

### **5.5.1 Assessment of the quality of seed produced post a La Niña rain fall event**

My findings that the majority of the sampled seed from all five *Acacia* species was overwhelmingly viable, disproves previous speculation that mating systems may have been compromised beyond a point where viable seed might be expected to be produced. My finding that the viability and survival rates of seed from the threatened *Acacia* species were significantly higher than for *A. ligulata* seed and seedlings sourced from the same region, was surprising since there is an abundance of this species across the region. Further to this, my finding that the fitness of offspring did not differ significantly between the many stands from which they were surveyed, irrespective of how small / isolated of they were, was also surprising. However, as my tests of offspring fitness were conducted under relatively benign coastal conditions, caution should be taken in interpreting these results. Seed / seedling cohorts from different plants that seem equally fit under non stressful conditions may reveal significant differences in fitness when placed under stressful conditions likely to be felt in their natural arid environment.

While my investigation into the quality of the seed produced by the threatened species revealed high levels of seed parasitism, the high numbers of viable seed produced on *A.*

*melvillei*, *A. homalophylla* and *A. loderi* plants meant that there were theoretically large numbers of viable seed available for recruitment. In contrast however, with only a few seed produced in some rare *A. carneorum* stands, the overall number of viable seed available for recruitment of *A. carneorum* seedlings was far less , even though seed viability rates were equivalent with *A. melvillei*, *A. homalophylla* and *A. loderi*. It is easy to assume that such low numbers of seed represents sub optimal reproduction, but it is possible that these numbers represent suitable levels for a species that has recruited almost exclusively through asexual reproduction (suckering) historically, and likely requires little recruitment by way of sex (Auld, 1993; Auld & Denham, 2001; O'Brian et al., 2013; Roberts et al., in review; Chapter 9, thesis).

Allocating a proportion of seed to a soil stored seed bank can bet hedge, against situations where water from huge rain events may not persist in the landscape long enough to support seedlings long term, and provides plants with multiple chances to recruit (Slatkin, 1974; Seger & Brockman, 1987; Roff, 2002; Evans et al., 2007). The proportion of a seed cohort that is dormant and destined for the soil stored seed bank versus the proportion that is allocated for immediate germination can reveal important information about the reproductive strategy of species, and the challenges their natural environments impose on reproduction (Venable & Lawlor, 1980; Cohen, 1966; Kemp, 1989; Thompson, 2000). In harsh environments such as those of arid and semi arid Australia, germination comes with risk, as rainfall that is sufficient for germination may be insufficient for seedling establishment (Jurado & Westoby, 1992). Many arid zone species, including Australian *Acacia* spread the risk of germination by keeping a high proportion of dormant seeds within the seed bank to avoid depletion following a single large rainfall event (Grice & Westoby, 1987; Jurado & Westoby, 1992; Phillipi, 1993; Auld, 1995; Ooi et al. 2009). The finding that less than 30% of seed set by *A. melvillei* and *A. loderi* plants was dormant seed available for a long lived soil stored seed bank, suggests that the strategy of these species is to recruit new seedlings as soon as conditions are suitable. However, it was clear from my seed burial experiments that a small portion of dormant *A. melvillei* and *A. loderi* seeds can remain viable for several years in the ground. This concurs with previous studies that show *A. loderi* seed can last up to 13 years in the seed bank and play an important role in the life history of these species (Auld, 1995). As such we should not underestimate the importance of such seed banks in the overall reproductive strategy of these *Acacia* species, or the potential contribution of soil stored seed to the recruitment I observed across the landscape. Indeed, seed in the soil that is available to germinate as soon as sufficient rain falls may be better placed to take full advantage of the conditions / water resources than those that are set after a rain event, and are delayed in recruiting by

comparison (Lesica & Steele, 1994; Miller et al., 2004; Shefferson et al., 2005; Lesica & Crone, 2007; Shefferson, 2009). The faster a seedling establishes in favourable conditions, the better chance it has of surviving when conditions worsen, especially within an arid environment where access to water for growth is ephemeral (De La Cruz et al., 2008).

### **5.5.2 Survey of recruitment in the field following a La Niña rain fall event**

Complicating assessments of reproductive health of iteroparous plants is the fact that reproduction and recruitment can vary greatly across seasons (Miller et al., 2006). Nevertheless, it might be safe to assume that conditions for seedling recruitment would likely be optimal after a large and sustained scale rain event and the setting of copious amounts of seed as occurred (Letnic & Dickman, 2006; Wardle et al, 2013).

The finding of at least some natural recruitment in the majority of *A. melvillei* and a substantial minority of *A. homalophylla* and *A. loderi* stands after a La Niña rain event, demonstrates that however diminished, the capacity to recruit seedlings still remains in many of these stands. In contrast, the total lack of sexual recruitment in any *A. carneorum* stands suggests that either sexual recruitment is not important for these species anymore, that it is still important but is highly episodic, or that they are in decline.

The significant variance in the levels of recruitment between stands of each of the four *Acacia* species that recruited seedlings, as well as between plants within the same stands of each species, could be viewed as either natural variance that may or may not reverse temporally, or else an indication of varying ground conditions between stands. Without historic data on recruitment rates over multiple decades prior to anthropogenic disturbance of these populations, conclusions about the reproductive health of these stands based on recruitment after one rain event could be misleading. In stands where only a handful of seedlings were detected however, it would be reasonably safe to assume that they are unlikely to contribute to any effective long term recruitment, as the expected mortality rates of seedlings would be high (Smith et al., 1997; Valentine, 1989; De La Cruz et al., 2008). Even in stands that have produced the highest numbers of recruits observed, we may not expect long term survival of recruits due to the intense grazing regimes currently in place in the region (Batty & Parsons, 1992; Auld, 1995b; Porteniers, 1998; Auld & Denham, 2001; Porteniers, 2001). Indeed, considering the voracity and numbers of feral grazers in the region, the number of

recruits would theoretically need to be even higher than the numbers produced prior to European settlement to garner the same natural levels of recruitment.

The detection of seedlings that came from a soil stored seed bank within these stands was the first documented evidence of their contribution to the recruitment of these threatened Acacia species. The presence of these recruits could be thought of as a mechanism for these species to take full advantage of major rain events by having seed ready to germinate as soon as water is available, therefore aligning their most vulnerable period of growth with optimal environmental conditions. Indeed, these recruits would be more likely to survive when conditions inevitably become drier as they will be more established and hence more resilient to harsh local conditions, and grazer damage, than those originating from the current canopy cohort.

The presence of seedlings originating from a soil stored seed bank introduces a challenge when trying to determine their contribution to overall recruitment levels. Given the natural variance in seed coat condition expected in any seed cohort, the variance in the depth at which seed would be buried, and the contribution from multiple generations, it is expected that dormant soil stored seed will germinate over a temporally wide range (Gepts, 2004). For this reason, it is possible that my estimates of the proportion of seedling recruits originating from the soil stored seed bank were underestimates given that some soil stored seed may have germinated at the same time as seed from the most recent canopy cohort, and were counted as canopy seed. Nevertheless, my estimates of the contribution from soil stored seed banks in many stands, highlights the importance of soil stored seed in many of these species, even after twenty years of drought.

Whilst many *A. melvillei* and *A. ligulata* seedlings came from the soil stored seed bank, relatively few *A. loderi* seedlings were obviously from a soil stored seed bank ,which may reflect an exhaustion of *A. loderi* seed banks across the region. This might be a reasonable assumption given the lifespan of *A. loderi* seed in soil has been demonstrated to be only up to 13 years (Auld, 1995) and seed may not have been set over the last 20 years (Porteniers, 2001). Whilst it may be that *A. melvillei* seed naturally persist longer within seed banks than those of *A. loderi*, this interspecies difference might also be explained by higher levels of *A. melvillei* seed set historically, by differences in local ground conditions, or simply by interspecies differences in seed coat characteristics.

### **5.5.3 Assessment of the driving factors for initial recruitment success and failure in stands**

Natural variation in recruitment rates between stands of the same species is expected, especially if they are at opposite ends of the species' geographic range. However in relatively closely situated stands, with more comparable topography, local environmental conditions and climate, we would expect similar recruitment rates. This was not found to be the case here however, with relatively close stands producing equivalent numbers of seed displaying vastly different recruitment success. Even vast differences in the physical structure of stands and condition of plants within these stands did not explain this variance, nor did differences in the numbers of fruit set, or any differences in the levels of viable seed produced. This suggests that differences in the quality of seed / seedling cohorts and / or local environmental conditions are the driving force behind these differences.

Differences in the fitness of seed between populations of plants can account for differences in recruitment rates between populations (Whelan et al., 2000; Mustajärvi et al., 2001; Goverde et al., 2002; Aizen & Feinsinger, 2003; Peterson et al., 2008; Andrieu et al., 2009; González-Varo et al., 2010). Despite not finding any obvious differences in the viability / fitness of seeds and seedlings originating from different stands across the region in this study, cryptic differences in their fitness may yet exist. My assessment of seed / seedling fitness conducted within benign coastal conditions may hide any cryptic differences in the fitness of seed which may become far more obvious under harsher semi arid conditions. In few other environments would relatively small weaknesses between seed and seedlings become more obvious than in arid environments where species already survive on a physiological knife edge. Considering the relatively favourable conditions experienced region wide during the period of La Niña rain, roughly equal numbers of recruits in stands where local conditions and levels of seed set were comparable, were expected. Differences in grazing pressures between sites, or differences in the fitness of offspring produced in different stands, are unlikely to explain differences in inter stand recruitment rates, as my observations were made soon after seedlings would have emerged, and while conditions on the ground (water availability) were favourable to seedling survival. More likely, these differences in inter stand recruitment rates are likely to reflect difference in local ground conditions between these stands, such as natural differences in micro-topographical features at different sites, as has been shown to be important for other species (Chauhan et al., 2006; Mayer & Erschbamer, 2011; Tokuoka et al., 2011; Song et al., 2013). Whilst it was not quantified in this study, I observed that many seedlings recruited in ditches in the earth that had clearly been made by cattle or farm equipment.

Some environmental features which are important for effective seed lodging and burial in many other species, such as a high percentage of ground understory vegetation cover within stands (Ludwig et al., 1994; Ludwig & Tongway, 1995; Ludwig & Tongway, 1996; Padilla & Pugnaire, 2006; Gul et al., 2007; Jankju, 2013), did not seem to affect recruitment levels in stands of any of the species studied here. It could be argued however, that this may be a function of the fact that the structure of the understory in these remaining *Acacia* stands is all unnatural. An increase in bare ground and increased edge effects have meant that ephemeral plant species including weeds, from surrounding agricultural lands, can exploit the understory of stands to a degree that might otherwise never occur. Without barriers to this domination by opportunistic species, held back under natural conditions by pre-existing natural understory communities and lower light levels in naturally denser stands, we might expect competition for bare soil to increase. Indeed native forest remnants embedded within agricultural matrices in the rural landscape have been found to be particularly susceptible to invasion (With, 2002; Eschtruth & Battles, 2009; Vila & Ibáñez, 2011). Whilst a proliferation of unnaturally dense undergrowth may not prevent seed from being removed in run off as may occur given bare soil (Dardel et al., 2014), it may ultimately serve to inhibit recruitment by competing for space and resources (Saunders et al., 1991; Hobbs et al., 2003; Shoo & Catterall, 2013; Aguirre-Acosta et al., 2014). Indeed, a depleted understory may be just as detrimental as an unnatural understory comprised of exotic weeds that out compete native seedlings for space and resources (Kearns et al., 1998; Yates et al., 2004).

My finding that recruitment was also highly variable among *Acacia* species, even between plants that produced equivalent numbers of seed and were located in the same stand, suggests that variance in local microhabitats, or topographical features over very small geographic scales may drive different recruitment rates. Indeed, several parentage-analysis studies of other plant species have found that the number of sampled seedlings assigned to different mother trees was typically highly unequal (Aldrich & Hamrick, 1998, Schnabel et al., 1998, Sezen et al., 2007, Nakanishi et al., 2009, Gaino et al., 2010, Hampe et al., 2010). Seedling recruitment has been shown to be affected by differences in local environmental factors acting over a small spatial scale including soil disturbance (Chauhan et al., 2006), soil moisture (Iacona et al., 2010), nutrition (Bisigato & Bertiller, 2004; Iacona et al., 2010; Peng et al., 2011) and local topography (Chauhan et al., 2006; Mayer & Erschbamer, 2011; Tokuoka et al., 2011; Song et al., 2013). Such local micro topographical features as well as the type and quality of soil in the area, would be crucial in determining whether seeds remain and become buried locally or not during periods where run off causes local soil erosion. A more likely

explanation for the variance in recruitment at such a local scale observed here might be a difference in the seed's ability to penetrate the soil, and/ or compete with existing understory vegetation for space (Kearns et al., 1998; Yates et al., 2004). The result of my seed planting experiment lends some support to this theory by highlighting the proportionally higher levels of initial recruitment found for *A. melvillei* and *A. loderi* when seeds were manually assured proper burial, compared with natural recruitment rates. Indeed if we expect that the majority of the viable, unparasitised seed would at least germinate to a detectable point, provided they lodged and were buried sufficiently, then we would expect to have seen far higher levels of recruitment than were observed, even in the most unsuccessful stands. This was apparent in one *A. loderi* stand surveyed here, where recruitment en masse was observed only in patches of ground that contained grooves and divots where seeds moved by water may settle and where water pools.

#### **5.5.4 Assessing the long term survival of recruits**

My finding of large numbers of largely viable seed coupled with noticeable levels of sexual recruitment in some stands was encouraging, however it would be premature to expect that this translates to population replacement or growth. It is still possible, and arguably probable, that seedlings are not fit enough, or local ground conditions too poor, to expect many to survive long term. Many *A. melvillei*, *A. loderi* and *A. ligulata* seedlings that were tagged and monitored, survived and flourished up to at least three years of age, but this was not the case in all stands. In some stands, especially those with low numbers of recruits to begin with, most or all of the recruits died, or were consumed, before three years of age. This highlights the need for high levels of initial recruitment in order to expect any long term recruitment. Indeed, the long term survival of the seedlings observed in this survey cannot be assured because observations were conducted over a very small window in the recruits' journey to adulthood and during relatively benign conditions, rather than in the harsh conditions which will characterize the majority of their future. Whilst for these threatened *Acacia* species, recruitment coincided with a period of water availability that may have ameliorated the worst effects of fragmentation, the long term persistence of seedlings that do take advantage of such favourable temporal conditions is still unlikely. Moreover, considering the intense levels of grazing observed on established seedlings during times of relative plenty (Auld, 1993, 1995; Cohn & Bradstock, 2000; Auld & Denham, 2001), grazing on these *Acacia* seedlings during drier times when ephemeral plants die off and vegetation drastically thins, is expected to be even

higher (Auld 1993, 1995; Cohn & Bradstock, 2000; Auld & Denham, 2001; Hein, 2006). Observation through the next dry period would give a better indication of the survivability of these recruits.

### **5.5.5 Driving factors for long term survival of recruits**

Understanding which local environmental factors are key to the survival of seedlings during the most vulnerable periods of their development can inform effective conservation strategies aimed at increasing a recruit's long term chance of survival. In arid and semi arid environments, desiccation is a major cause of seedling mortality. The presence of suitable micro climates / habitats can mean the difference between seedlings surviving particularly dry periods or hot days, and perishing (Niering et al., 1963; Bertness & Callaway, 1994; Bronstein, 2009). The presence of overstory canopies in particular can have a significant impact on reducing solar radiation and wind, and increasing relative humidity beneath the canopy (Conard & Radosevich, 1982; Carlsson & Callaghan, 1991; Chen et al., 1995), leading to lower soil temperatures and increased soil moisture, therefore reducing the risk of desiccation for seedlings (Conard & Radosevich, 1982; Chen et al., 1995; Callaway & Walker, 1997; Holmgren et al., 1997). The accumulation of plant litter under these canopies may also facilitate seedling establishment although too much may hinder it (Shaw, 1968; Myster & Pickett, 1993; Wan et al., 1999). In hot and dry environments, seedlings located under the shade of a canopy are thought to have some survival advantage over those located outside the canopy (Niering et al., 1963; Bertness & Callaway, 1994; Bronstein, 2009). The fact that I found the opposite was true of *A. melvillei* and *A. loderi* seedlings was surprising, however this is likely to reflect the favourable conditions of high water availability prevailing during the period my observations were made. Moreover, seedlings that grow under trees in now sparsely vegetated agricultural regions are more likely to be trampled by livestock seeking shelter from the sun, or else disproportionately grazed for the same reason. As overstory *Acacia* trees are often maintained as 'shade trees' by farmers, the higher grazing rates generally observed on *A. loderi* seedlings located under canopies of parent plants, compared to those outside of them, is also likely to be explained this way. Regardless, the shade provided by the canopy of all local plant species in the region is likely to become a more important factor in the persistence of seedlings once water availability decreases in the region and desiccation becomes a more significant threat.

The importance of understory plants acting as nurse plants for recruits is well documented in many systems (Bertness & Callaway, 1994; Gómez-Aparicio et al., 2004; Rousset & Lepart, 2000; Sanchez-Velaquez et al., 2004; Soliveres et al., 2010). The importance of understory plants to the survival of *A. ligulata* plants in Western NSW has also been demonstrated previously (Whitney, 2005a). This was also supported by my findings of higher average three year survival for *A. melvillei*, *A. loderi* and *A. ligulata* seedlings situated under the cover of nurse plants, compared to seedlings located outside the protection of nurse plants. Indeed, according to the “stress-gradient” hypothesis (Bertness & Callaway, 1994), we should expect nurse plants to be more important in arid and semi arid environments than temperate ones. Where grazing pressures are intense, the protective effect of nurse plants for young vulnerable seedlings is further emphasized (Callaway, 1995; Rousset & Lepart, 1999; García et al., 2000; Gómez et al., 2003). Maintenance of a healthy understory is clearly critical to improving the chances of these threatened *Acacia* seedlings surviving, especially when the landscape dries out and grazing pressures are expected to intensify. Higher survival rates of *A. carneorum* suckers located under nurse plants compared to those totally exposed, has also been observed in the region (Auld, 1993; *pers obs.*).

There are often tradeoffs between protection from the sun and grazers facilitated by canopy shade and nurse plants and a seedlings growth (Kitajima, 1994; Osunkoya et al., 1994; Veenendaal et al., 1996). In conditions of ample water, growth rates of seedlings are often dependent on the availability of light, with seedlings under shade generally growing more slowly than those in full or partial light (Alexander & Maggs, 1970; Popma & Bongers, 1988; Bush & Van Auken, 1990; Seiwa, 2007). I found this to be the case for *A. melvillei* and *A. loderi* seedlings. In complete contrast, *A. ligulata* seedlings located outside the canopy shade were on average shorter after three years than those under or at the edge of these canopies. While the shade and protection provided under canopies and nurse plants will likely benefit the survival chances of *A. melvillei*, *A. loderi* and *A. ligulata* seedlings in the future, it is clear this reduced access to sunlight comes at a cost, with these seedlings much slower growing than those located outside nurse plant and canopy protection.

It must be acknowledged that the higher levels of seedling survivorship I found to be associated with understory nurse plants could in theory be the result of local site specific influences unrelated to the protection provided by nurse plants. For instance, those areas supporting understory plants may have a different soil structure or higher nutrient levels compared with those areas that lack such plants. Whilst there is precedence to believe that a

nurse plant effect may be occurring in this system, further studies of the soil chemistry in areas supporting nurse plants versus those areas lacking them would be useful in more confidently assigning the phenomenon I discovered to the nurse plant effect itself or not.

While the benefits of shade for seedling survival are seemingly obvious in hot dry environments, it may be that quicker growth associated with higher access to light is advantageous as a survival tactic. Seedlings that can grow tap roots and become larger and more resilient before access to water dries up and grazing pressures increase, would be expected to have a survival advantage over those that grow slower. The higher survival rates I found for seedlings that germinated earlier from the seed bank, compared with those that germinated later from the canopy seed, is indicative of this. Seedlings that may initially be protected by the presence of understory vegetation ('nurse plants'), may end up competing for limited water with this understory for dwindling water resources as the landscape dries out (Bush & Van Auken, 1990; Shoo & Catterall, 2013). Of course for plants situated under nurse plants, such tradeoffs must be weighed up in respect to the decreased chance of being consumed by grazers. Historically semi arid *Acacia* species may have benefited from seedlings emerging in a range of microhabitats, such as under and outside canopy shade and the protection of nurse plants, to ensure against particularly hot spells, or peaks in local grazing pressures soon after recruitment. Under the unnaturally high grazing regime since European colonization of the region, this balance may have become skewed towards favouring faster growth of seedlings however, to increase resilience to herbivory damage.

### **5.5.6 Future persistence of semi arid *Acacia* in the region**

For plant species such as *A. carneorum* that can maintain stands largely through suckering alone, an ongoing lack of sexual recruitment is unlikely to be of immediate consequence to their structure, however the voracious consumption of suckers is of great concern. For species such as *A. melvillei* *A. homalophylla* and *A. loderi* however, a clear reliance on sexual recruitment for stand replacement and maintaining genetic diversity (Roberts et al., 2013; Forrest et al., 2015; Roberts et al., 2016; Forrest et al., unpublished work) means reductions in seedling numbers are of critical concern. With fewer seedlings contributing to these stands, and therefore a disproportionate number of suckers making up stands, we can expect not only a reduction in stand numbers, but a steady reduction in genetic diversity of stands as adult plants die off and genetic drift becomes more prominent. As suckers are

generally far more resilient to desiccation and grazing pressures than seedlings, since they are serviced by their more stable parent plants which provide both water resources and resources for repairing damage sustained by grazing (Piquot et al. 1998; Honnay & Bossuyt 2005), under conditions of heavy grazing we might expect suckers to disproportionately survive to adulthood. This effect is likely to be exacerbated by agricultural practices and road verge maintenance disturbing the roots of plants, which promotes suckering (Batty & Parsons, 1992). Even if suckers were to compensate for the lack of sexual recruitment in stands, as has been found in many road side *A. homalophylla* stands (Chapter 2, thesis), the consequent homogenization of these stands will leave them with a reduced adaptive capacity. The importance of maintaining sexual recruitment in these stands to maintain genetic resilience in the face of future climate change should be of upmost importance to managers. Follow up surveys of these tagged *A. melvillei*, *A. homalophylla* and *A. loderi* seedlings in years to come, preferably until they reach reproductive maturity, would of course be the most conclusive way to assess the long term contribution of these seedlings to the structure of these stands.

## 5.6 Appendix

**Appendix 5.6.1 a. Relationship between local structural and climatic variables characterizing semi arid *Acacia* stands and the proportion of mature plants within stands recruiting seedlings after a rare region wide rain event:** Single regressions and Hierarchical multiple regressions between the percentage of 10 plants within each of 47, 10, 26 and 20 *A. melvillei*, *A. homalophylla*, *A. loderi* and *A. ligulata* stands spread across the semi arid region of far western NSW and the: 1. Number of plants per stand, 2. Distance of a stand from the nearest neighbouring stand, 3. Average distance of neighbouring plants within stands, 4. Average height of mature (independent) plants, 5. Average width of mature plants, 6. Average # of suckers per immature (independent) plant, 7. Percentage of local ground covered in understory vegetation, 8. Average % of mature plants covered in healthy foliage, 9. Average % of plants leaf surface area consumed by herbivores, 10. Average % of mature plants canopies parasitised by epiphytes, 11. Average annual local rainfall averaged over two consecutive years, 12. Average local temperature averaged over two consecutive years.

	V % of plants with seedlings			
	Relationship R2, F, P			
	<i>A. melvillei</i>	<i>A. homalophylla</i>	<i>A. loderi</i>	<i>A. ligulata</i>
Regressions- structural condition of stand				
1. Size of stand (# plants per stand)	0.101, 5.074, <b>0.029*</b>	<0.000, <0.000, 1.000	0.184, 5.399, 0.029	<0.000, 0.001, 0.979
2. Connectivity (distance from nearest neighbour stand)	0.056, 2.680, 0.109	0.061, 0.452, 0.523	0.219, 6.718, 0.016	0.058, 1.107, 0.307
3. Density (average distance of plants from the nearest neighbour plant)	0.065, 3.145, 0.083	0.020, 0.144, 0.716	0.002, 0.041, 0.842	0.009, 0.160, 0.694
4. Height of plants (average height of independent plants)	0.007, 0.325, 0.572	0.006, 0.042, 0.844	0.012, 0.289, 0.596	0.089, 1.768, 0.200
5. Width of plants (average width of independent plants)	0.070, 3.304, 0.076	0.244, 2.256, 0.177	0.009, 0.227, 0.638	0.028, 0.527, 0.477
6. # of suckers (average number of suckers per independent plant)	0.002, 0.088, 0.769	0.573, 9.410, 0.018	0.038, 0.959, 0.337	
7. Understory cover (% of understory vegetation)	0.037, 1.716, 0.197	0.062, 0.466, 0.517	0.086, 2.255, 0.146	0.041, 0.765, 0.393

Regressions- condition of plants				
8. <b>Canopy condition</b> (average % covered in healthy foliage)	0..082, 4.008, 0.051	0.666, 13.975, 0.007	0.003, 0.063, 0.804	0.042, 0.784, 0.388
9. <b>Leaf herbivory</b> (average % of plants leaf surface area consumed)	0.006, 0.273, 0.604	0.002, 0.012, 0.917	0.029, 0.720, 0.405	0.070, 1.360, 0.259
10. <b>Epiphyte infection</b> (average % of canopy parasitised by epiphytes)	0.009, 0.387, 0.537	0.441, 5.526, 0.051	0.003, 0.077, 0.784	n/a
Regressions- local climatic conditions				
11. <b>Local rainfall</b> (average annual local rainfall over 2 years (2010/2011))	<0.000, 0.003, 0.960	0.028, 0.200, 0.668	0.162, 4.641, 0.051	0.035, 0.646, 0.432
12. <b>Local temperatures</b> (annual average over 2 years (2010/2011))	0.028, 1.296, 0.261	0.271, 2.609, 0.150	<0.000, 0.006, 0.941	<0.000, 0.008, 0.931
Hierarchical multiple regressions				
Model 1: Measures 1-7	0.300, 2.325, <b>0.045*</b>	n/a	0.365, 1.229, 0.347	0.271, 0.805, 0.584
Model 2: Measures 1-10	0.327, 1.703, 0.119	n/a	0.444, 0.960, 0.519	0.533, 1.567, 0.240
Model 3: Measures 1-12	0.329, 1.348, 0.240	n/a	0.515, 0.884, 0.586	0.725, 2.370, 0.105

**Appendix 5.6.1 b. Relationship between local structural and climatic variables characterizing semi arid *Acacia* stands and the number of seedlings recruiting within stands, after a rare region wide rain event:** Single regressions and Hierarchical multiple regressions between the # of seedlings recruited within each of 47, 10, 26 and 20 *A. melvillei*, *A. homalophylla*, *A. loderi* and *A. ligulata* stands spread across the semi arid region of far western NSW and the:

1. Number of plants per stand, 2. Distance of a stand from the nearest neighbouring stand, 3. Average distance of neighbouring plants within stands, 4. Average height of mature (independent) plants, 5. Average width of mature plants, 6. Average # of suckers per immature (independent) plant, 7. Percentage of local ground covered in understory vegetation, 8. Average % of mature plants covered in healthy foliage, 9. Average % of plants leaf surface area consumed by herbivores, 10. Average % of mature plants canopies parasitised by epiphytes, 11. Average annual local rainfall averaged over two consecutive years 12. Average local temperature averaged over two consecutive years.

	Relationship R <sup>2</sup> , F, P			
	<i>A. melvillei</i>	<i>A. homalophylla</i>	<i>A. loderi</i>	<i>A. ligulata</i>
<b>Regressions- structural condition of stand</b>				
<b>1. Size of stand (# plants per stand)</b>	0.009, 0.399, 0.531	<0.000, <0.000, 1.000	0.209, 5.829, 0.025	0.012, 0.016, 0.647
<b>2. Connectivity (distance from nearest neighbour stand)</b>	0.002, 0.074, 0.787	0.049, 0.359, 0.568	0.200, 5.492, 0.029	0.033, 0.607, 0.446
<b>3. Density (average distance of plants from the nearest neighbour plant)</b>	0.007, 0.313, 0.578	0.040, 0.295, 0.604	0.005, 0.114, 0.739	0.045, 0.846, 0.370
<b>4. Height of plants (average height of independent plants)</b>	0.001, 0.038, 0.846	0.073, 0.554, 0.481	0.002, <0.000, 0.993	0.006, 0.101, 0.754
<b>5. Width of plants (average width of independent plants)</b>	0.034, 1.539, 0.221	0.183, 1.573, 0.250	0.013, 0.301, 0.589	0.050, 0.954, 0.342
<b>6. # of suckers (average number of suckers per independent plant)</b>	0.005, 0.238, 0.628	0.411, 4.880, 0.063	<0.000, 0.003, 0.954	N/A
<b>7. Understory cover (% of understory vegetation)</b>	0.004, 0.198, 0.658	0.111, 0.874, 0.381	0.050, 1.168, 0.291	0.123, 2.518, 0.130
<b>Regressions- condition of plants</b>				
<b>8. Canopy condition (average % covered in healthy foliage)</b>	0.001, 0.029, 0.866	0.825, 32.999, 0.001	0.004, 0.097, 0.758	0.049, 0.931, 0.347

9. Leaf herbivory (average % of plants leaf surface area consumed)	0.004, 0.202, 0.656	0.020, 0.145, 0.715	0.005, 0.104, 0.750	0.082, 1.614, 0.220
10. Epiphyte infection (average % of canopy parasitised by epiphytes)	<0.000, 0.001, 0.976	0.214, 1.902, 0.210	0.426, 16.330, 0.001	n/a
Regressions- local climatic conditions				
11. Local rainfall (average annual local rainfall over 2 years (2010/2011))	0.001, 0.057, 0.812	0.002, 0.016, 0.901	0.006, 0.140, 0.712	0.044, 0.826, 0.375
12. Local temperatures (annual average over 2 years (2010/2011))	<0.000, 0.006, 0.939	0.201, 1.759, 0.226	0.007, 0.150, 0.702	0.017, 0.305, 0.588
Hierarchical multiple regressions				
Model 1: Measures 1-7	0.102, 0.618, 0.738	n/a	0.226, 0.583, 0.759	0.186, 0.496, 0.801
Model 2: Measures 1-10	0.151, 0.621, 0.785	n/a	0.244, 0.429, 0.895	0.332, 0.684, 0.699
Model 3: Measures 1-12	0.153, 0.498, 0.901	n/a	0.251, 0.305, 0.968	0.427, 0.672, 0.728

**Appendix 5.6.2. Relationship between the fecundity of semi arid *Acacia* stands and the number of seedlings recruited within stands of five semi arid *Acacia* species in far western NSW:** # of seed produced on 10 mature (independent) plants within 47, 10, 26 and 20 *A. melvillei*, *A. homalophylla*, *A. loderi* and *A. ligulata* stands spread across the semi arid region of far western NSW, and the number of seedlings recruited within close proximity to those plants.

<i>Acacia</i> Species	<i>A. melvillei</i>	<i>A. homalophylla</i>	<i>A. loderi</i>	<i>A. ligulata</i>
Linear relationship ( $r^2$ , F statistic, p value)	0.008, 0.383, 0.539	0.094, 0.828, 0.390	0.032, 0.792, 0.382	0.015, 0.292, 0.595

**Appendix 5.6.3. Long term persistence and condition of semi arid *Acacia* seedlings recruited after a rare large scale rain event across far western NSW:** Comparisons of the survival rates (% of seedlings surviving after three years of observation), grazing rates (0-5 scale) and growth (height of seedlings after three years of monitoring) between *A. melvillei*, *A. homalophylla*, *A. loderi* and *A. ligulata* seedlings recruited under 30 plants respectively, as well as between five separate stands of each species, after a large scale rain event in 2011.

		Long term persistence of seedlings (not with relation to mature canopy or nurse plants)					
			Survival			Height	
Tests between species	Seedling type	All	2011 canopy seed cohort (new)	Seed bank (old)	Canopy	Seed bank	
	Kaplan-Meier (Log rank test) survival analysis Kruskal-Wallis tests						
	Comparison between species	$\chi^2(2, n=1541) = 356.661, p<0.000$	$\chi^2 (2, n=1135)=145.264, p<0.000$	$\chi^2(1, n=406)=64.067, p<0.000$	$\chi^2 (2, n=1135)=139.064, p<0.000$	$\chi^2 (2, 412)=0.027, p=0.870$	
	Post hoc tests ((A), (B), (C))						
	Average	<i>A. melvillei</i>	(A) 84.34	(A) 75.57 (SD± 5.254)	(A) 95.27 (SD±	(A) 6.061 (SD± 0.163)	(A) 4.077 (SD±

	mortality rates (%)		(SD± 13.71)		55.246)		1.001)
		<i>A. loderi</i>	<b>(B)</b> 38.56 (SD± 7.90)	<b>(B)</b> 37.87 (SD± 4.661)	<b>(A)</b> 100.00 (SD± 0.00)	<b>(B)</b> 11.244 (SD± 0.580)	<b>(B)</b> 3.082 (SD± 0.5)
		<i>A. ligulata</i>	<b>(C)</b> 65.63 (SD± 8.02)	<b>(C)</b> 58.26 (SD± 15.322)	<b>(B)</b> 67.65 (SD± 10.271)	<b>(C)</b> 19.952 (SD± 1.914)	<b>(C)</b> 5.149 (SD± 3.136)
Tests within species	<i>A. melvillei</i>	Testing for differences / similarities between 5 stands	$\chi^2$ (4, n=579)=4.349, p=0.361	$\chi^2$ (4, n=307)=1.913, p=0.384	$\chi^2$ (4, n=272) =0.101, p<0.951	$\chi^2$ (4, 307)=1.913, p=0.384	$\chi^2$ (4, 272)=20.086, df=4, p<0.000
	<i>A. loderi</i>		$\chi^2$ (4, n=718)=73.904, p<0.000	$\chi^2$ (4, n=713)=18.488, p<0.000	N/A	$\chi^2$ (4, n=713)=18.488, p<0.000	N/A
	<i>A. ligulata</i>		$\chi^2$ (4, n=244)=29.357, p<0.000	$\chi^2$ (4, n= 115)=13.064, p<0.011	$\chi^2$ (4, n=134) =6.458, p=0.167	$\chi^2$ (4, 115)= 13.064, p=0.011	$\chi^2$ (4, 134)=25.154, p<0.000

\*\* No significant P values were found when Bonferroni adjustments were made to account for potential 'Type 1 errors' as a result of multiple testing

Appendix 5.6.4 a. **Effect of proximity to canopy shade of mature plants on the persistence and condition of *Acacia* seedlings:** Comparisons of the survival rates (% of seedlings surviving after three years of observation), grazing rates (0-5 scale) and growth (height of seedlings after three years of monitoring) between *A. melvillei*, *A. homalophylla*, *A. loderi* and *A. ligulata* seedlings located under, at the edge and outside the canopy cover (shade) of the closest potential mature plant, after a large scale rain event in 2011. 30 mature plants divided equally across five separate stands for each *Acacia* species were used.

Effect of proximity to mature canopy							
		Survival (Kaplan-Meier ( <i>Log rank test</i> ) survival analysis)			Growth (Kruskal-Wallis tests)		
Origin of seed		All	2011 Canopy Cohort (New)	Soil stored seed bank (Old)	2011 Canopy Cohort (New)	Soil stored seed bank (Old)	
<i>A. melvillei</i>	Comparison between species		$\chi^2$ (2, n=579)= 2.882, p=0.237	$\chi^2$ (2, n=307)= 0.486, p= 0.784	$\chi^2$ (2, n=272)= 1.271, p=0.530	$\chi^2$ (2, 307)=33.687, p=0.000	$\chi^2$ (2, 272)= 10.297, p=0.006
	Position with respect to mature plant	Under	N/A	N/A	N/A	5.23 SE± 1.28	4.19 SE± 0.77
		Edge	N/A	N/A	N/A	6.35 SE± 0.97	5.24 SE± 0.46
<i>A. loderi</i>	Comparison between species		$\chi^2$ (2, n=718)= 37.394, p<0.000	$\chi^2$ (2, n=713)= 36.925, p<0.000	N/A	$\chi^2$ (2, 713)= 3.405, p=0.182	N/A

	Position with respect to mature plant	Under	23.81 SE $\pm$ 1.87	72.31 SE $\pm$ 18.52	N/A	8.63 SE $\pm$ 1.9	N/A
		Edge	40.43 SE $\pm$ 6.69	79.25 SE $\pm$ 8.75	N/A	14.18 SE $\pm$ 3.12	N/A
		Outside	41.67 SE $\pm$ 11.45	71.88 SE $\pm$ 4.68	N/A	11.69 SE $\pm$ 3.1	N/A
<i>A. ligulata</i>	Comparison between species		$\chi^2$ (2, n=244)= 4.055, p=0.132	$\chi^2$ (2, n=115)= 3.026, p=0.220	$\chi^2$ (2, n=134)= 9.886, p=0.007	$\chi^2$ (2, n=115)=10.644, p=0.005	$\chi^2$ (2, 134)=10.309, p=0.006
	Position with respect to mature plant	Under	N/A	55.56 SE $\pm$ 21.925	45.96 SE $\pm$ 17.14	19.67 SE $\pm$ 5.73	7.59 SE $\pm$ 1.43
		Edge	N/A	83.33 SE $\pm$ 12.50	75.00 SE $\pm$ 10.71	20.81 SE $\pm$ 5.8	5.37 SE $\pm$ 1.12
		Outside	N/A	70.83 SE $\pm$ 18.52	61.43 SE $\pm$ 14.46	13.29 SE $\pm$ 1.5	4.65 SE $\pm$ 0.89

Appendix 5.6.4b. **Effect of proximity to nurse plants on the persistence and condition of *Acacia* seedlings:** Comparisons of the survival rates (% of seedlings surviving after three years of observation), grazing rates (0-5 scale) and growth (height of seedlings after three years of monitoring) between *A. melvillei*, *A. homalophylla*, *A. loderi* and *A. ligulata* seedlings located under and outside the cover of a nurse plant, after a large scale rain event in 2011. 30 mature plants divided equally across five separate stands for each *Acacia* species were used.

Effect of proximity to nurse plants						
			Survival (Kaplan-Meier ( <i>Log rank test</i> ) survival analysis)		Growth (Kruskal-Wallis tests)	
Origin of seed			All	2011 Canopy Cohort (New)	Soil stored seed bank (Old)	2011 Canopy Cohort (New)
<i>A. melvillei</i>			$\chi^2 (1, n=579)=0.192$ , p=0.661	$\chi^2 (1, n=307)= 0.884$ , p=0.347	$\chi^2 (1, n=272)= 1.849$ , p=0.174	$\chi^2 (1, n=307)= 13.537$ , p<0.000
		Under	N/A	N/A	N/A	4.89 SE± 0.21
	Position with respect to nurse plant	Outside	N/A	N/A	N/A	6.56 SE± 0.19
<i>A. loderi</i>			$\chi^2 (1, n=718)= 34.398$ , p<0.000	$\chi^2 (1, 713)= 31.628$ , p<0.000	N/A	$\chi^2 (1, n=713)= 1.380$ , p=0.240
		Under	44.53 SE± 17.93	39.91 SE± 17.94	N/A	8.9 SE± 0.1
	Position with respect to nurse plant	Outside	36.23 SE± 3.48	31.44 SE± 2.493	N/A	10.34 SE± 0.75
<i>A. ligulata</i>			$\chi^2 (1, n=244)= 2.982$ , p=0.225	$\chi^2 (1, n=115)= 1.792$ , p=0.180	$\chi^2 (1, n= 134)=0.997$ , p=0.318	$\chi^2 (1, n=115)= 22.419$ , p<0.000
						$\chi^2 (1, 134) = 10.731$ , p=0.001

	Position with respect to nurse plant	Under		N/A	N/A	3.85 SE± 0.62	5.6 SE± 0.79
		Outside		N/A	N/A	21.33 SE± 1.98	6.37 SE± 1.31

## **Chapter 6: Is the threatened *A. carneorum* pollen or pollinator limited?**

### **6.1 Abstract**

Pollination can often be disrupted by the fragmentation of plant populations such that they may suffer reproductive failure, inbreeding depression or outbreeding depression. For extant stands of threatened *A. carneorum* in western NSW, the failure of seed set in most stands, despite ample rain, is not understood but may reflect pollen limitation or the delivery of pollen of poor quality to flowers. I utilized a combination of pollinator observations and pollen tube analysis to assess the types, numbers, efficiency and foraging behaviour of pollinators servicing plants in four stands of threatened *A. carneorum* and four stands of the co occurring but thriving *A. ligulata* within Kinchega National Park. I found a diverse assemblage of 17 and 23 native insects visiting *A. carneorum* and *A. ligulata* flowers respectively that carried *Acacia* pollen on their bodies. Whilst *A. ligulata* flowers were visited more regularly than *A. carneorum* flowers, the presence of large numbers of introduced honeybees (*Apis mellifera*) visiting *A. ligulata* flowers accounted for a large proportion of this difference. Analysis of flowers for pollen tubes suggest viable pollen was deposited onto a large proportion of *A. carneorum* flowers (37.3% SE ± 0.3) and the majority of *A. ligulata* (55.1% SE±0.1) flowers in all stands surveyed. Whilst the vast majority of native pollinators' foraging behaviours are likely to facilitate self pollen transfer in both *Acacia* species, the domination of *A. ligulata* plants pollination by honeybees may lead to reduced outcrossing, loss of genetic diversity and inbreeding depression. Importantly, I found no obvious differences in the pollinator services received by *A. carneorum* stands, with and without a history of setting seed. Taken together, these results suggest that while deficits in pollination are unlikely to explain the total lack of seed set in most *A. carneorum* stands in the region, increased inbreeding by honey bees may have long term negative consequences for the structure of currently thriving *A. ligulata* stands.

## 6.2 Introduction

For long lived plants existing within highly anthropogenically fragmented populations, a lack of seed set over a prolonged period, often viewed as human induced reproductive failure, may simply be natural reproductive torpor during unfavourable climatic conditions. A lower or higher investment in sexual and asexual reproduction, or a natural transition from investment in sexual reproduction to an asexual lifestyle can also be misinterpreted as reproductive failure (Silander, 1985; Caraco & Kelly, 1991; Stuefer et al., 1996; Piquot et al., 1998; Honnay & Bossuyt, 2005). The drivers of reproductive failure in long lived plants however, have rarely been investigated.

While previous investigations of this thesis into the reproductive capacity of several long lived overstory *Acacias*, existing as highly fragmented stands in the semi arid region of western NSW, highlighted the importance of large scale rain events for sexual reproduction in most of these species, the lack of seed set found in the majority of *A. carneorum* stands existing under the same seemingly optimal conditions, was puzzling and requires explanation. It would appear the mating system of this species is suboptimal, or that the genotypes of these stands are almost obligately clonal (O'Brian et al., 2013; Roberts et al., in review). Although here and in a previous study (Gilpin et al., 2014), it has been shown that *A. carneorum* produces far fewer flowers than co-occurring *A. ligulata* and other *Acacia* species in the region, investigations of the pollinator services of *A. carneorum* plants in the region have uncovered a diverse range of native insect pollinator species capable of carrying *Acacia* pollen on their bodies (Gilpin et al., 2014). The effectiveness of these flower visitors as pollinators has not been thoroughly investigated from the perspective of pollen deposition, nor has there been any investigation of the pollen available to flowers.

Pollination crucial for sexual reproduction can be disrupted and lead to reduced fecundity, weakened offspring or in extreme cases reproductive failure, if pollinators are sufficiently unable to locate small stands, or their foraging behaviour is sufficiently altered such that they provide incompatible pollen (Goverde et al., 2002; Aguilar et al., 2006; Peterson et al., 2008; González-Varo et al., 2010).

Here I take advantage of altered environmental conditions produced by a region wide rain event that are likely to be favourable to pollinators, and utilize a combination of methods including pollinator observations, pollen counts on captured insects and pollen tube analysis of flowers, to compare and contrast the pollination services received by *A. carneorum* stands

with and without a history of setting fruit. I also study co-occurring *A. ligulata* stands, in parallel, that are thought to be thriving in contrast. Specifically, I test the hypothesis that there is a significant difference in the pollination of *A. carneorum* and *A. ligulata* plants, as well as between *A. carneorum* stands, with and without a history of setting seed by investigating:

1. The types of potential pollinators (flower visitors) visiting flowers.
2. The rate of visitation from potential pollinators.
3. The effectiveness of potential pollinators to carry pollen on their bodies.
4. The effectiveness of pollinator assemblages to deposit viable pollen on flowers.
5. The foraging behaviours of pollinators.

## **6.3 Methods**

### **6.3.1 Choice of stands and plants**

Four *A. carneorum* stands and four *A. ligulata* stands within or bordering Kinchega National Park in far western NSW, were selected to survey flower visitors (potential pollinators) in January 2012 for *A. carneorum* and September 2011 for *A. ligulata*. While all four stands of *A. ligulata* were chosen randomly, I selected two *A. carneorum* stands that had not been recorded as setting fruit for the past two decades, and the only two stands in the area which had been known to set fruit consistently in the recent past. Three plants in each stand were randomly chosen to be the subjects of observation.

### **6.3.2 Sampling method**

For both *A. carneorum* and *A. ligulata* plants, one branchlet was sampled on the north, south, east and west sides of the canopy of each plant at mid distance from the base and highest point of each canopy. Branchlets supported between 14 and 22 inflorescences consisting of approximately 20-30 flowers per inflorescence, for both species. For each branchlet the abundance of flower visitors and their foraging behaviour (described in detail below) was observed by a single observer for 15 minutes within each of three different periods of the day (0600-1100, 1200-1600 and 1700-2000). Taken together each branchlet was observed for a total of 45 minutes in a day. This was carried out for a total of three consecutive days for *A. carneorum* and then for *A. ligulata* during a week of very stable climatic conditions

without cloud cover or rain, and with average daily temperatures over the six days varying by only 2°C. The order in which the different stands were observed within each of the three time periods was varied among days. Pollinator observations were not conducted at night given preliminary observations on multiple nights indicated few to no potential pollinators visiting flowers.

I used butterfly nets to capture as many of each species of flower visitor in each stand surveyed as possible over three days, to analyse the amount of *Acacia* pollen they carried on their bodies (their effectiveness at carrying pollen). This was undertaken at the conclusion of pollinator observations at each of the three periods of the day, so as to minimise the effect my presence may have on pollinator behaviour. 10-20 flower visitors (potential pollinators) of most species were caught for analysis from within each stand surveyed. Where less than ten individuals of a species were able to be caught I used all that were caught.

### **6.3.3 Characterising the assemblage of flower visitors**

Insects were identified to the lowest possible taxonomic level by Dr David Britton from the Australian Museum. To decide which species were potential pollinators I placed each specimen under a microscope and looked for the presence of *Acacia* pollen. Polyads taken from flowers of both *Acacia* species were used as reference material so as to easily identify the presence of the correct type of pollen within a mixed pollen load. Where polyads of the target *Acacia* species were found on a flower visitor it was classed as a potential pollinator.

### **6.3.4 Quantifying the abundance of potential pollinators in stands**

The number of each type of flower visitor visiting branchlets of *A. carneorum* and *A. ligulata* plants was tallied to obtain an estimate of the rate of visitation, as well as an estimate of the proportional contribution of the different potential pollinator species to the overall assemblage.

### **6.3.5 Assessing the effectiveness of potential pollinators to carry and deposit pollen**

#### *(i) Effectiveness of individual pollinator species in carrying *A. carneorum* and *A. ligulata* pollen*

*A. carneorum* and *A. ligulata* flower visitors were assessed to determine their effectiveness as carriers of pollen by measuring the number of pollen grains found on their bodies at the time of capture. This method was used as a crude indicator of a flower visitor's efficiency at transporting pollen despite not guaranteeing successful deposition of pollen onto flowers.

Captured insects were inspected under a microscope for the presence of pollen using reference pollen. The average number of pollen grains/polyads on individual insects was then counted and each species of pollinator in the assemblage was assigned an average value (number of polyads carried per insect). Due to the possibility that some species of pollinators are relatively ineffective at carrying pollen individually but significantly more abundant than more individually effective species, and thus potentially responsible for transport of more pollen as a species group, I also calculated the effectiveness of pollinator species to carry pollen as a group. This was achieved by multiplying the average effectiveness values calculated for each pollinator species described above, by the abundance of each pollinator species calculated earlier.

*(ii) The effectiveness of pollinator assemblages at depositing viable pollen on stigmas*

In the middle of the flowering season of both *Acacia* species (January for *A. carneorum* & September for *A. ligulata*), 10 randomly selected plants within each of the four *A. carneorum* and *A. ligulata* stands used above (a total of 20 inflorescences per plant taken in lots of 5 from four sides of each plant (N, S, E, W)), were collected to test for evidence of a) pollen tube initiation and b) pollen tube growth to the ovules of flowers. From each inflorescence three flowers were haphazardly selected, ovaries and stigmas were removed and these were stained using aniline blue (Hough et al., 1985). The stained stigma and ovaries were set on slides and viewed under a UV light source to look for pollen tubes which fluoresce under these conditions. Given that I found no examples of stigmas with pollen attached but without pollen tubes at least initiating (suggesting pollen likely falls off stigmas in these instances), I scored each flower as either having pollen tubes which terminate prior to reaching the ovary of flowers or containing pollen tubes that reached ovaries.

### 6.3.6 Assessing foraging behaviour of potential pollinators

Observations of the foraging behaviour of insects visiting plants, and estimates of the abundance of flower visitors, were carried out simultaneously. For each insect that landed on an inflorescence under observation during these time periods I recorded a) the length of time that a pollinator spent foraging among flowers on the focal branchlet (this was done until foraging ceased or for a maximum of 10 minutes), and b) the proportion of times the pollinator was confirmed to move to another branchlet on the same plant after moving from the focal branchlet. The characteristic foraging behaviour of each pollinator species as a 'species group' was also calculated by taking into account each species' characteristic foraging style, characterised above, as well as the frequency with which they visited flowers, calculated above in **6.3.4**. This was achieved by multiplying the average visit frequency for each pollinator species with i) the average proportion of time each pollinator species spent foraging on branchlets before leaving and ii) the average proportion of movements a species was confirmed to make to another branchlet within the same plant after visiting the branchlet under observation.

### 6.3.7 Statistical analysis of data

I used two way analysis of variance (2 way ANOVA) with stands nested within species and Tukey's post hoc tests to compare the abundance of pollinators and the effectiveness of pollinators to transport and deposit pollen between the two *Acacia* species, as well as between the four stands of each species. Specifically, predictor variables are 1. Species (*A. carneorum* and *A. ligulata*), 2. Fruiting history (fruit present / absent) and 3. Pollinator species shared / not shared between species, whilst response variables are 1. Abundance of potential pollinators, 2. Effectiveness of individual insect species to carry pollen, 3. Effectiveness of flower visitors to carry pollen as a group and 4. The effectiveness of pollinator assemblages at depositing viable pollen on stigmas. Whilst the assumptions of ANOVA were not always met as specified by the Shapiro-Wilks test of normality and Levine's tests of equal variances for all sets of data analysed, advice from statistical consultants indicated that this approach was still appropriate given the normally distributed nature of these data after square root transformation. Moreover (Underwood, 1981 1997) have argued that ANOVA is insensitive to even large deviations from normality and inequality of variances, especially if the sample sizes are not very small (less than 5) and designs are balanced, as was the case here.

## **6.4 Results**

### **6.4.1 Characterising the assemblage of flower visitors**

A wide range of largely native insect species were observed to visit the flowers of both *A. carneorum* and *A. ligulata*, with at least one individual of each species analysed found to be carrying at least some pollen of these *Acacia* species on their bodies (Figure 6.1; Table 6.1). I found 17 different putative potential pollinator species representing five different orders (*Coleoptera, Diptera, Hymenoptera, Lepidoptera, and Apidae*) visiting the flowers of *A. carneorum* plants. I found 22 different potential pollinator species, also representing the same five different orders, visiting the flowers of *A. ligulata* plants.

For *A. carneorum*, Hymenoptera species (wasps) accounted for 10 of the 17 species comprising the assemblage, while Hymenoptera and Coleoptera species together made up 14 of the 22 species of the *A. ligulata* pollinator assemblage. I found 15 pollinator species were common pollinators of both plant species, however there were some species found in only one or the other assemblage. One unidentified species of wasp and one unidentified species of beetle was found on *A. carneorum* only, and 5 species of beetle, one species of fly and most notably the European honeybee (*Apis mellifera*) were found only visiting *A. ligulata* plants (Table 1). Whilst there were noticeably more beetle species visiting *A. ligulata* stands, it is possible these beetles are also consumers of flowers and they were not seen moving between branchlets like all the other flower visitors (Young, 1986). The species of pollinators visiting each *A. carneorum* stand were identical between the four different stands, irrespective of their history of reproductive success. This was also the case for *A. ligulata*.



**Figure 6.1. Examples of flower visitors of *A. carneorum* and / or *A. ligulata* flowers:**  
 (clockwise from top left) *Apis mellifera* on *A. ligulata*, *Nacaduba biocellata*, *Vespidae Delta* and *Sphecidae Prionyx*.

**Table 6.1. Identification of potential pollinators of *A. carneorum* and *A. ligulata* plants within Kinchega National Park, NSW:** Insects identified to as close to the species level as possible with the type of pollen (*A. carneorum* / *A. ligulata*) detected being carried on their bodies identified.

Order/family	Tribe/Genus/species	Code name	Flower visitors of:		Carrying pollen of:	
			<i>A. carneorum</i>	<i>A. ligulata</i>	<i>A. carneorum</i>	<i>A. ligulata</i>
Coleoptera	Unidentified spA	Beetle 1		*		*
	Unidentified spB	Beetle 2	*		*	
	Chrysomelidae	Beetle 3		*		*
	Curculionidae	Beetle 4		*		*
	<i>Melyridae</i> <i>Dicranolaius</i>	Beetle 5		*		*

	<i>Coelophora inaequalis</i>	Beetle 6		*		*
Diptera	<i>Calliphoridae</i>	Blowfly	*	*	*	*
	<i>Chrysomya rufa</i>					
	<i>Lucilia cuprina</i>	Small Fly 1	*	*	*	*
	<i>Lucilia sericata</i>	Small Fly 2		*		*
	<i>Syrphidae Erastilini</i>	Hover Fly	*	*	*	*
Hymenoptera	<i>Vespidae Delta</i>	Wasp 1	*	*	*	*
	<i>Mutillidae spA</i>	Wasp 2	*	*	*	*
	<i>Sphecidae Prionyx</i>	Wasp 3	*	*	*	*
	<i>Scoliidae Pseudotrielis</i>	Wasp 4	*	*	*	*
	<i>Vespidae</i>	Wasp 5	*	*	*	*
	<i>Chuemonidae</i>	Wasp 6	*	*	*	*
	<i>Pompillidae Hemipepsis</i>	Wasp 7	*	*	*	*
	<i>Mutillidae spB</i>	Wasp 8	*	*	*	*
	<i>Scoliidae Radumens</i>	Wasp 9	*	*	*	*
	Unidentified	Wasp 10	*		*	
Lepidoptera	<i>Nacaduba biocellata</i>	Moth	*	*	*	*
Apidae	<i>Apis mellifera</i>	Honeybee		*		*
	Amegilla (Sp 1)	Native Bee 1	*	*	*	*
	Amegilla (Sp 2)	Native Bee 2	*	*	*	*

#### 6.4.2 Quantifying the abundance of potential pollinators in stands

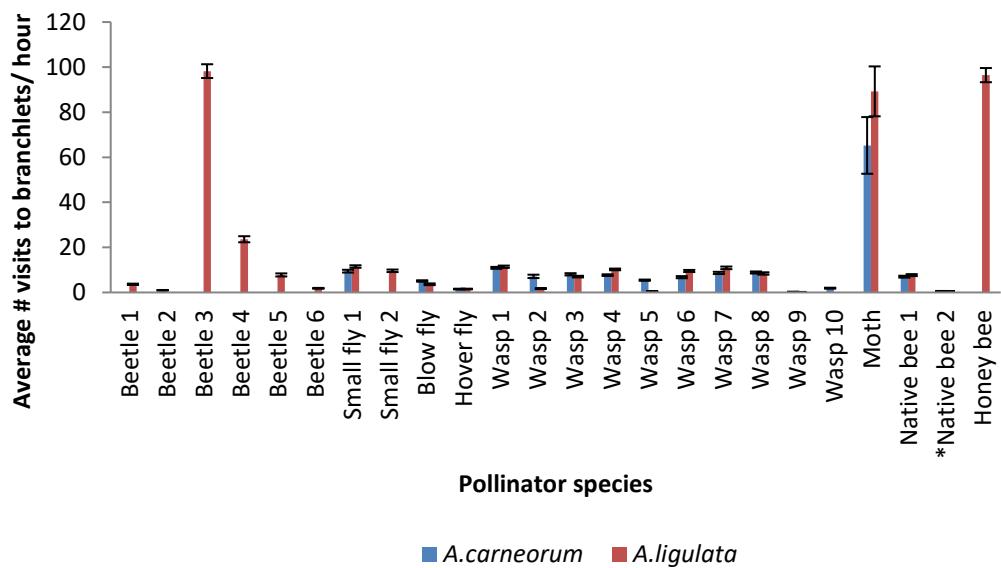
Visitation rates varied greatly between some of the insect species visiting both *Acacia* species (Figure 6.2 a). For *A. carneorum*, one species of moth found to be carrying pollen (*Nacaduba biocellata*) and 10 native species of wasp accounted for 84% of the total number of visits recorded to these plants, with the remaining six species accounting for the remaining

16% of visits (Figure 6.2 a). In contrast, while I found similar visitation rates by the 15 native insect species shared by both the *A. carneorum* and *A. ligulata* assemblages, flower visitation of *A. ligulata* was seemingly dominated by European honeybees which accounted for 30% of the total number of visits recorded to *A. ligulata* branchlets, or 51% of the total number of visits to *A. ligulata* branchlets when the contribution from the relatively immobile beetle species visiting these plants was excluded.

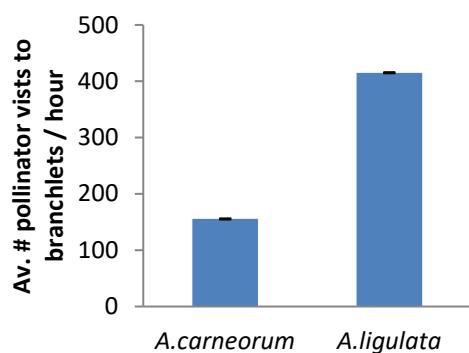
The two beetle species (*Coleoptera Chrysomelidae* and *Coleoptera Curculionidae*) not found on *A. carneorum* flowers were highly abundant on *A. ligulata*, and together with honeybees (*Apis mellifera*) accounted for the relatively greater numbers of pollinator visits to *A. ligulata* (Figure 6.2 a). Indeed, two way analysis of variance found significantly higher overall numbers of insects visiting *A. ligulata* plants as compared to *A. carneorum* plants, even when the beetle species were excluded ( $F(1, 16) = 230.431, p < 0.001$ ) (Figure 6.2 b). No effect of site on the abundance of these pollinators was found for either plant species ( $F(3, 14) = 0.454, p = 0.715$ ), including between the *A. carneorum* stands with a history of setting seed and those without (Figure 6.2 c).

While an independent samples t-test revealed no significant difference in the visit rates of the 15 native insect species that visited both plant species ( $t(29) = -0.789, p = 0.438$ ), there were markedly more visits to *A. ligulata* flowers by the insect species not shared with *A. carneorum* than those of the few species that only visited *A. carneorum* (Figure 6.2 d). There were significantly more visits to *A. ligulata* plants by *Apis mellifera* alone than visits to *A. carneorum* plants by those insects that only visited *A. carneorum* ( $t(2) = -56.686, p < 0.001$ ). Moreover, the number of flower visits to *A. ligulata* plants by species of insect not shared with *A. carneorum* was noticeably higher than the average number of visits to *A. ligulata* plants by any of the species that are shared with *A. carneorum* (Figure 6.2 d). This was also true even when the beetle species were excluded, with significantly more visits to *A. ligulata* plants by *Apis mellifera* alone than by the 15 insect species shared with *A. carneorum* ( $t(15) = -17.317, p < 0.001$ ). These trends were consistent across all four stands of both plant species (Appendix 6.6.1 a& b).

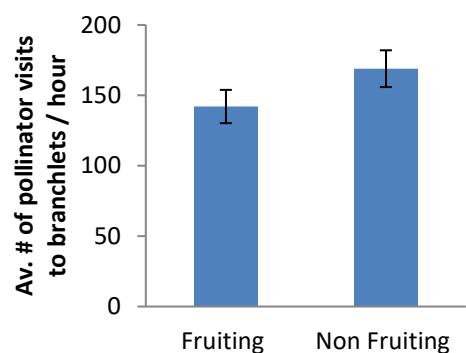
a)



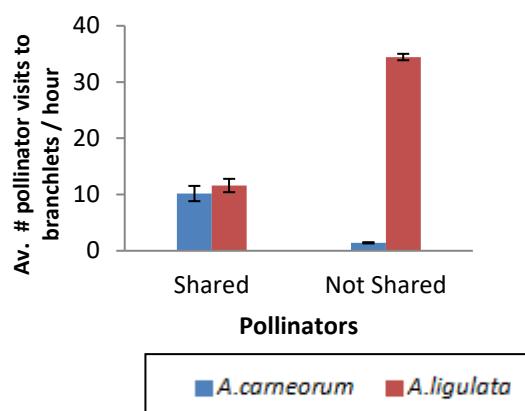
b)



c)



d)



**Figure 6.2. Abundance of potential pollinators visiting flowers on *A. carneorum* and *A. ligulata* in and around Kinchega National Park in western NSW:** a) Average # of each flower visitor species visiting branchlets per hour, b) Average number of flower visitors to *A. carneorum* and *A. ligulata* per hour, c) Average number of flower visitors to plants in stands of *A. carneorum* that produce and those that do not, d) Average number of visits per hour by shared flower visitors compared with those species not shared (endemic). \*Averages abundances represent average findings of all four branchlets, on each of three plants, within each of four stands.\* SE bars represent variation between stands of each plant species.

#### 6.4.3 Assessing the effectiveness of potential pollinators to transport and deposit pollen

##### (i) Effectiveness of individual insect species to carry pollen

I found a great level of variance in the amount of pollen different insect species transported on their bodies (Figure 6.3 a). Of the insects comprising the *A. carneorum* pollinator assemblage, the fly, moth and beetle species transported relatively few pollen grains on their bodies; on average less than three polyads per insect. The wasp species, with the exception of two species, were found transporting a moderate amount of pollen on average, with the most effective wasp species (*Hymenoptera vespidae delta*) carrying on average 9 (SE± 1) polyads per insect. While too few of the larger native bee species (*Apidae Amegilla Sp 2*) were able to be caught to form a confident estimate of their capacity to carry *Acacia* pollen, the smaller native bee species (*Apidae Amegilla Sp 1*) was found to transport on average 10 (SE± 2) pollen grains per insect which was the most of any of the species in the assemblage. The vast majority of pollinator species were found to carry a similar amount of pollen on their bodies irrespective of the plant or stand they were captured in. There were noticeable differences however, in the average numbers of pollen grains found to be transported on wasp 1 (*Hymenoptera vespidae delta*), wasp 9 (*Hymenoptera scoliidae radumens*) and the native bee 2 (*Apidae Amegilla*) between both the four *A. carneorum* and four the *A. ligulata* stands surveyed (Appendix 6.6.3 a & b).

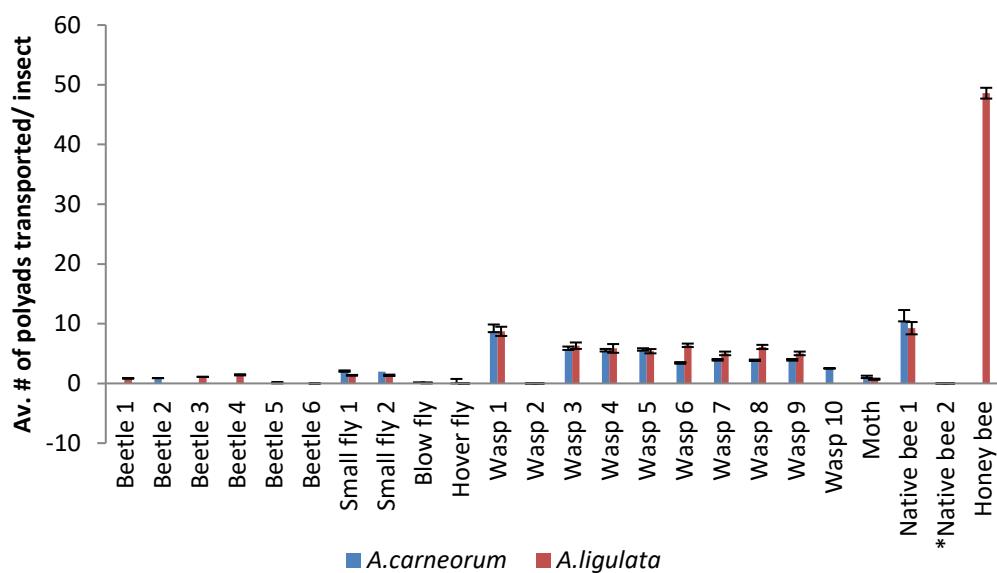
The amount of pollen carried by the average insect visiting *A. ligulata* plants was considerably higher than for *A. carneorum* plants, even when all the beetle species were removed from the analysis (Figure 6.3 b). Two way analysis of variance confirmed this statistically ( $F (1, 16) = 128.873, p < 0.001$ ), whilst also finding a significant variance between the four stands of both species ( $F (3, 14) = 7.553, p < 0.001$ ). Surprisingly, Tukey's post hoc analysis revealed that the average pollinator visiting the two *A. carneorum* stands with a history of setting fruit (Middle Camp stand and KNP Fruiting stand) carried significantly less pollen than

those visiting the two stands without a history of setting fruit (South Dune stand & Big Dune stand) (Figure 6.3 c).

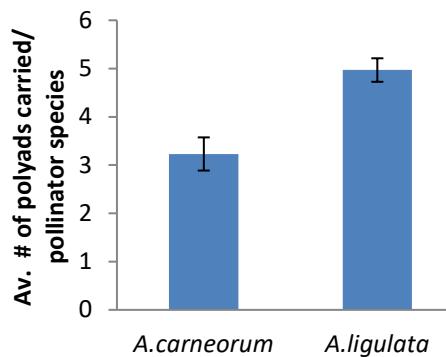
The 15 insect species that visited flowers of both plant species were found on average to carry more *A. ligulata* pollen than *A. carneorum* pollen (Figure 6.3 d), with independent t-tests revealing that this difference was statistically significant  $t(29)=-3.427$ ,  $p=0.002$ . The insect species found visiting only *A. ligulata* flowers carried noticeably more pollen on average than those insect species that only visited *A. carneorum* plants (Figure 6.3 d). Even when the beetle species which may be consuming flowers were removed from the analysis, honeybees were found to carry significantly more pollen than the insects that only visited *A. carneorum* plants ( $t(2) =-23.581$ ,  $p<0.001$ ) (Figure 6.3 a). They also carried significantly more on average, than the other flower visitors shared with *A. carneorum* ( $t(15)=-7.170$ ,  $p<0.001$ ). These trends were consistent across all four stands of both plant species surveyed (Appendix 6.6.2 a & b).

When the abundance of each insect species visiting flowers was also taken into account, some of the species that were relatively poor at carrying pollen as individuals, carried as much if not more pollen as a species group, owing to their sheer abundance. Of particular note were the abundant beetles, flies and moth species, which transported considerably more pollen on their bodies as a species group, than the majority of species that were individually far more effective pollen carriers (Figure 6.3 e). While the 15 native pollinator species common to both the *A. ligulata* and *A. carneorum* pollinator assemblages were comparable in their effectiveness at transporting pollen grains as a group, the high abundance of honeybees, in combination with their effectiveness at carrying pollen individually, assured their dominance in the pollination of *A. ligulata* plants (Figure 6.3 e). I found that 88% of the total amount of pollen carried on all insect visitors of *A. ligulata* was accounted for by honeybees alone. Moreover, the five beetle species, also exclusively found moving between *A. ligulata* flowers carried a significant amount of pollen as a group owing to their relatively large numbers (Figure 6.3 e). With the exception of two wasp species (*Vespidae delta* & *Scoliidae radumens*), these trends were similar across all four stands of both *A. carneorum*, irrespective of their history of setting seed, and the four *A. ligulata* stands (Appendix 6.6.2 c & d).

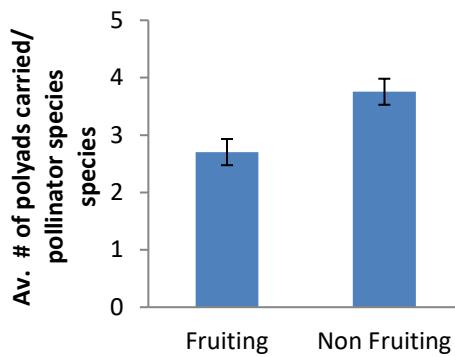
a)



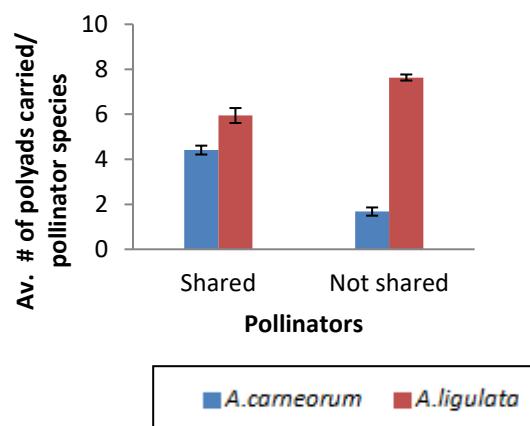
b)



c)



d)



e)

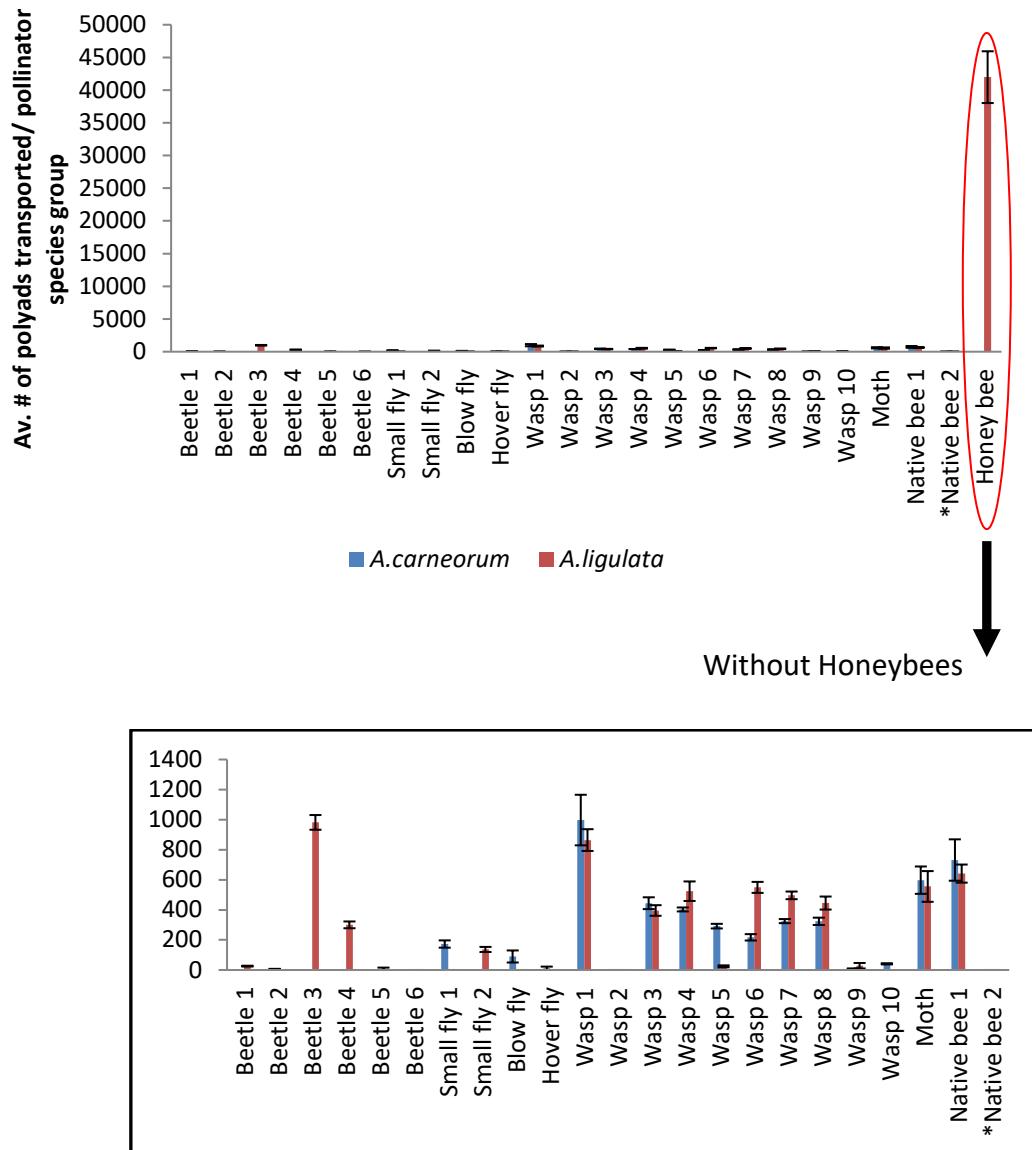


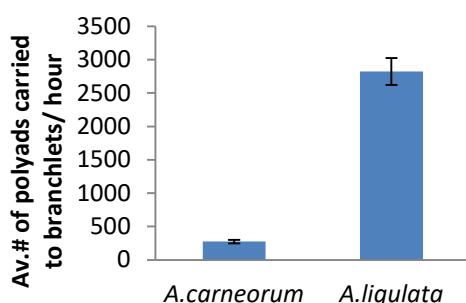
Figure 6.3. Effectiveness of potential *A. carneorum* and *A. ligulata* pollinators at transporting pollen in and around Kinchega National Park in western NSW: a) Average number of polyads transported to branchlets per hour by each flower visitor species, b) Average number of polyads transported by the average of all flower visitor species to branchlets per hour, c) Average number of polyads transported by the average of all flower visitors to branchlets of *A. carneorum* plants within stands that produce fruit and those that do not, d) Average number of polyads transported to branchlets per hour by the flower visitor species shared by both plant species and those not shared (endemic), e) Average number of polyads transported to branchlets per hour by each species of flower visitor, with and without honeybees included respectively. \*Averages represent average numbers of polyads found on 10-20 individuals of each potential pollinator species captured on all four branchlets, on each of three plants, within each of four stands. Standard error bars represent variation between stands of each plant species for all figures.

(ii) Effectiveness of flower visitors to carry pollen as a group

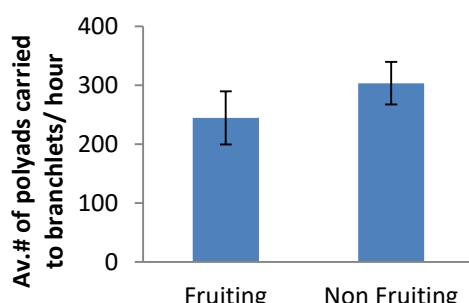
Flower visitors to *A. ligulata* plants carried nearly eight times as much pollen on their bodies as a group than those visiting *A. carneorum* plants (Figure 6.4 a). Two way analysis of variance revealed that this difference was statistically significant, even when the beetle species were removed from the analysis ( $F(1, 16) = 1046.738, p < 0.001$ ). Moreover, a significant effect of site on the effectiveness of these insect assemblages to transport pollen was found ( $F(3, 14) = 8.952, p < 0.001$ ). Surprisingly, Tukey's post hoc analysis revealed that the average effectiveness of the insect assemblage visiting the two *A. carneorum* stands with a history of setting seed was significantly lower than for the other two stands without a history of setting fruit (Figure 6.4 b).

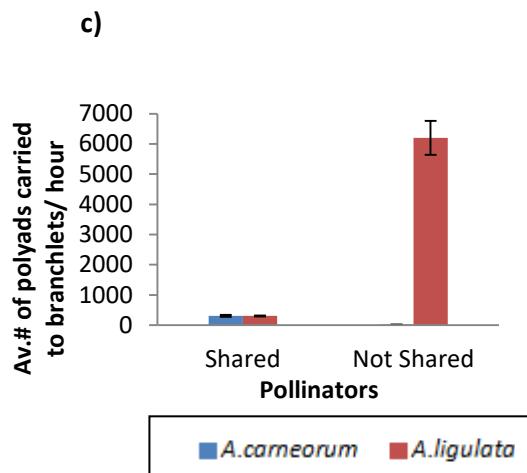
Independent samples t-tests revealed no significant difference in the effectiveness of the 15 native insect species shared by both plant species to transport pollen when the abundance of each species was also taken into account ( $t(29) = -0.602, p = 0.569$ ). When viewed as a group, noticeably larger total quantities of pollen were found to be transported by insect species that were found to visit *A. ligulata* plants but not *A. carneorum* plants however, than on those insect species that were only found to visit *A. carneorum* flowers (Figure 6.4 c). Again, this was driven largely by the relative effectiveness and abundance of honeybees. When all beetles were excluded from the analysis, honeybees were found to carry on average significantly more pollen than the remaining insect species that only visited *A. carneorum* plants ( $t(2) = -9.828, p < 0.001$ ). Further to this, the mean number of pollen grains found to be transported on the bodies of honeybees was significantly higher than that found to be carried by pollinators visiting both *A. ligulata* and *A. carneorum* plants ( $t(15) = 2.622, p = 0.002$ ). These trends were consistent across all four stands of both plant species surveyed (Appendix 6.6.2 c & d).

a)



b)

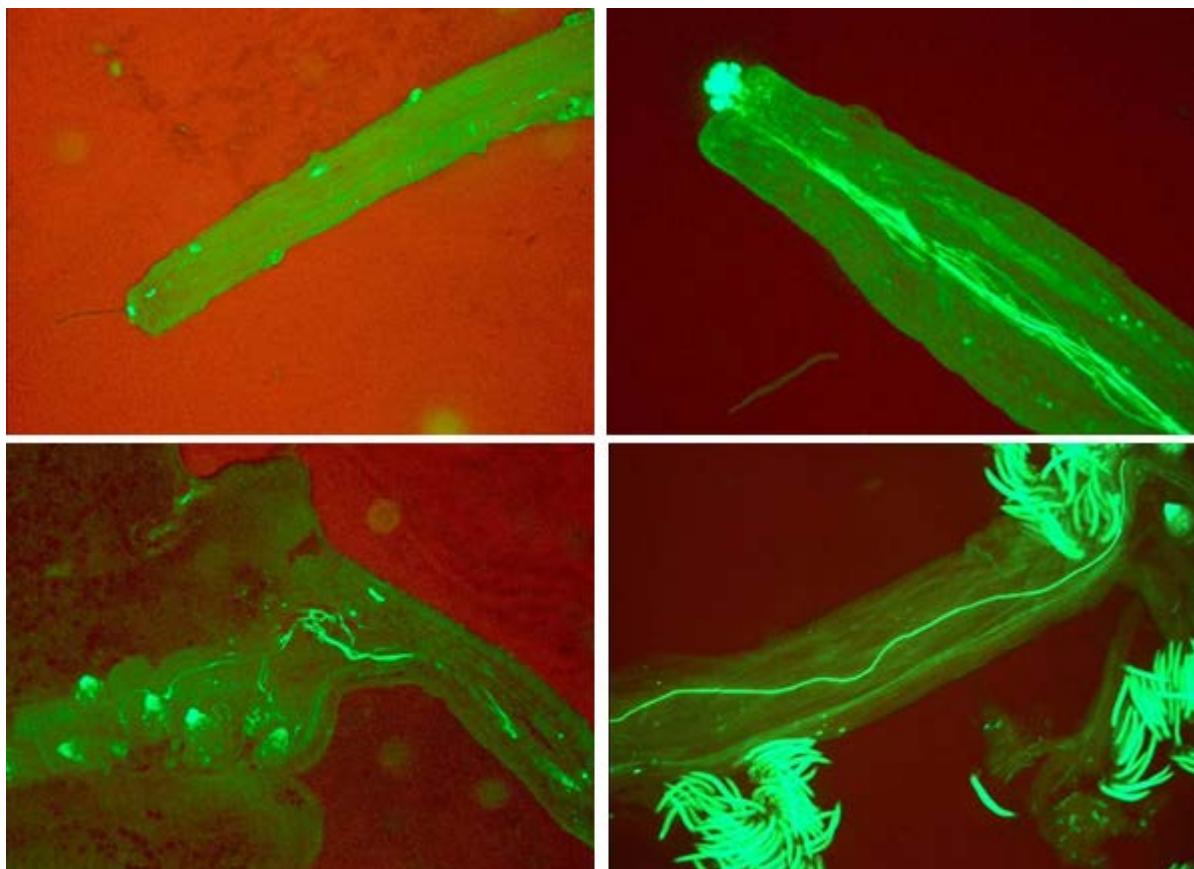




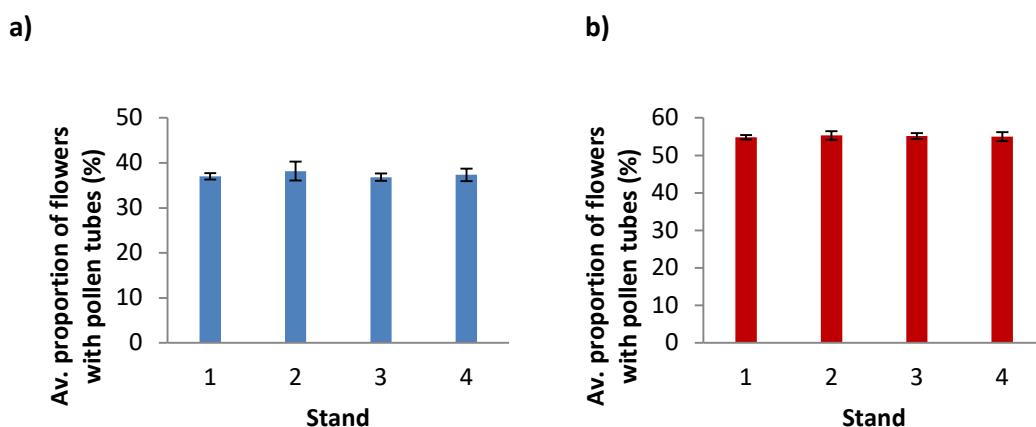
**Figure 6.4. Effectiveness of the *A. carneorum* and *A. ligulata* pollinator assemblages at transporting pollen in and around Kinchega National Park in western NSW:** a) Average number of polyads transported by all flower visitors to branchlets per hour, b) Average number of polyads transported by all flower visitors to branchlets of *A. carneorum* plants within stands that produce fruit and those that do not, c) Average number of polyads transported to branchlets per hour by the portion of the pollinator assemblage shared by both plant species compared with the portion not shared (endemic). \*Averages represent average numbers of polyads estimated to be carried by all potential pollinators visiting flowers on all four branchlets, on each of three plants, within each of four stands surveyed. \*Error bars (SE) represent variation between stands of each plant species for all figures except Figure 3 d) where SE bars represent variation between the two fruit setting and two non fruit setting *A. carneorum* stands surveyed.

### (iii) The effectiveness of pollinator assemblages at depositing viable pollen on stigmas

In each of the stands where pollinator observations were carried out, I found seemingly viable pollen, and pollen tubes, in a large proportion of *A. carneorum* and *A. ligulata* flowers collected from all 6 plants (Figure 6.5). I found a noticeably higher proportion of flowers with pollen tubes initiating in flowers of *A. ligulata* plants than flowers of *A. carneorum* plants (Figure 6.6 a & b). Two way analysis of variance confirmed that this difference was statistically significant ( $F(1,18) = 10.011$ ,  $p < 0.001$ ). No effect of stand was found ( $F(3, 20) = 220.203$ ,  $p < 0.001$ ) indicating there was no difference in the average proportion of flowers with pollen tubes in each of the four *A. ligulata* stands or *A. carneorum* stands, irrespective of their capacity to set fruit.



**Figure 6.5. Examples of *A. carneorum* flowers with and without pollen tubes stained with aniline blue and viewed with fluorescence microscopy with and without pollen tubes growing:** (clockwise from top left) Style without pollen tubes, Style with pollen tubes, Style with pollen tubes reaching the ovary, Pollen tubes reaching individual ovules.



**Figure 6.6: Proportion of *A. carneorum* and *A. ligulata* flowers with pollen tubes initiating:** a) & b) Average % of *A. carneorum* and *A. ligulata* flowers respectively with pollen tubes growing down stiles. \*Averages represent average number of 2400 flowers per *Acacia* species with pollen tubes initiating down styles, across four branchlets on each of ten plants within each of four stands surveyed. \*\*Standard error bars (SE) represent variation between the three plants in each of the four stands surveyed.

#### **6.4.4 Assessing foraging behaviour of potential pollinators**

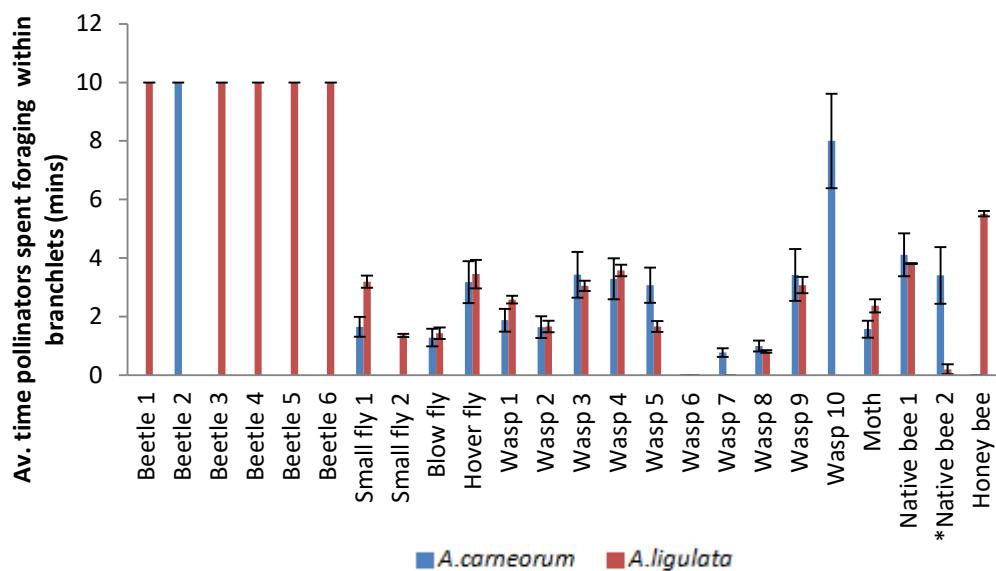
##### *(i) Characterizing the foraging behaviour of pollinator species*

I found considerable variance between the amount of time the different species of flower visitors to *A. carneorum* and *A. ligulata* plants spent foraging between flowers within branchlets, before moving away (Figure 6.7 a). For the flower visitors of *A. carneorum*, foraging times on the same branchlet ranged from less than one minute on average for the wasp (Mutillidae species B) to greater than 10 minutes for the beetle species (Figure 6.7 a). Similarly, for the *A. ligulata* assemblage this foraging time ranged from less than one minute on average for the wasp (Mutillidae species B) to over 10 minutes for all five beetle species, with the dominant honeybees (*Apis mellifera*) also spending considerably longer than the rest of the insects, foraging between flowers within the same branchlet (Figure 6.7 a). On average insects spent a considerably longer time visiting *A. ligulata* flowers within a branchlet before moving away than the average insect visiting *A. carneorum* flowers (Figure 6.7 b). Two way analysis of variance revealed that this difference was statistically significant, even when all the beetle species were removed from the analysis ( $F(1, 16) = 396.008, p < 0.001$ ). Moreover this was the trend at all four of the stands of each species surveyed (Appendix 6.6.3 a & b) with no effect of site found ( $F(3, 14) = 1.007, p = 0.396$ ). Of particular note, no noticeable differences in the average intra branchlet foraging times of any of the potential pollinator species was observed between the two *A. carneorum* stands with a history of setting fruit and those without (Figure 6.7 c).

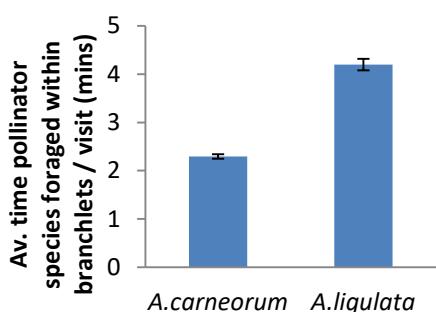
An independent samples t-test revealed that the average amount of time the 15 insect species common to both plant species spent foraging within branchlets was on average significantly higher within *A. ligulata* plants compared with *A. carneorum* plants ( $t(29) = -8.521, p < 0.001$ ) (Figure 6.7 d). The insect species found visiting *A. ligulata* plants, but not *A. carneorum* plants, also spent on average significantly more time foraging within branchlets than the insect species found only visiting *A. carneorum* plants (Figure 6.7 d). Even when the beetle species visiting these plants were excluded, visits to *A. ligulata* branchlets by the honeybees alone were on average significantly longer than by the visitors to *A. carneorum* plants only ( $t(2) = -2.366, p = 0.027$ ). Moreover, insect species that only visited *A. ligulata* plants also spent noticeably more time foraging within branchlets than those species of flower visitor shared by both plant species (Figure 6.7 d). When beetles were removed from the analysis, honeybees still spend on average significantly more time foraging within branchlets than the

rest of the flower visitors ( $t(15)=-3.445$ ,  $p<0.001$ ). These trends were consistent across all four stands of both plant species (Appendix 6.6.3 a & b).

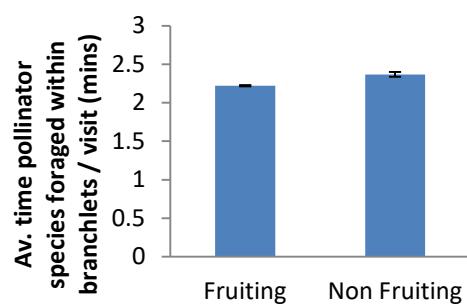
a)



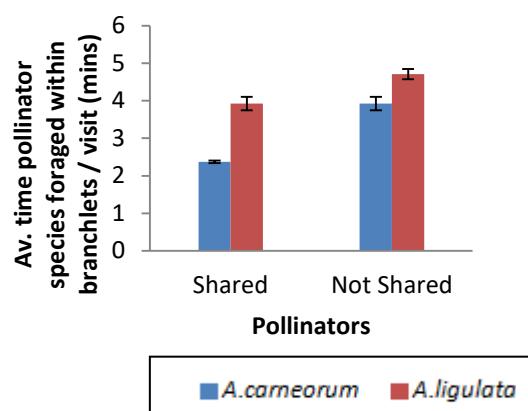
b)



c)



d)



**Figure 6.7. Average time (mins) insects spent foraging between flowers within branchlets of *A. carneorum* and *A. ligulata* before moving away in and around Kinchega National Park in western NSW:** a) Average amount of time flower visitors spent foraging within a branchlet under observation, b) Average amount of time the average flower visitor spent foraging between flowers within branchlets, c) Average amount of time the average flower visitor spent visiting *A. carneorum* plants within stands that produce fruit and stands that do not, before moving away, d) Average amount of time the average pollinator species shared, and not shared by both *A. carneorum* and *A. ligulata* assemblages, spent foraging within branchlets  
*\*Averages represent average amount of time each potential pollinator species visiting four branchlets, on each of three plants, within each of four stands spent foraging within a branchlet before moving away. \*Error bars (SE) represent variation between stands of each plant species for all figures.*

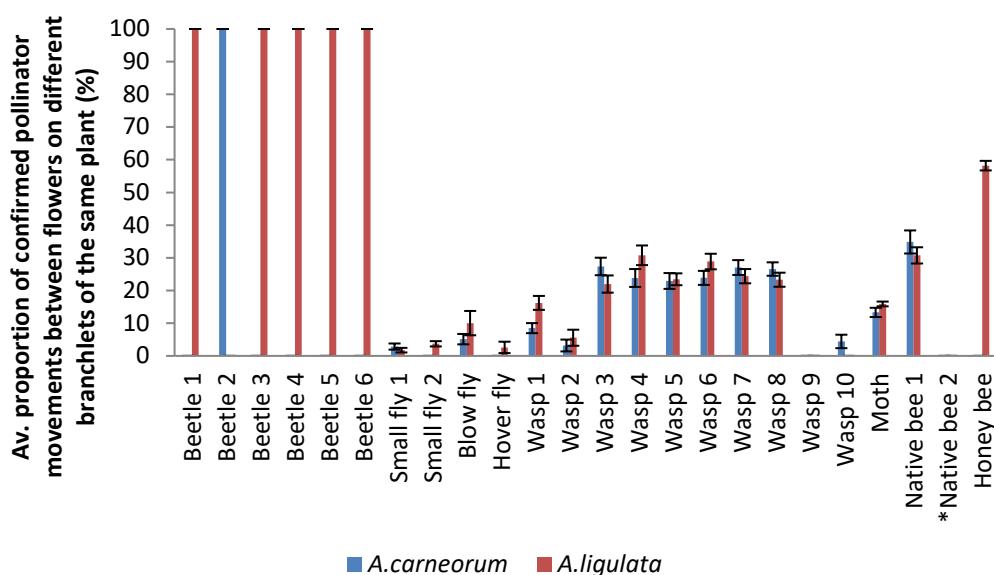
I could not verify where the vast majority of flower visitors flew after a foraging bout due to their speed. For many species however most movements were to other branchlets on the same plant suggesting that these species are likely to move between plants less frequently during a foraging bout. On *A. carneorum* plants, while only 3% of the fly (*Syrphidae Erastilini*) visits were able to be observed with certainty to move to flowers on other branches of the same plant, six of the ten wasp species were observed to make these local movements between 22 and 28% of the time. The native bee species (*Amegilla sp1*) made these local movements 35% of the time. The beetle species were never witnessed to move from a branchlet during a ten minute period of observation, but were seen to move between flowers on a branchlet (Figure 6.8 a).

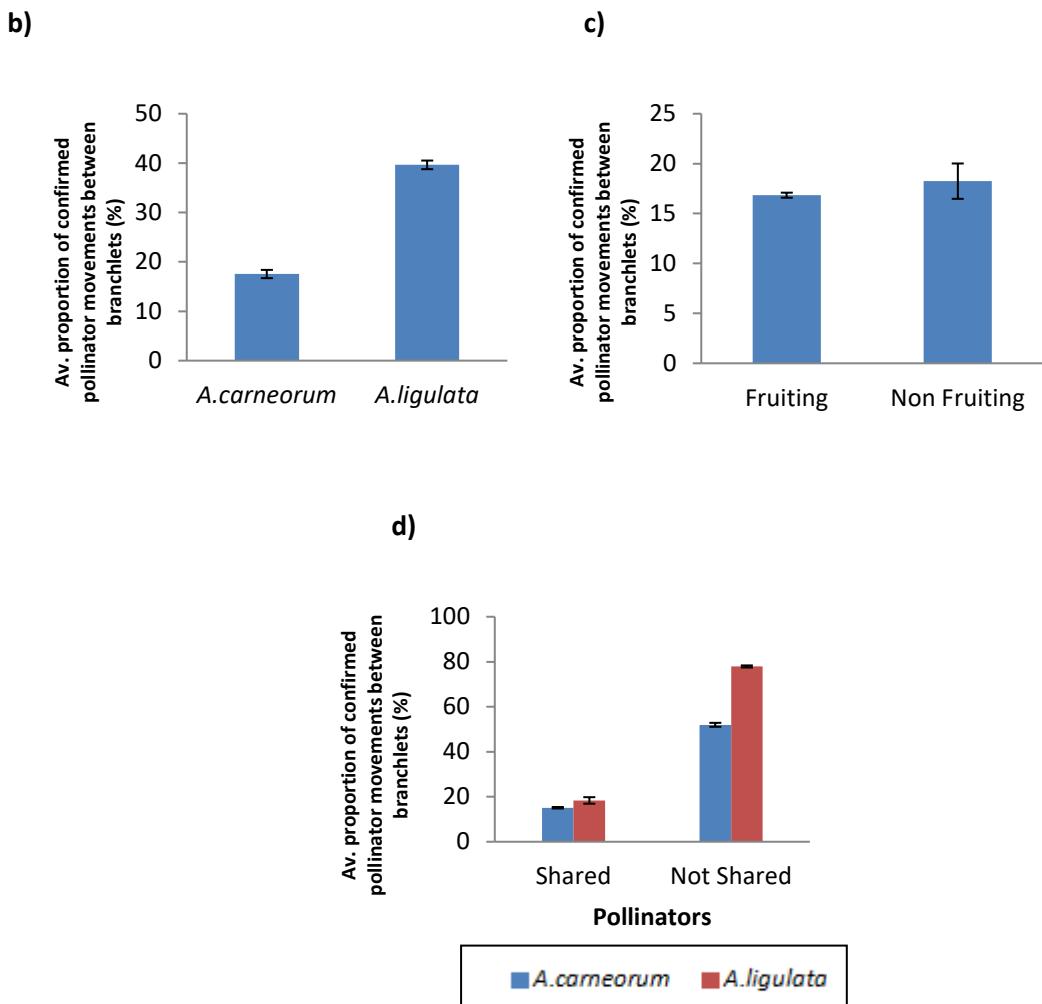
For *A. ligulata*, the proportion of movements by the average flower visitor being made between branchlets of the same plant was noticeably higher than for *A. carneorum* plants (Figure 6.8 b). Two way analysis of variance revealed this difference to be statistically significant, even when all the beetle species were removed from the analysis ( $F (1, 16) = 408.299, p < 0.001$ ). For *A. ligulata*, this ranged from similarly low numbers for fast moving flies such as *Syrphidae Erastilini* at 2%, between 21% and 31 % for six of the nine wasp species and 58% for slow moving honeybees. Again, none of the five beetle species found on *A. ligulata* flowers were ever witnessed moving from a branchlet but were seen moving between inflorescences (Figure 6.8 a). These trends were consistent across all four stands of both plant species surveyed (Appendix 6.6.4 a & b). Within an observed branchlet, the average proportion of times each insect species moved to another branchlet on the same plant after foraging was roughly equivalent at all plants. Two way analysis of variance revealed that there was no statistically significant effect of site on the proportion of times the average flower visitor was

observed to move to another branchlet within the same plant after foraging ( $F(3, 14)=0.868$ ,  $p=0.462$ ).

Independent samples t-tests revealed that the proportion of times the 15 flower visitor species shared by both *Acacia* species were observed to move to other branchlets within the same plant, was significantly higher on *A. ligulata* plants compared with *A. carneorum* plants ( $t(29) = -2.195$ ,  $p=0.048$ ). Species of flower visitor that were not shared by both *Acacia* species, moved noticeably more often to branchlets on the same plant after foraging within a branchlet in general, or were not observed to move at all (Figure 6.8 c). Insect species visiting only *A. ligulata* plants however were observed to move to other branchlets within the same plant significantly more compared to insect species that visited only *A. carneorum* plants (Figure 6.8 d). Even when the beetle species visiting these plants were excluded, honeybees alone moved within the same plant significantly more often than the remaining flower visitor species that only visited *A. carneorum* plants ( $t(2)=-26.367$ ,  $p<0.001$ ). Furthermore, honeybees were observed to move to other branchlets of the same *A. ligulata* plant significantly more often than the average flower visitor shared by both *Acacia* species ( $t(15)=-39.190$ ,  $p<0.001$ ) (Figure 6.8 d). These trends were also consistent across all four stands of both plant species surveyed (Appendix 6.6.4 a & b).

a)





**Figure 6.8. Average proportion of confirmed movements potential pollinators make between branchlets of the same on *A. carneorum* and *A. ligulata* plant after foraging, in and around Kinchega National Park, in western NSW :** a) Average proportion (%) of confirmed movements of each species of flower visitor to branchlets within the same plant after foraging, b) Proportion of movements the average species of flower visitor was confirmed to make between branchlets after foraging within a branchlet, c) Proportion of confirmed movements the average pollinator species made between branchlets of *A. carneorum* stands that produce fruit and those that do not, d) Proportion of confirmed movements the average flower visitor shared, and not shared (endemic), by both the *A. carneorum* and *A. ligulata* assemblages, made between branchlets of the same plant.\*Averages represent the average proportion of confirmed movements by each potential pollinator species visiting four branchlets, on each of three plants, within each of four stands between branchlets of the same plant after foraging within a branchlet. \*Error bars (SE) represent variation between stands of each plant species for all figures.

ii) Overall style of foraging behaviour of the assemblage

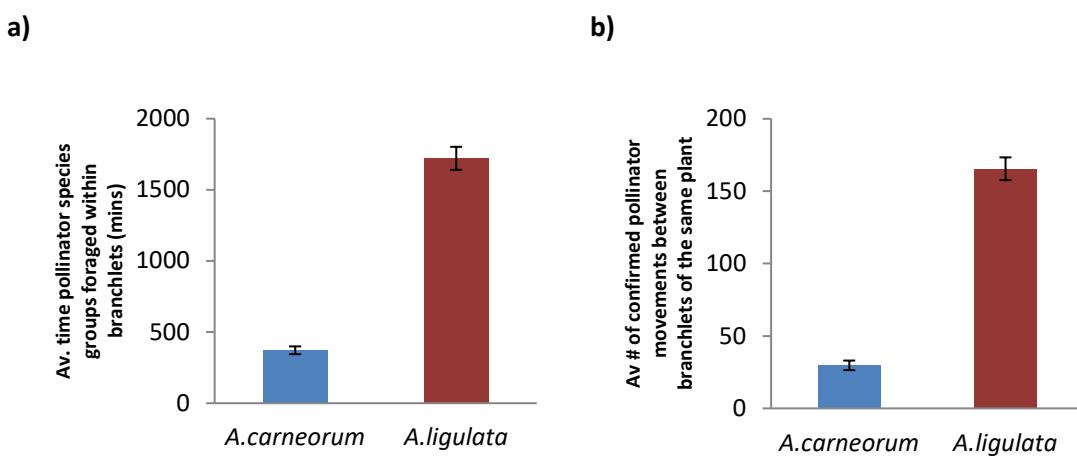
When the abundance of flower visitors was taken into account, the total length of time flower visitors of *A. ligulata* spent foraging within branchlets under observation prior to moving away, was considerably longer than the total amount of time flower visitors spent visiting *A. carneorum* branchlets, even when all beetle species visiting either species were removed from the analysis (Figure 6.9 a). Two way analysis of variance revealed that this difference was statistically significant ( $F(1, 16) = 335.35, p < 0.001$ ). Likewise, two way analysis of variance also revealed that the total number of insect movements between branchlets of the same plant after a foraging bout was higher on average on *A. ligulata* plants compared with *A. carneorum* plants, even when beetles were removed from the analysis ( $F(1, 16) = 362.77, p < 0.001$ ) (Figure 6.9 b).

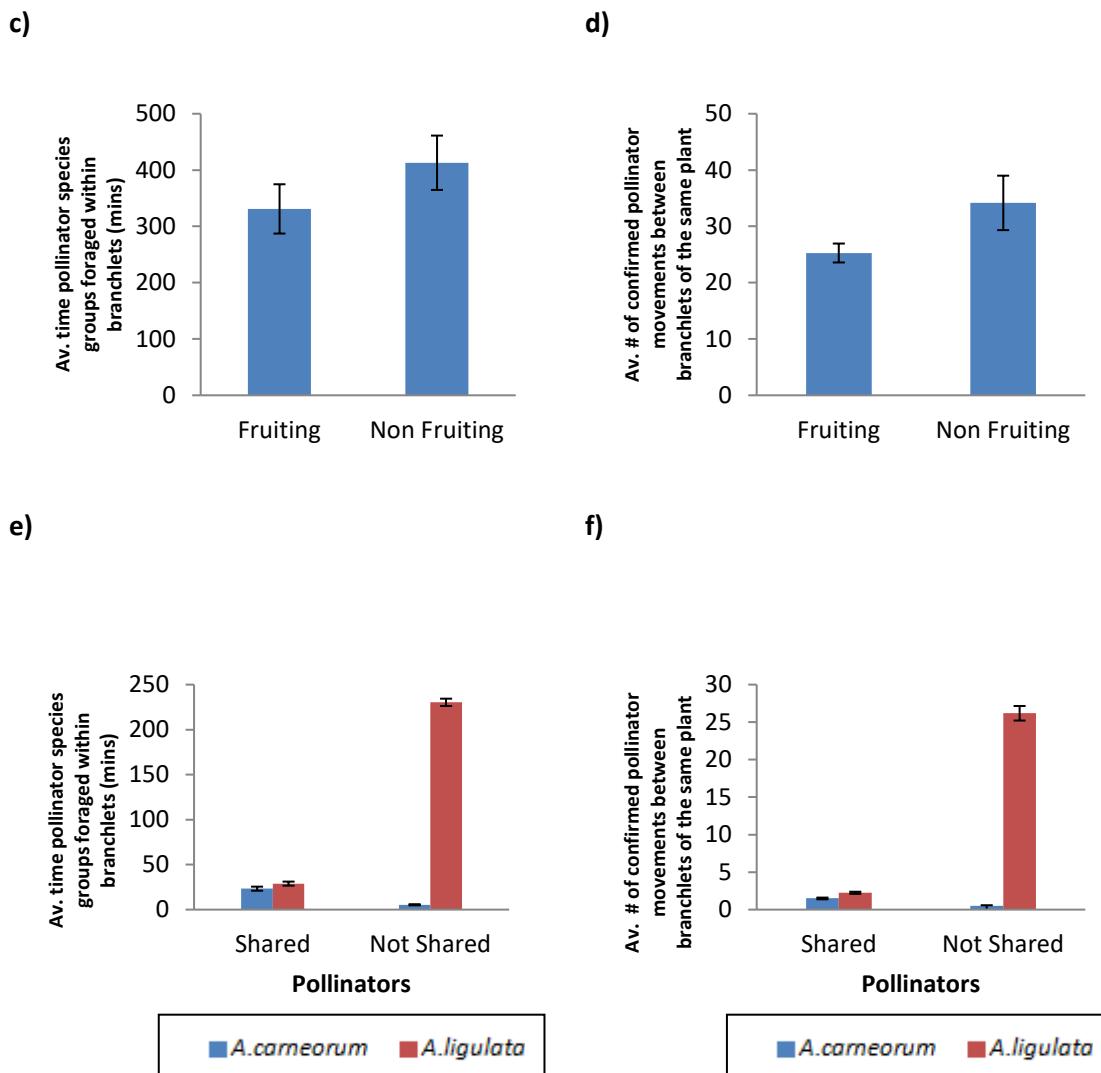
The foraging behaviours of flower visitors varied very little between the four sites of each species, suggesting a general uniformity in the foraging behaviours of each insect species. As such, the behaviours of flower visitors on *A. carneorum* plants within stands with a history of setting fruit and those without, differed very little (Figure 6.9 c & d). Two way ANOVA also revealed there was no statistical difference in the total amount of time flower visitors spent foraging within branchlets of plants, located in the four different *A. carneorum* or *A. ligulata* stands ( $F(3, -14) = 1.007, p = 0.396$ ). Similarly, between the four different *A. carneorum* and *A. ligulata* stands, there was no statistical difference in the total proportion of movements flower visitors made to other branchlets on the same plant after they finished foraging on a branchlet under observation ( $F(3, 14) = 0.919, p = 0.437$ ).

As a whole, the relatively more local foraging behaviour of the *A. ligulata* assemblage was again largely driven by introduced honeybees as well as the five beetle species which were exclusive to *A. ligulata*, rather than differences in the behaviour of the mostly shared native flower visitors (Figure 6.9 e & f). An independent samples t-test revealed that the total amount of time the 15 shared insect species spent foraging within branchlets was on average significantly higher on *A. ligulata* plants than on *A. carneorum* plants ( $t(29) = -1.762, p = 0.092$ ), whilst the flower visitors that visited only *A. ligulata* also spent on average significantly more time foraging within branchlets than the insect species found to visit only *A. carneorum* plants (Figure 6.9 e). Even when the beetle species visiting these plants were excluded, honeybees alone spent significantly longer foraging within branchlets as a species group than the only remaining two flower visitors that visited only *A. carneorum* plants ( $t(2) = 54.620, p < 0.001$ ). Furthermore, those insect species that only visited *A. ligulata* plants spent on average

noticeably more time in total foraging within branchlets than those species of flower visitor shared by both plant species (Figure 6.9 e). Again, when beetles were removed from the analysis, honeybees still spent on average significantly more time foraging within branchlets as a species group than the rest of the flower visitors combined ( $t(15)=-18.445$ ,  $p<0.001$ ). These trends were consistent across all four stands of both plant species surveyed (Appendix 6.6.3 a & b).

The majority of flower visitor species common to both *A. carneorum* and *A. ligulata* assemblages were observed to move to other branchlets on the same plant significantly more often in *A. ligulata* plants than in *A. carneorum* plants ( $t (29)=-4.344$ ,  $p<0.001$ ). Species of flower visitor that visited *A. ligulata* only were observed to move to other branchlets within the same plant far more often than species of flower visitors that visited *A. carneorum* only (Figure 6.9 f). Even when the beetle species visiting these plants were excluded, honeybees alone moved within the same plant significantly more often in total than remaining flower visitor species that only visited *A. carneorum* ( $t (2) =-26.367$ ,  $p<0.001$ ). Moreover, honeybees were observed to move to other branchlets of the same *A. ligulata* plant significantly more often as a species group than the average flower visitor shared by both *Acacia* species ( $t (15)=-49.100$ ,  $p<0.001$ ). These trends were also consistent across all four stands of both plant species surveyed (Appendix 6.6.4 a & b).





**Figure 6.9. Overall foraging style of the *A. carneorum* and *A. ligulata* pollinator assemblages in and around Kinchega National Park in western NSW:** a) Average amount of time (mins) the *A. carneorum* and *A. ligulata* assemblages spend foraging between flowers within branchlets, b) Average # of movements the pollinator assemblage was confirmed to make between branchlets within the same plant, c) Average amount of time (mins) flower visitors of *A. carneorum* plants spend foraging within branchlets before moving away within stands that produce fruit and those that do not, d) Average # of confirmed movements flower visitors made between branchlets within the same *A. carneorum* plants, within stands that produce fruit and those that do not, e) Average amount of time (mins) the shared, and not shared (endemic) flower visitors spent foraging within branchlets before moving away, f) Average # of movements shared and not shared (endemic) flower visitors, made between branchlets of the same plant after foraging within a branchlet. \*Averages represent the average amount of time that potential pollinators spent visiting flowers on all four branchlets, on each of three plants, within each of four stands surveyed. \*Standard error bars represent variation between the four stands of each plant species for all figures except Figure 7 e & f where SE bars represent variation between the two fruit setting and two non fruit setting *A. carneorum* stands surveyed.

## **6.5 Discussion**

### **Evaluating the pollination services of stands**

While it cannot be confirmed whether important pollinators of either *A. carneorum* or *A. ligulata* have been lost from the region, or even whether all the species of flower visitor were found to be carrying pollen are true pollinators able to effectively deposit pollen they may be carrying, or whether they may be carrying too little to expect pollination to occur (Ivey et al., 2003; Lsadler, 2006; Orford & Memmott, 2015), frequent visitation by a suite of native insects carrying viable pollen suggests that pollen limitation is unlikely to explain the lack of seed set in the majority of *A. carneorum* stands. Whilst I found an almost identical set of native insects visiting *A. ligulata* flowers, the finding of large numbers of introduced honeybees visiting *A. ligulata* plants, as found the previous year (Gilpin et al., 2014), is likely to have significant consequences for their mating systems, genetic structure and adaptive capacity as has been found for many other native plant species (Taylor and Whelan, 1988; Taylor and Whelan, 1988; Vaughton, 1992; Vaughton, 1996; and England et al., 2001).

#### **6.5.1 Comparing and contrasting fruit setting and non fruit setting *A. carneorum* stands in and around Kinchega National Park.**

The difference in sexual function between the majority of *A. carneorum* stands that have continuously failed to set seed and the minority that have been observed to set seed, could be theoretically explained in several ways ranging disruption of pollinators (Turner et al., 1982; Hedrick, 1985; Charlesworth & Charlesworth, 1987; Levin et al., 2009), to natural differences among stands (Wyatt, 1986; Sartor et al., 2011; Hardion et al., 2015). My finding of equivalent numbers of the same potential pollinators, displaying similar foraging behaviours, as well as my finding of an equivalent proportion of flowers with pollen tubes reaching the ovary, highlights that hypotheses for reproductive failure, other than insufficient pollination need to be explored. Previous surveys (Chapter 3, thesis) found no significant differences between the number of flowers produced in stands with a history of setting fruit and not setting fruit, ruling out differences in flowering effort as an explanation. Whilst it is possible that there are simply natural differences in the importance of sexual reproduction between different *A. carneorum* stands, as found in many other plant species (Wyatt, 1986; Rathcke & Real, 1993; Fishman & Wyatt, 1999; Sartor et al., 2011; Hajrudinović et al., 2015; Hardion et al., 2015), it is also possible that the quality of pollen being supplied to each stand differs between stands. If a

minority of stands are receiving compatible pollen whilst the majority are not, the dichotomy of maternally successful and unsuccessful stands that we observe would be explained neatly (Goverde et al., 2002; Aguilar et al., 2006; Peterson et al., 2008; Gonzalez-Varo et al., 2010). Recent studies have shown that these same *A. carneorum* stands are all clonal (O'Brien, et al 2014; Roberts et al., *in review*), and therefore each stand has a very limited number of genotypes with which they may mate. If most isolated stands happen to be situated too far from other compatible stands then the recorded lack of seed set would be expected (Whelan et al., 2009 Mustajärvi et al., 2001; Goverde et al., 2002; Andrieu et al., 2009; González-Varo et al., 2010). Nevertheless, the possibility that higher levels of isolation might encourage inter stand movements within a foraging bout must also be considered. If stands are small and isolated, pollinators can exhaust local resources and be forced to forage over larger distances than they might otherwise, potentially increasing the diversity of pollen supplied to plants (White et al., 2002; Côrtes et al, 2013; Dick et al., 2003; Byrne et al., 2008; Hanson et al., 2008).

Distinguishing whether differences in reproductive capacity of some *A. carneorum* stands is explained by innate natural differences in the reproductive capacity of the different genets, or are a result of differences in compatibility with the pollen being provided, might only be investigated through active manipulation of the types of pollen delivered to these plants. By manually controlling the types of pollen delivered to these plants we might be able to uncover whether they require more divergent pollen than is currently being supplied by pollinators. Ideally, such experiments would provide pollen from a variety of sources both locally and from distant stands, that may have been better connected previous to anthropogenic modification of the region (Waser and Price, 1983, 1989 & 1991; Waser et al. 1987; Forrest et al. 2011).

### **6.5.2 Pollination of thriving *A. ligulata* stands in Kinchega National Park.**

My finding that the assemblage of insects visiting *A. ligulata* flowers differed to *A. carneorum* only by the presence of large numbers of European honeybees as well as several native beetle species, supports findings from the previous year (Gilpin et al., 2014). Considering the stark differences in the reproductive performance of these two co-occurring *Acacia* species, it was somewhat surprising to find here that *A. carneorum* and *A. ligulata* were pollinated by an almost identical and diverse assemblage of native pollinators. Whilst I found the proportion of flowers with pollen tubes higher on *A. ligulata* plants than *A. carneorum* plants, this was almost certainly largely driven by the many honeybees and beetles visiting *A.*

*ligulata* flowers, but not *A. carneorum*. It is uncertain whether the beetles serve as pollinators of *Acacia* flowers, however, several beetle species found on other flowers have been shown to be accidental pollinators, as well as consumers of flowers (Baker & Hurd, 1968; Crowson, 1981; Young, 1986). Honeybees have been shown to be effective pollinators of both agricultural species (Delaplane & Mayer, 2005) and many native species (Taylor and Whelan, 1988; Ramsey, 1988; Taylor and Whelan, 1988; Paton, 1990, 1993; Vaughton, 1996; England et al., 2001). My findings here that honeybees carried far more pollen on their bodies than any of the other native pollinators lends further support to this theory.

Observations of foraging behaviour of pollinators in both *A. carneorum* and *A. ligulata* stands, confirmed suspicions that pollen dispersal occurs largely within stands. Pollinators in both assemblages clearly spent the vast majority of their time foraging between flowers on the same plant as is typical of many insect pollinators (Paton and Turner, 1985; Ramsey, 1988; Vaughton, 1996; Richardson et al., 2000; England et al., 2001). As a result, I would expect the vast majority of pollen that is being deposited to be self pollen, with a lesser amount coming from neighbouring plants in the same stand. This is especially likely to be the case within these *A. ligulata* stands given that honeybees have been shown to increase the levels of inbreeding in many native plant species (Taylor and Whelan, 1988; Ramsey, 1988; Vaughton, 1996; and England et al., 2001). While honeybees' foraging behaviours are relatively local, and hence serve to mainly move self and local pollen to flowers, their effectiveness at picking up pollen means they can exhaust pollen stocks on flowers that would otherwise be transported by the native pollinators over larger distances (Taylor and Whelan, 1988). This tends to be exacerbated in smaller isolated stands where those foraging behaviours intensify within such stands (Cunningham, 2000).

Given the genetic diversity I found characteristic of at least some *A. ligulata* stands (Forrest et al., 2015), and the preference for initiating and setting outcrossed seed (Whitney, 2005), we would expect the mating system to be threatened by the domination of locally foraging honeybees now present in the system. While every *A. ligulata* plant observed produced seed despite being primarily visited by honeybees, the reduced average growth rates of seedlings produced by selfed seed, compared with manually outcrossed seed, suggests a cost in fitness for increased inbreeding in this species. Additionally, high levels of fecundity maintained in these *A. ligulata* stands, despite a potential increase in levels of inbreeding, may be a consequence of the large numbers and efficiency of honeybees as pollinators counteracting the naturally low levels of

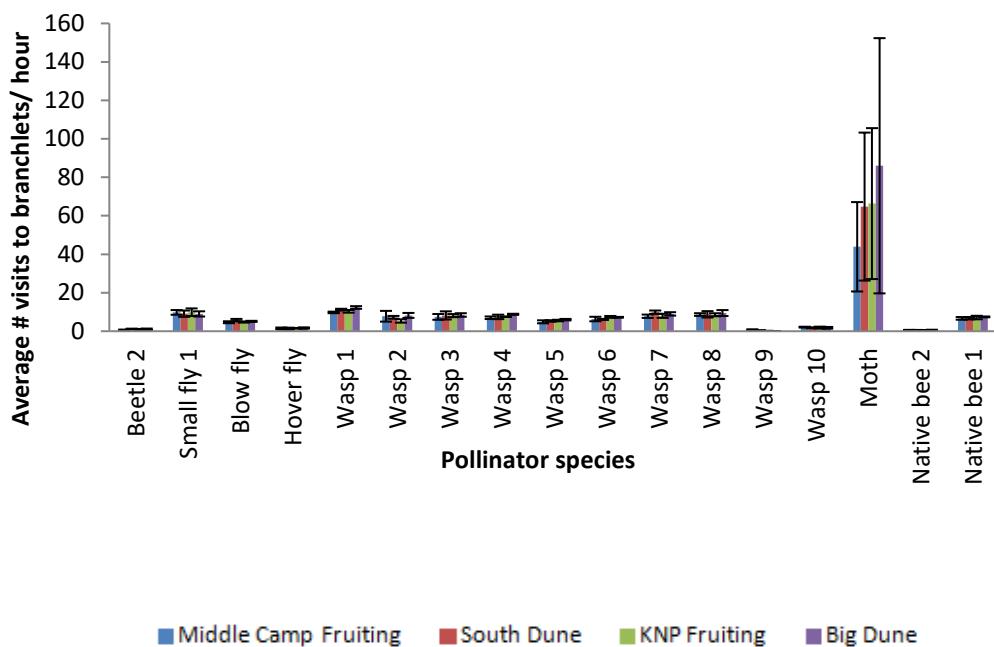
self compatibility in this species. Indeed, in a previous study self pollinations were found to result in only 6% of the *A. ligulata* seed that outcross pollinations produced (Whitney, 2005). Nevertheless, high levels of inbreeding are expected to have some negative long term effects on stand viability, especially within fragmented systems, even if only manifested as loss of genetic diversity and adaptive capacity (Kolreuter, 1761; Fischer & Matthies, 1998; Ghazoul, 2005; Leimu et al., 2006; Bowman et al., 2008; Schleuning *et al.* 2009). However, considering the high levels of genetic diversity remaining in at least some stands (Forrest et al., 2015), coupled with a mate choice mechanism favouring outcrossed pollen (Whitney, 2005b), the loss of genetic diversity within these stands may be slow.

Whether honeybee pollination is now a uniform feature across *A. ligulata* stands in the region, and whether this only occurs during periods of high rainfall or only in stands located close to standing bodies of water, all remains to be uncovered. It is important to note that all pollinator observations undertaken here have been on Kinchega National Park during periods of high water availability and while large local bodies of water such as Menindee lakes were full. Observations during drier periods are required to determine whether honeybees dominate in general or only during a time of water excess. Indeed, it is well known that honeybees need access to a permanent source of water when they are in environments with high ambient temperatures (Seeley & Visscher, 1985; Heinrich, 1996). In this sense my findings for *A. ligulata* should only be taken as how these plants respond to La Niña conditions, given they reproduce annually even in drier years. Even if honeybee pollination is only associated with these periods of high water availability, or tied to agriculture or managed hives in the region, we might still expect a significant proportion of stands (and their genetic structure) to be a product of matings facilitated by honeybees given recruitment levels during these periods of high water availability are likely higher than during drier periods (Guttermann, 2000).

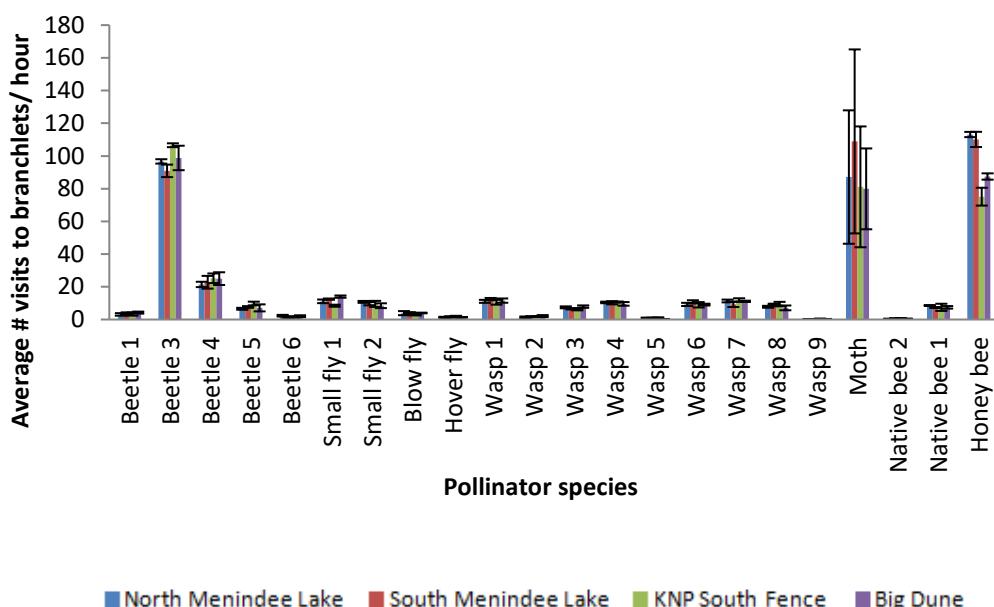
## 6.6 Appendix

Appendix 6.6.1: **Abundance of each potential pollinator species visiting a branchlet in each of four stands surveyed in and around Kinchega National Park in western NSW:** Average number of flower visitors visiting a) *A. carneorum* and b) *A. ligulata* branchlets per hour.

a)

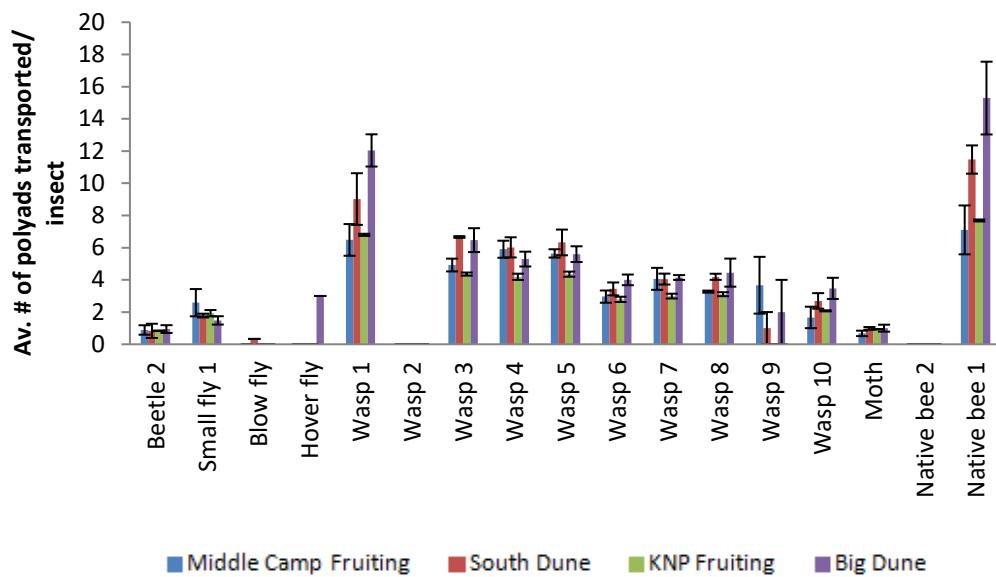


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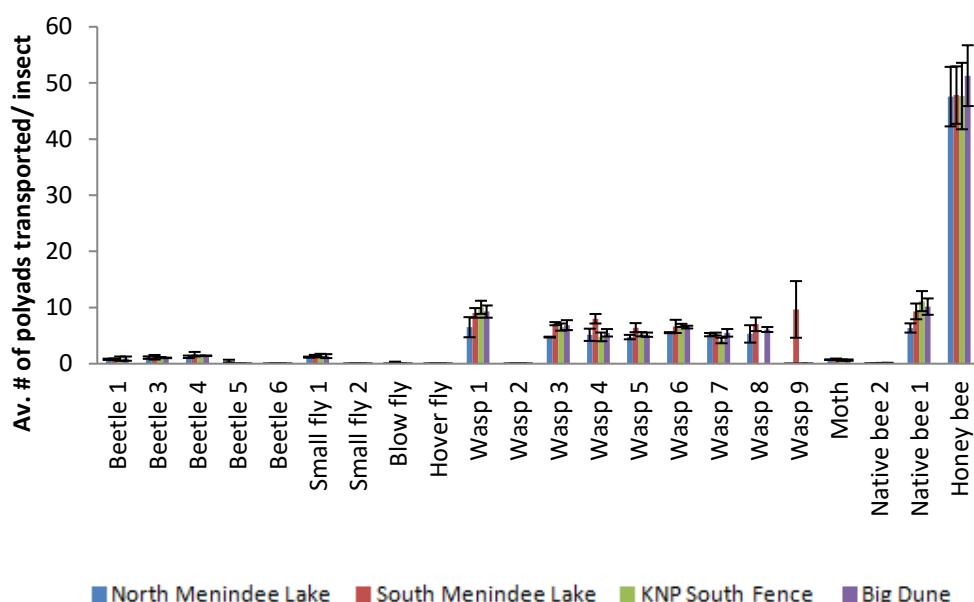


**Appendix 6.6.2: Effectiveness of flower visitors at carrying pollen in each of the four *A. carneorum* and *A. ligulata* stands surveyed in and around Kinchega National Park in western NSW:** Average number of polyads carried by each species of flower visitor to branchlets of a) *A. carneorum* and b) *A. ligulata* assemblages per hour, c) & d) Average number of polyads transported by each species of flower visitor as a species group visiting a branchlet of *A. carneorum* and *A. ligulata* (including and excluding honeybees for the latter) per hour respectively.

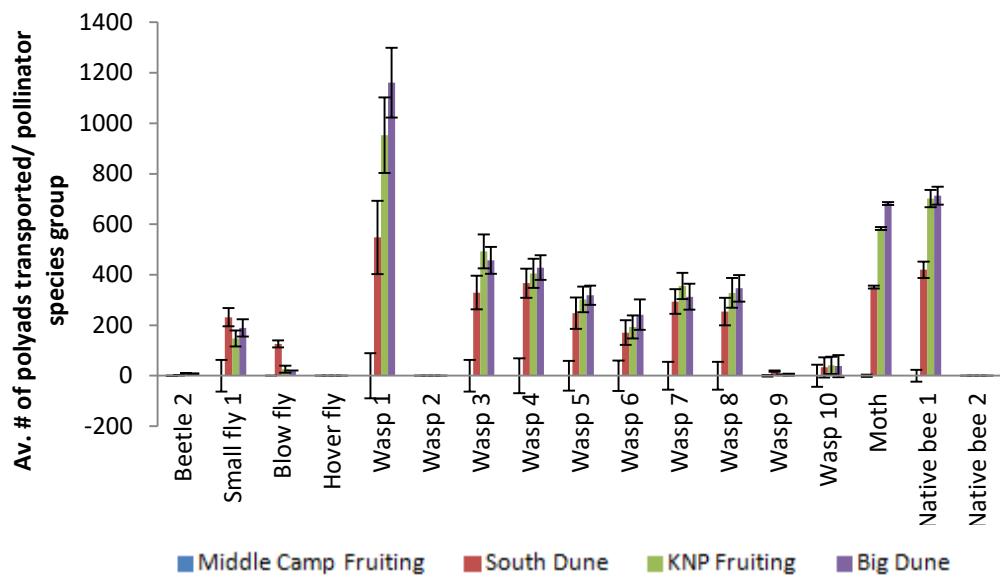
a)



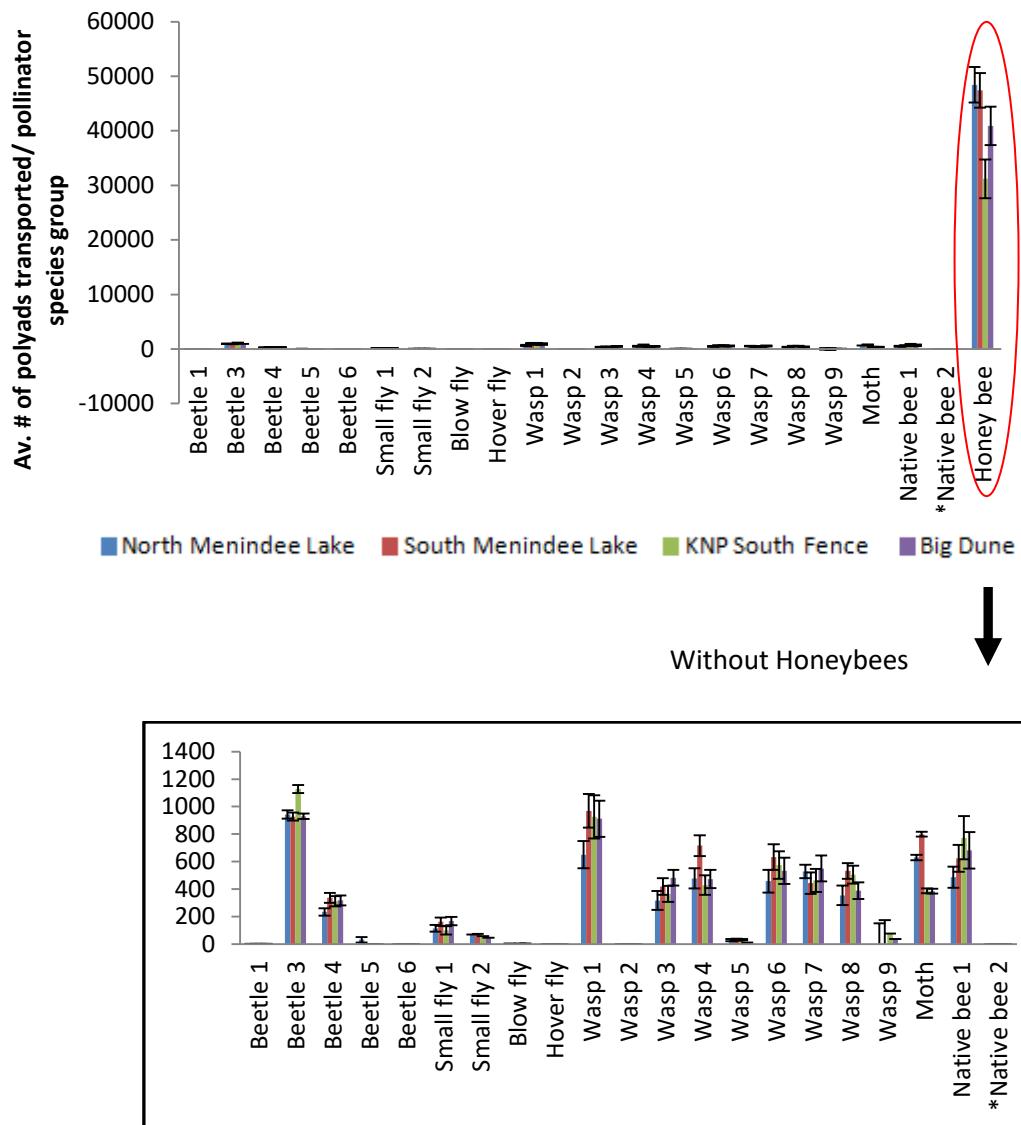
b)



c)

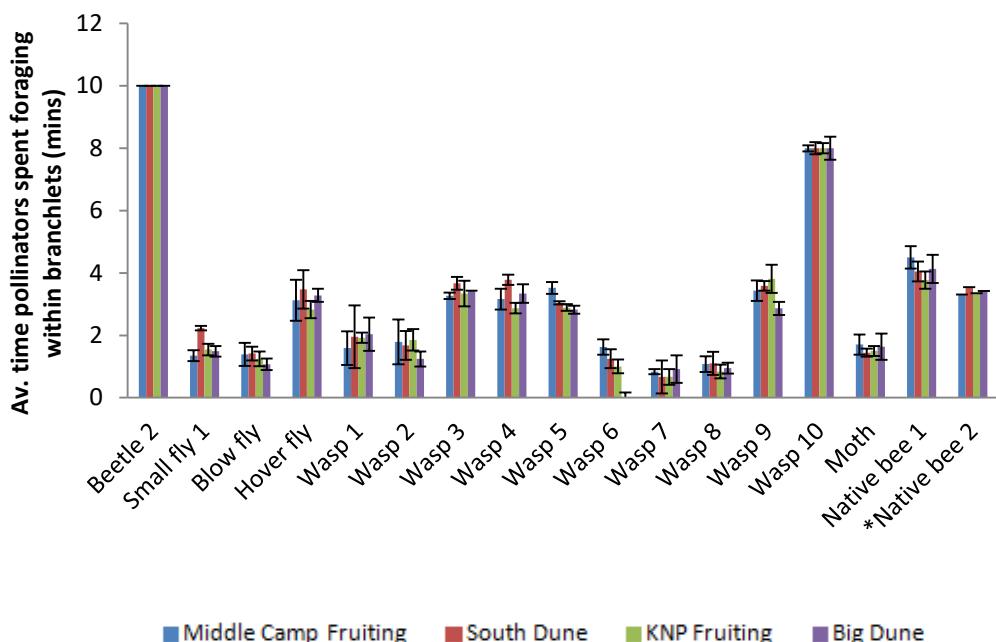


d)

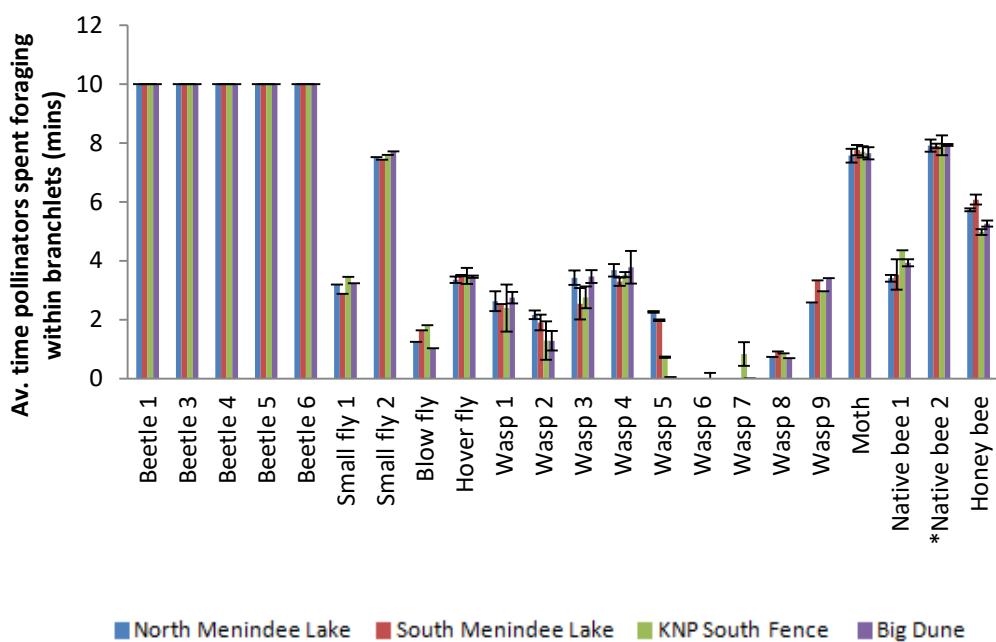


**Appendix 6.6.3. Foraging intensity of flower visitors to *A. carneorum* and *A. ligulata* plants within each of four stands surveyed in and around Kinchega National Park in western NSW:**  
 Average amount of time (mins) each flower visitor species spent foraging on a branchlet of a) *A. carneorum* and b) *A. ligulata* before moving away. \*Standard error bars represent variation between the three plants surveyed within each stand.

a)

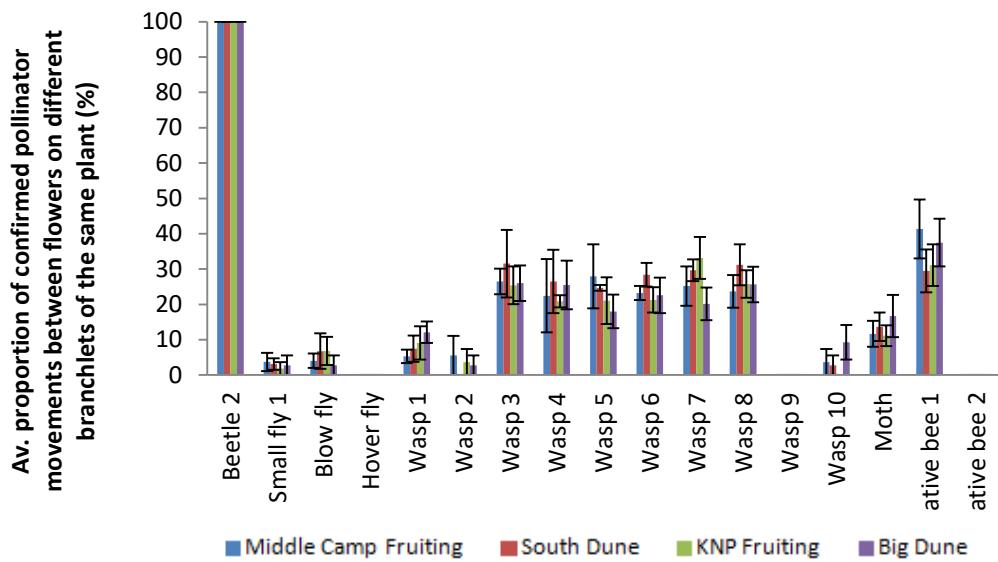


b)

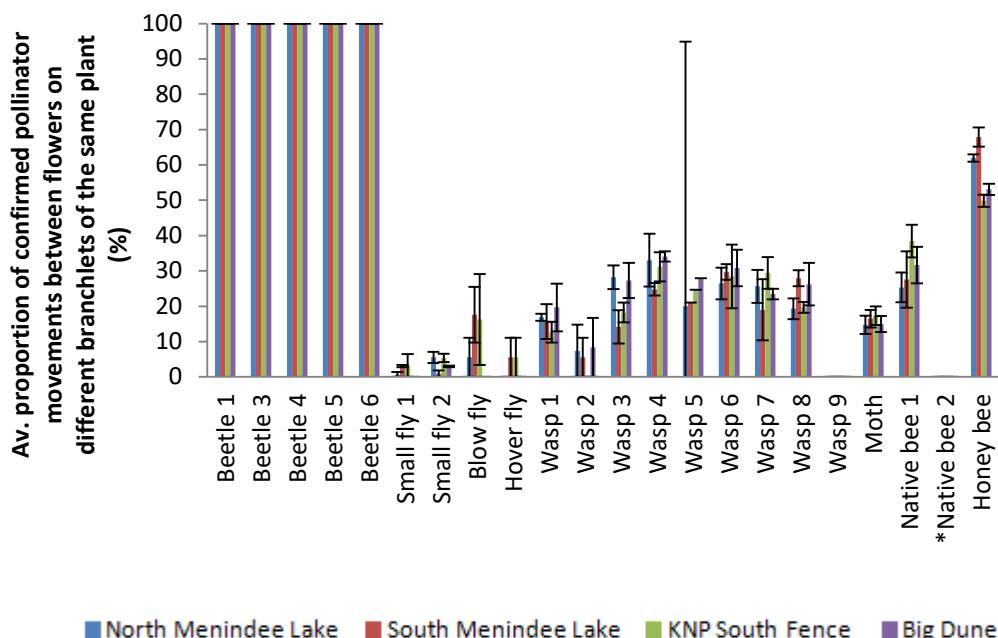


**Appendix 6.6.4. Movement of flower visitors to *A. carneorum* and *A. ligulata* plants in each of four stands in and around Kinchega National Park, in western NSW : a) & b) Average proportion (%) of confirmed movements each species of flower visitor makes to branchlets within the same a) *A. carneorum* and b) *A. ligulata* plants.**

a)



b)



## **Chapter 7: Effect of pollen quality on the reproductive output and fitness of offspring of a threatened semi arid *Acacia* species (*A. carneorum*) and a thriving and co-occurring *Acacia* species (*A. ligulata*) in far western NSW.**

### **7.1 Abstract**

Determining whether a prolonged lack of reproduction in long lived plants is true reproductive failure rather than a response to unfavourable climatic conditions, or an evolutionary loss of sexual or asexual reproduction, requires careful consideration. In chapters 3 & 6 of this thesis, I showed that pollen quantity was unlikely to explain prolonged failure to set seed within *A. carneorum* populations of western NSW. Nevertheless, because these *A. carneorum* stands contain only one or two clones, it is possible that the pollen reaching stigmas lacks sufficient genetic diversity. Here I performed manual pollination experiments and analysed naturally produced seed and seedlings to compare the effect of pollen quality on plants of *A. carneorum* and *A. ligulata*, on pollen tube germination, seed set and several measures of offspring fitness, over two consecutive years in Kinchega National Park following the large scale rain event of 2010 / 2011. Manual crosses ranged from self and local crosses to crosses between plants located hundreds of metres, several kilometres and tens of kilometres away. Although no *A. carneorum* seed was set through manual pollination, regardless of the source of the pollen applied or the stands' history of seed set, this was likely to be due to low natural seed set levels, even within stands with a history of seed set. Indeed up to 73% of flowers on plants in some *A. carneorum* stands that set no seed, were found to have pollen tubes via natural pollination. In contrast seed set levels were predictably high on *A. ligulata* plants, irrespective of the source of outcrossed pollen. Self pollen however, set less than a third as much *A. ligulata* seed by comparison in both years the experiment was run. Interestingly, naturally produced *A. ligulata* seed grew on average 18 % and 26% slower than seed produced through manual outcrossing in the first and second years the experiment was run, irrespective of the source of the outcrossed pollen applied. Moreover, manually sourced self pollen also produced seed that grew slower than the average outcrossed seed (36% & 57% slower in consecutive years). Taken together these results suggest that sexual reproduction in *A. carneorum* stands is rare at best irrespective of the quality of pollen it receives, whilst

seemingly healthy sexual reproduction in *A. ligulata* stands may hide losses in the quality of seed currently being set. While *A. ligulata* plants may be able to cope with fluctuations in the quality of pollen supplied to plants, managers may also need to consider the long term health of these seemingly thriving populations given their isolation and the presence of introduced honeybees pollinating them.

## 7.2 Introduction

Plants can fail to reproduce for a variety of reasons related to physiological stresses (Jennersten 1988; Ouborg et al., 1991; Goverde et al., 2002; Steffan-Dewenter & Westphal, 2008) and compromised mating systems (Charlesworth et al., 1987; Aizen & Feinsinger, 1994; Jacquemyn et al. 2003; Aguilar et al. 2006). For long lived plants, assessments of reproductive health can be complicated by the fact that a lack of seed set over a prolonged period may simply be a natural response during unfavourable climatic conditions, a low investment in sexual reproduction (Kearney, 2003; Honnay & Jacquemyn, 2008; Vallejo-Marín et al., 2010), or a natural transition from sexual to asexual reproduction (Silander, 1985; Caraco & Kelly 1991; Piquot et al. 1998; Honnay & Bossuyt 2005). However, the drivers of reproductive failure in long lived plants that reproduce irregularly have rarely been investigated owing to the difficulty of predicting when reproduction will occur. *A. carneorum* stands in far western NSW, which now exist as often highly fragmented and aging stands as a result of land clearing and prolonged reproduction and recruitment failure (Auld 1993, 1995, Auld & Denham, 2001; Porteniers, 2001), provide an ideal model system to investigate prolonged reproductive failure of acutely fragmented long lived overstory species.

Previous findings that prolonged sexual reproductive failure in the majority of *A. carneorum* stands across western NSW was unlikely to be the result of drought or senescence (Chapter 3, thesis), or limitation in pollen quantity (Chapter 6, thesis), suggests that the lack of seed set reflects sterility or is a consequence of incompatible pollen being supplied to plants (Crow & Kimura, 1970; Oostermeijer et al. 1995; Fischer & Matthies, 1998; Aguilar et al. 2006). Here I take advantage of rare conditions produced by a region wide rain event, likely to be optimal for reproduction of arid plants (Büsgen & Münch 1929; Norton & Kelly 1988; Letnic & Dickman, 2006; Wardle et al., 2013) to present *A. carneorum* plants with pollen from a variety of local and distant sources, to test the latter hypothesis that historic failure to set seed in most stands is a function of plants receiving incompatible pollen. I also run these experiments on *A. ligulata* simultaneously, as a comparison to assess the effect of pollen source on the fecundity and fitness of offspring produced in a thriving and readily reproducing co-occurring *Acacia* species.

## **7.3 Methods**

To ascertain whether pollen of different quality affects the reproductive outcomes of *A. carneorum* and *A. ligulata* plants, as well as to compare the reproductive outcomes of outcrossing between various stands with natural pollination, pollen was collected from several sources ranging in distance from the experimental plants (recipient plants) and applied to these recipient plants. The quantity and quality of offspring produced was measured and compared with naturally pollinated flowers.

### **7.3.1 Pollination treatments**

Multiple sources of pollen were selected to capture a range of distances from recipient plants, beginning with self pollen to pollen from distant stands that are unlikely to be connected by pollinators regularly, if at all. The effect of natural pollination was assessed by simply allowing select flowers to be exposed to local pollinators. The specific pollination treatments that recipient *A. carneorum* and *A. ligulata* plants were exposed to in consecutive years are listed in Table 7.1 and detailed descriptions of the source of those pollination treatments are listed in Table 7.2.

### **7.3.2 Design of pollination experiments**

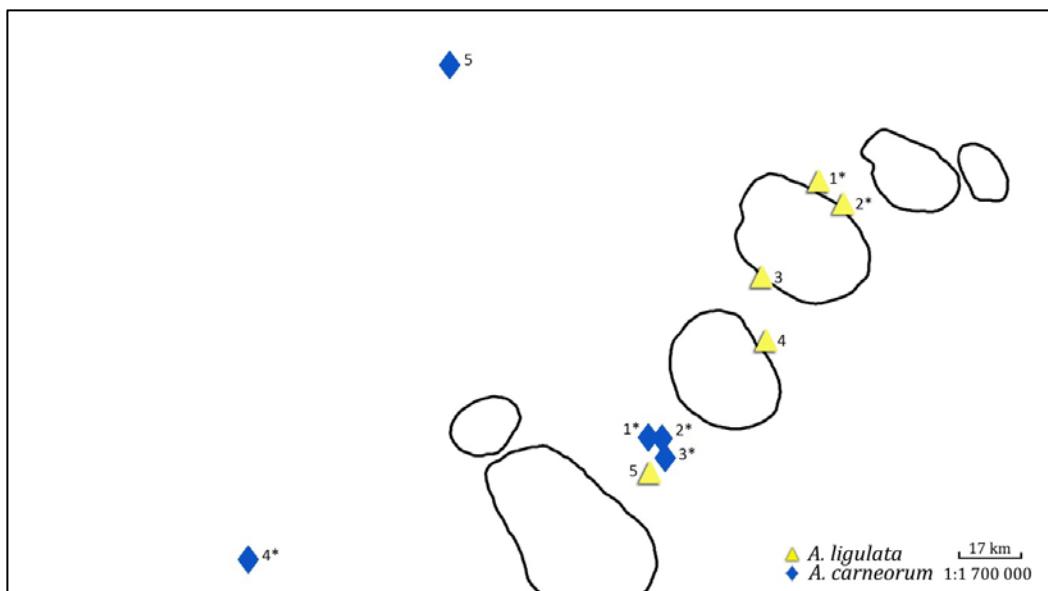
- i) *Selection of recipient and donor stands and plants*
- a) *Recipient sites and plants*

During the peak of flowering seasons of January 2011 and 2012, I used two and three *A. carneorum* stands within Kinchega National Park as recipient stands to assess the effects of natural and experimental pollen additions (Figure 7.1). For *A. carneorum* I chose both fruiting and non-fruiting stands with sites varying between the two years the experiment was run (Table 7.1, Figure 7.1). For *A. ligulata* I used the same two stands, located on the edge of Lake Menindee in both 2010 and 2011 (Figure 7.1). In each case these stands acted as recipient stands to assess the reproductive outcomes of both natural insect facilitated pollinations and experimental pollen additions using pollen from both local and distant sources.

Multiple recipient plants were selected haphazardly within each stand such that they were all within a 200 m radius (see Table 7.1 for numbers). An equal number of plants within recipient stands were also haphazardly chosen at least 10 m away from any of the recipient plants to act as controls. These plants were deemed far enough away from the bagged recipient plants for pollinators to be undeterred from pollinating branchlets left bagless due to the presence of bags on recipient plants.

*b) Donor sites and plants*

Multiple sites were chosen to be pollen donor sites from which to harvest pollen for transfer to recipient sites. I tried to capture a range of stands located near and far from the recipient stands (Figure 7.1 & Table 7.1) in an attempt to source pollen from plants with differing levels of genetic divergence from the recipient stands, presuming such diversity exists. As within recipient stands, multiple donor plants (Table 7.1) were selected haphazardly within each donor stand such that they were all within a 200 m radius so as to be readily accessible.



**Figure 7.1. Location of *A. carneorum* and *A. ligulata* stands used as recipient and donor stands for manual pollination experiments conducted over two consecutive years in and around Kinchega National Park in far western NSW:** *A. carneorum* stands (1. Kinchega National Park (KNP) Fruiting, 2. Big Dune, 3. South Dune, 4. Middle Camp (MC) Fruiting, 5. Quandong stands). *A. ligulata* stands (1. North Menindee Lake, 2. South Menindee Lake, 3. South West Menindee Lake, 4. Cowndilla Lake and 5. South West Kinchega). \*Indicates stands that were used as recipient stands in either one or both of the consecutive years.

ii) *Set up of pollination experiment*

a) *Recipient plants*

I randomly selected multiple branchlets spread throughout the canopy of recipient plants. Each branchlet contained between 10 and 20 inflorescences which in turn each contained approximately 20-30 unopened flowers. All already opened flowers on these branchlets were removed and the branchlets were bagged in organza to prevent the natural pollination of then unopened flowers. Bagged branchlets were given a coloured and numbered tag, with the randomly assigned colour representing a different pollination treatment that newly opened flowers would receive. As all treatments were replicated on recipient plants and the number of treatments and replicates were increased in the second year of the experiment for both plant species, multiple branchlets per plant were assigned for each treatment (Table 7.1).

On the set of control plants, located within the same stands as the recipient plants (at least 10 m from the recipient plants), the same number of branchlets that were assigned that year for ‘open treatments’ on recipient plants (Table 7.1), were bagged in the same way. These acted as an alternative set of branchlets left open to pollinators to pollinate (‘open distant treatment’) once the experiment began.

b) *Donor plants*

Four times as many branchlets as bagged on recipient plants were bagged on donor plants after flowers on those branches, that were already opened, were similarly removed, to ensure there was enough freshly opened flowers available over consecutive days to pollinate all the flowers that opened on all recipient plants that day. Recipient plants also doubled as donor plants. On each recipient plant at least four times the number of branchlets chosen to receive pollen were also bagged in the same manner (after already open flowers were removed) to provide a source of donor pollen to be transferred to flowers within the same plant (selfing), between the recipient plants within the same stand (‘intra cross’) and between plants in different stands (‘inter cross’) (Table 7.1).

**Table 7.1. Details of experimental pollination of *A. carneorum* and *A. ligulata* plants in Kinchega National Park:** Numbers, names and fruiting status of recipient and donor stands and plants, including the use and approximate distance of donor stands from recipient stands, and the number and types of all treatments applied to recipient plants specifying the overall number of branchlets, inflorescences and flowers (estimate) pollinated over two consecutive years following a La Niña rain event across western NSW.

(a) *Acacia carneorum*

Year	Recipient stands and plants			Donor plants and stands				Treatments			
	Stand name (Number of plants used)	Sexual history	# plants/ stand	Donor stands (6 plants from each)	Sexual history	Approximate Distance to recipient plants	# treatments/ recipient plant	Types	No. bagged branchlets/ treatment	Recipient stands	# inflorescences pollinated (approx 25 flowers/ inflorescence)
2011	KNP Fruiting site(KNP(F))	Non fruiting	6	Big Dune	Non fruiting	10-50m	5	Open	6	KNP(F)	540
				South Dune	Non fruiting	1km		SD		SD	480
				KNP Fruiting	Fruiting	400-500m		Open distant		KNP(F)	516
				Quarry	Fruiting	50km		SD		SD	504
				Big Dune	Non fruiting	10-50m		Intra	6	KNP(F)	462
								SD		SD	474
								Inter		KNP(F)	558
								SD		SD	528

				South Dune	Non fruiting	1km		Inter- Distant	6	KNP(F)	528
				KNP Fruiting	Fruiting	400-500m			SD	540	
				Quarry	Non fruiting	50m					
2012	Big Dune (BD)	Non fruiting	6	Big Dune	Sterile	10-50m	5	Open	6	BD	534
				KNP Fruiting	Fruiting	400-500m				SD	558
				Middle Camp	Fruiting	30km				MC	492
				Quarry	Fruiting	50km		Intra	6	BD	552
				Big Dune	Non fruiting	10-50m				SD	510
	South Dune (SD)	Fruiting	6	KNP Fruiting	Fruiting	400-500m				MC	528
				Middle Camp	Fruiting	30km	Inter 1	6	BD	510	
				Big Dune	Non fruiting	10-50m			SD	432	
				KNP Fruiting	Fruiting	400-500m			MC	516	
				Middle Camp	Fruiting	30km	Inter 2	6	BD	450	

				Quarry	Non fruiting	50km				SD	480
Middle Camp (MC)	Fruiting			Big Dune	Non fruiting	30km				MC	480
				KNP Fruiting	Fruiting	30km				Inter-Distant	6
				Middle Camp	Fruiting	Approx 10-50m				BD	528
				Quarry	Non fruiting	Approx 80km				SD	516
										MC	492

(b) *Acacia ligulata*

Year	Recipient stands and plants			Donor plants and stands				Treatments			
	Stand name (Number of plants used)	Sexual history	# plants/stand	Donor stands (6 plants from each)	Sexual history	Approximate Distance to recipient plants	# treatments/recipient plant	Types	No. bagged branchlets/treatment	Recipient stands	# inflorescences pollinated (approx 25 flowers/inflorescence)
2011	North Menindee	Fruiting	3	North Menindee	Fruiting	Approx 2-10m	6	Open	6	NM(N)	238
										NM(S)	234

	Lk .(North site) (NM(N))			Lake							
				South Menindee Lake	Fruiting	Approx 1km			Open distant	6	NM(N) 394 NM(S) 352
				North Menindee Lake	Fruiting	Approx 1km			Intra	6	NM(N) 133 NM(S) 110
				South Menindee Lake	Fruiting	Approx 2-10m			Inter	6	NM(N) 136 NM(S) 130
									Selfed	6	NM(N) 124 NM(S) 133
									Autogamy	6	NM(N) 211 NM(S) 205
2012	North Menindee Lk. (North site) (NM(N))	Fruiting	4	North Menindee Lake	Fruiting	Approx 2-10m	9		Open	4	NM(N) 165 NM(S) 163
				South Menindee Lake	Fruiting	Approx 1km			Open distant	4	NM(N) 159 NM(S) 161
				Far South Menindee Lake	Fruiting	Approx 10km			Intra	4	NM(N) 265 NM(S) 203

North Menindee Lk. (South site) (NM(S))	Fruiting	Cowandilla Lake	Fruiting	Approx 20km		Inter	4	NM(N)	377	
		South Fence	Fruiting	Approx 35km		NM(S)	340			
		North Menindee Lake	Fruiting	Approx 1km		Selfed	4	NM(N)	219	
						NM(S)	377			
		South Menindee Lake	Fruiting	Approx 2-10m		Autogamy	4	NM(N)	163	
						NM(S)	238			
		Far South Menindee Lake	Fruiting	Approx 10km		Inter- Distant 1	4	NM(N)	158	
						NM(S)	167			
		Cowandilla Lake	Fruiting	Approx 20km		Inter- Distant 2	4	NM(N)	203	
						NM(S)	163			
		South Fence	Fruiting	Approx 35km		Inter- Distant 3	4	NM(N)	276	
						NM(S)	158			

**Table 7.2. Treatment types used for manual pollination experiments of *A. ligulata* and *A. carneorum* plants on Kinchega National Park in far western NSW:** Description of the seven different pollen treatments manually or naturally applied to flowers on designated branchlets.

Number	Treatment	Origin of pollen applied	Purpose of treatment
1	Local outcross ('Intra' treatment)	Pooled pollen from six haphazardly selected plants within each recipient stand, other than the recipient plants	Simulate local outcrossing between plants within the same stand
2	Outcross ('Inter' treatment)	Pollen pooled from six haphazardly selected plants was reciprocally applied to recipient plants in each recipient stand	Simulate outcrossing with movement among neighbouring stands
3	Distant outcross ('Distant' treatment)	Pollen pooled from six haphazardly chosen plants within stands distant to the recipient stands (>5km away) was transported and applied to recipient plants	Simulate outcrossing over distances that pollen transfer is likely to be less frequent than matings between local plants
4	Selfing ('Self' treatment)	Pollen collected from bagged flowers within each of the recipient plants was applied to flowers on those same plants	Simulate self pollination
5	Natural pollination ('Open' treatment)	Brought in by pollinators from unknown source/s (bags were removed once the first flower in each bag had opened)	Compare the matings of natural pollination against manual treatments
6	('Autogamy' treatment)	No pollen applied (bags were left on branchlets throughout the duration of the experiment)	Control for the handling of inflorescences and bags
7	Natural pollination control ('Open distant' treatment):	Brought in by pollinators from unknown source/s (identical to the open treatment but performed on four other plants no closer than 10 metres from a recipient plant)	Control for our presence and the presence of bags on natural pollinator foraging behaviour

c) *Running pollination experiments*

The bagged inflorescences on recipient plants were checked each day for flower opening. When all flowers on a large portion of inflorescences had opened, the experiment began. All donor plants were visited early in the morning. Bags were opened and all open inflorescences within these bags were picked and stored in Petri dishes. All inflorescences picked from plants in the same stand were then pooled and stored in insulated boxes for transport (to keep pollen fresh). Donor inflorescences were taken to each recipient stand for application of pollen grains to the flowers within bagged branchlets assigned for those treatments, within two hours of collection.

On arrival at recipient plants the bags on all the ‘open’ and ‘open distant’ treatments were then removed to allow natural pollinators access to these flowers. Each Petri dish was shaken to free polyads into the bottom of the dish and pollen was transferred to recipient inflorescences using small squares of paper attached to sticks. This was achieved by gently wiping the end of the pollen laden square over the stigmas of each inflorescence until the pollen was transferred to the stigmas of flowers and visibly removed from the device (Figure 7.2). When all flowers on each branchlet were exposed to the pollen type that was reserved for them at least twice, the bag was replaced. All pollen treatments were administered to all recipient plants in all recipient stands within one day. To ensure that each plant was pollinated at the same time of day, several people were used to apply pollen to inflorescences / flowers on branchlets assigned to the three manipulative treatments on each plant within each of the recipient stands. This process was repeated every day for four consecutive days until all opened inflorescences within each bag had been pollinated at least twice. The remaining unopened inflorescences were removed.

Each worker rotated daily to manually pollinate a different plant at each site such that they never pollinated the same plant two days in a row, to reduce the impact of variation in pollination technique (Figure 7.2). The order in which each treatment was applied to plants was also rotated each day to safe guard against collected pollen losing vigour in storage throughout the day.

On the third day of pollination, three inflorescences (approximately 60-90 flowers) were removed from each bag and stored to look for evidence of pollen tube growth. Once the last day of pollination was complete, bags were then replaced onto the branchlets allocated for both ‘open’ and ‘open distant’ treatments to halt any further access by pollinators, and all bags

on all treatments were firmly fastened. The bags on *A. carneorum* recipient plants were left on until May of the same year and those on *A. ligulata* recipient plants were left until January of the following year. This allowed time for fruit to mature fully at which time bags were opened and fruit within each bag collected for analysis.



**Figure 7.2. Manual pollination of an *A. ligulata* plant at Kinchega National Park in western NSW:** (clockwise from top left) Manual pollination of *A. carneorum* flowers, Pollen applicator applying pollen to an *A. carneorum* inflorescence, Bagging of *A. ligulata* branchlets with organza bags after manual pollination, Bagged *A. ligulata* branchlet left over night after manual pollinations.

*d) Measurements of reproductive success*

The reproductive outcomes of my experimental pollinations and those of the open treatments were measured in several ways (Table 7.3).

**Table 7. 3. Measurements of reproductive effort and offspring fitness in stands of *A. ligulata* and *A. carneorum*:** Description of four measurements of reproductive effort and offspring fitness and descriptions of these measurements.

Measurement	How it was measured
(i) Pollen tube growth	-Proportion of flowers with pollen tubes visible within a)the style and b) reaching the ovules.
(ii) Reproductive success	-Fruit set (% of inflorescences exposed to pollen treatments that set mature fruit)
(iii) Quality of fruit and seed	-Number of seed per fruit and seed weight (g)
(iv) Fitness of seedlings	-Average growth rate (cm/time) -Seedling survival (%)

The fitness of seedlings was tested by growing scarified seed in 10cm by 10cm by 20 cm deep punnets, outdoors, under coastal conditions (Sydney) with regular watering (every second day). Coastal soil was used in each punnet which was inoculated with soil from the area around where the seed were collected (20% of soil).

### **7.3.3 Statistical analysis of data**

I used two-way analysis of variance (2 way ANOVA) with sites (*Acacia* stands) nested in pollen treatments, and Tukey's post hoc tests to compare the response of plants to manual pollination with pollen from a variety of sources, as well as between the multiple stands of both *A. carneorum* and *A. ligulata*, in two consecutive years separately. Specifically, the predictor variable for these models is 'Pollen source', whilst the response variables are 1. Reproductive potential (Percentage of flowers that contained pollen tubes), 2. Reproductive success (Fruit set (%)), 3. Quality of fruit and seed (Number of seed per fruit and seed weight (g)) and 4. Fitness of seedlings (Average growth rate (cm/time) & Seedling survival (%)). Whilst my data did not always pass the Sahapiro-Wilks test of normality and Levine's tests of equal

variances, despite log, square and square root transformations, advice from statistical consultants indicated that ANOVA was still appropriate given the nature of these data being normally distributed, experimental designs being balanced and large sample sizes (Underwood, 1981 1997).

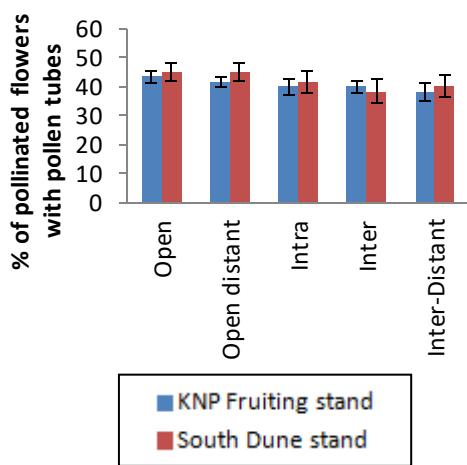
## 7.4 Results

### 7.4.1 Assessing the effect of pollen source on reproductive success

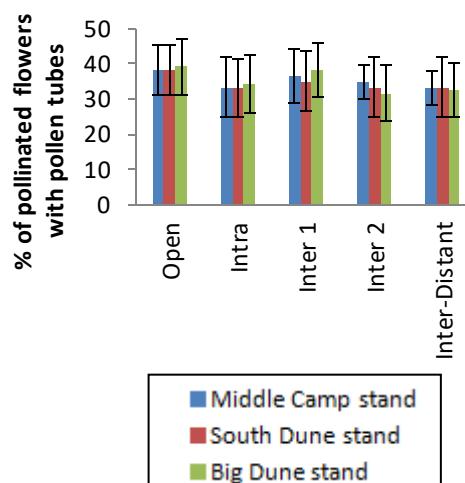
#### (i) Reproductive potential

I found little effect of pollen treatment on the proportion of flowers that were found to have pollen tubes initiating down the styles of flowers for both *Acacia* species. In each year, a similar proportion of both manually and naturally pollinated flowers of each species were found to possess pollen tubes initiating down the styles of flowers across all five treatments (Figure 7.3 a -d). Two-way ANOVA revealed no significant difference in the proportion of pollinated flowers growing pollen tubes between the five treatment types, in either year of the experiment on *A. carneorum* plants ( $F(4, 7)=1.159$ ,  $p=0.360$  for year 1 and  $F(4, 13)=0.316$ ,  $p=0.865$  for year 2), or the five and eight treatments (excluding the autogamy treatments) on *A. ligulata* plants ( $F(4, 7)=3.413$ ,  $p=0.068$  in year 1 and  $F(4, 7)=0.000$ ,  $p=0.984$  in year 2).

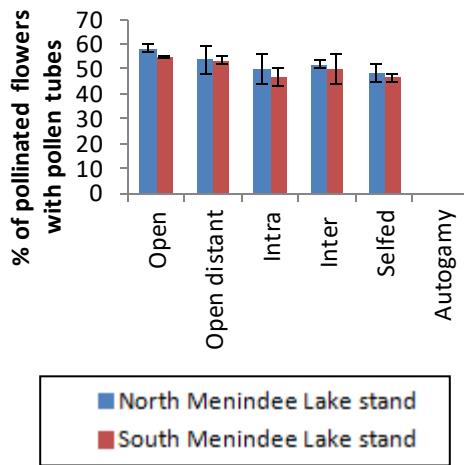
a) *A. carneorum* (2011)



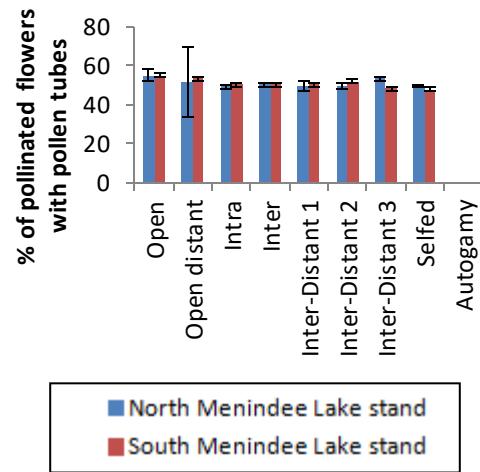
b) *A. carneorum* (2012)



c) *A. ligulata* (2010)



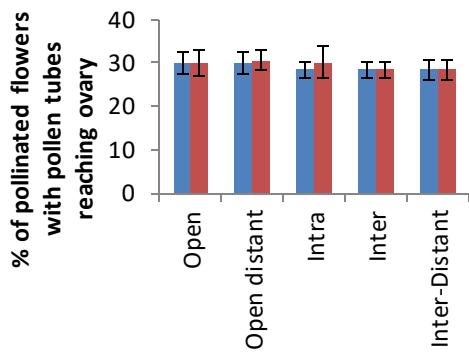
d) *A. ligulata* (2011)



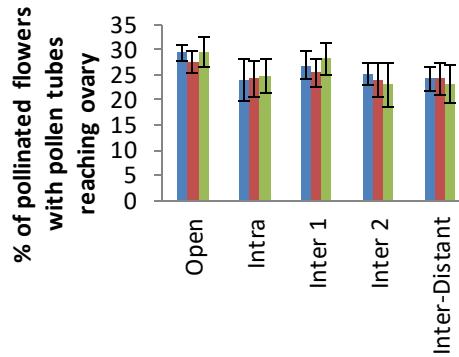
**Figure 7.3. Effect of several pollen addition treatments and open pollination on pollen tube growth in multiple stands of *A. carneorum* and *A. ligulata* within Kinchega National Park:** The ‘KNP Fruiting’ and ‘Middle Camp’ *A. carneorum* stands had a history of setting fruit naturally whilst the other *A. carneorum* stands did not. Only one *A. carneorum* stand ('South Dune') was used in consecutive years. \*Error bars represent variation (SE) between plants within recipient stands.

Of the flowers that contained pollen tubes, a great and roughly equivalent majority grew pollen tubes all the way to the ovaries of flowers, irrespective of the source of pollen (Figure 7.4 a-d). Two-way ANOVA revealed no significant difference in the proportion of pollinated flowers growing pollen tubes to the ovaries between the five treatments types, in either year of the experiment on *A. carneorum* plants ( $F(4, 7)=0.720$ ,  $p=0.587$  for year 1 and  $F(4, 13)=0.315$ ,  $p=0.865$  for year 2), or the five and eight treatments (excluding the spontaneous autogamy treatments) on *A. ligulata* plants ( $F(4, 7)=2.912$ ,  $p=0.091$  in year 1 and  $F(7, 4)=1.168$ ,  $p=0.391$  in year 2).

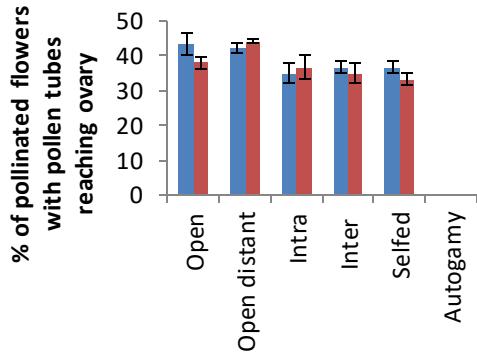
a) *A. carneorum* 2011



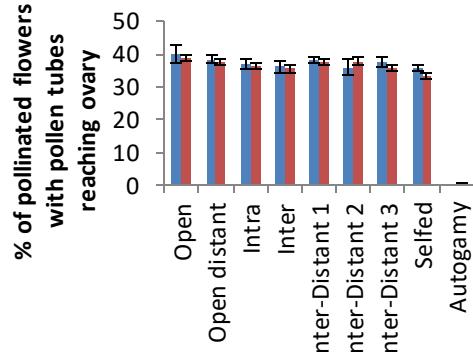
b) *A. carneorum* 2012



c) *A. ligulata* 2010



d) *A. ligulata* 2011



■ North Menindee Lake stand  
■ South Menindee Lake stand

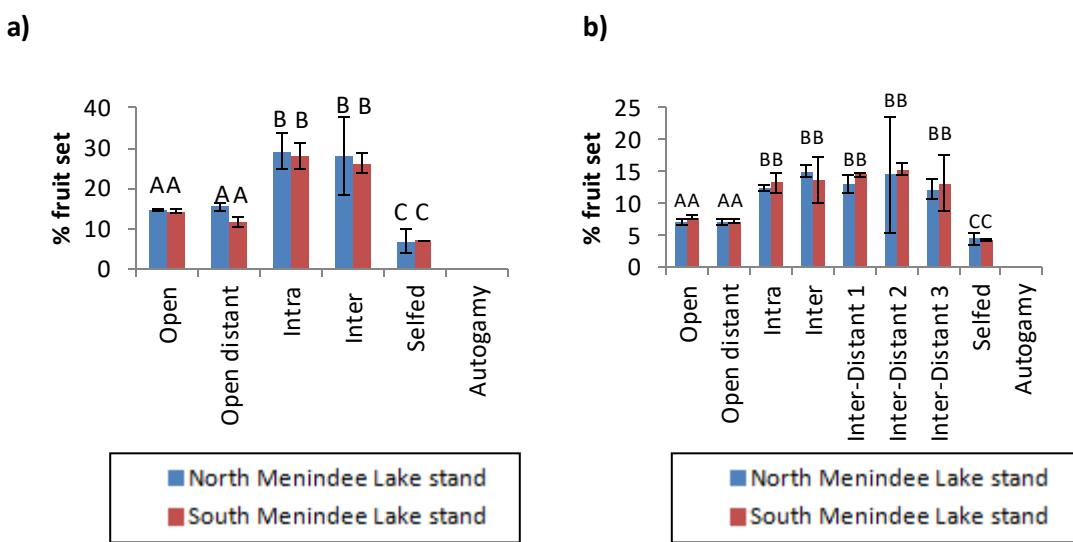
**Figure 7.4. Effect of several pollen addition treatments and open pollination on pollen tube growth to the ovules of flowers, in multiple stands of *A. carneorum* and *A. ligulata* within Kinchega National Park:** The ‘KNP Fruiting’ and ‘Middle Camp’ *A. carneorum* stands had a history of setting fruit naturally whilst the other *A. carneorum* stands did not. Only one *A. carneorum* stand (‘South Dune’) was used in consecutive years. \*Error bars represent variation (SE) between plants within recipient stands.

#### (ii) Reproductive success

##### Fruit set (%)

No fruit was set in response to any of the manually pollinated open pollination treatments, on any of the recipient *A. carneorum* plants, in either 2011 or 2012. However, in 2012 low levels of naturally set seed were observed on all of the recipient plants used in both of the stands

with a history of setting fruit (Middle Camp and KNP fruiting) (Average of 1 fruit per 1788 inflorescences). In contrast, *A. ligulata* plants set copious quantities of seed naturally and our manual pollinations also initiated seed. Irrespective of the source of the pollen, manual outcrossing produced noticeably more fruit per inflorescence than were produced through natural pollination, which in turn produced on average more than was set through selfing in both years the experiment was run (Figure 7.5a & b). No fruit was set in any spontaneous autogamy treatment in either year the *A. ligulata* pollination experiment was run, suggesting that flower fertilization does not occur, or is at least very rare without assistance from vectors. These findings also demonstrate that this species is unlikely to reproduce through parthenogenesis. Two way analysis of variance revealed a significant effect of treatment in both years the experiment was run, when the autogamy treatment was excluded ( $F(4,7)$  = 8.586,  $p=0.017$  for year 1 and  $F(7, 4) = 59.506$ ,  $p <0.001$  for year 2). Tukey's post hoc analysis confirmed that while all manual treatments supplying outcrossed pollen set equivalent numbers of fruit, flowers pollinated with self pollen as well as those left open to be pollinated naturally set a significantly lower proportion of fruit in both years.

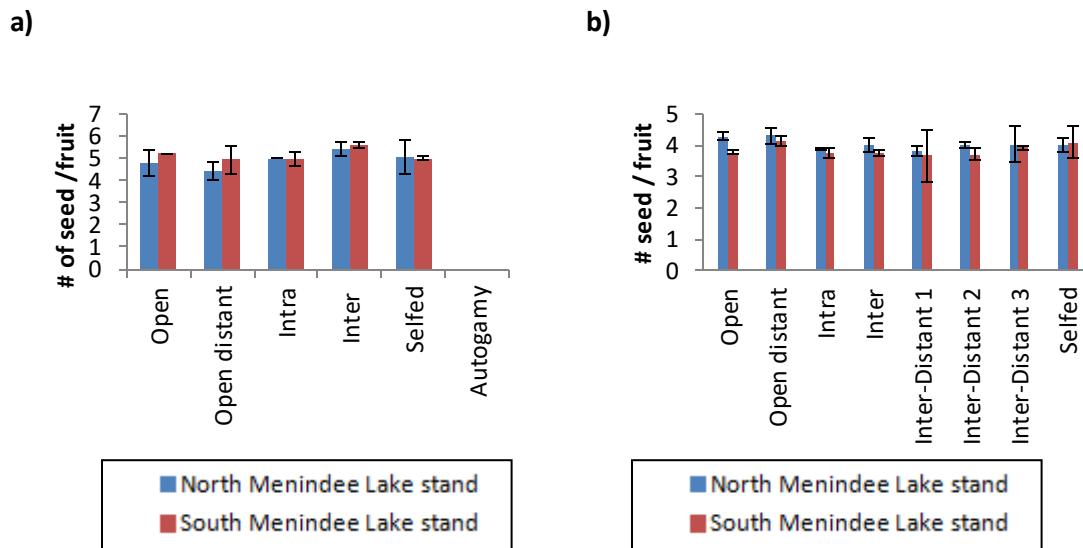


**Figure 7.5. The effect of pollen quality on fruit set on *A. ligulata* plants within two recipient stands in Kinchega National Park over two consecutive years:** The mean proportion of inflorescences exposed to experimental pollen addition and open pollination that set fruit in a) 2010 & b) 2011. \*Error bars represent variation (SE) between plants within recipient stands.

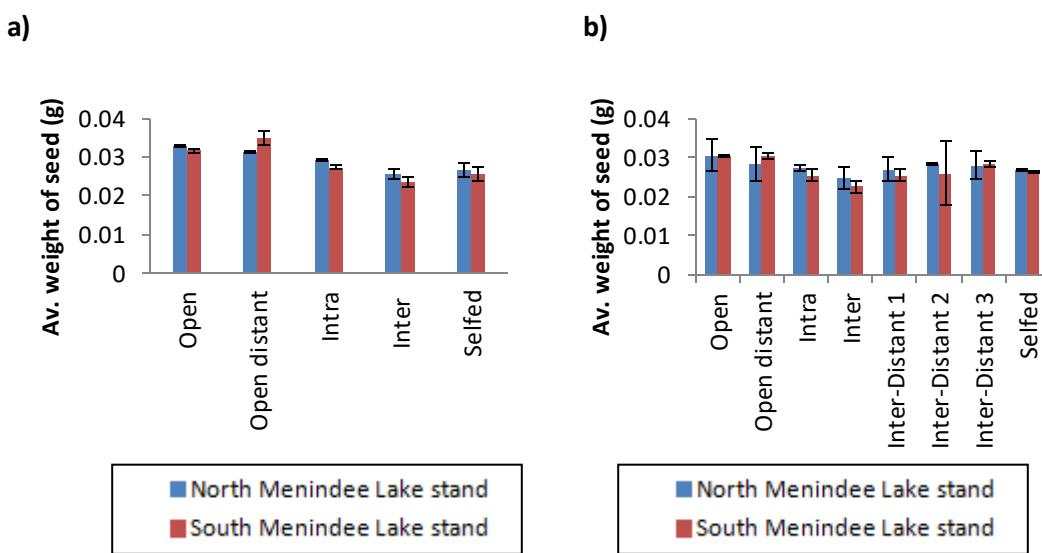
(iii) Quality of fruit and seed

Number of seed per fruit and seed weight (g)

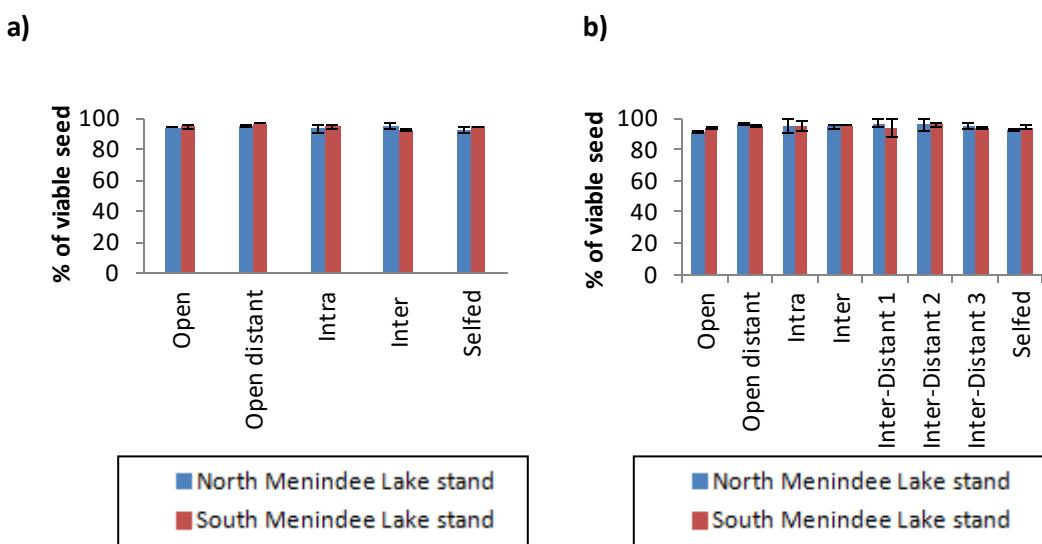
No noticeable difference in the quality of *A. ligulata* fruit produced from any of the manual treatments or naturally pollinated treatments (open treatments) was detected in either year the experiment was run as measured by (i) the average number of seed per pod (Figure 7.6 a & b), (ii) the average weight of seed (Figure 7.7 a & b). However, a significant effect of treatment on (iii) viability of seed (germination rates) was observed (Figure 7.8 a & b). With the autogamy treatment excluded, due to the lack of any seed produced in this treatment, analysis of variance confirmed that no significant difference between treatments in: (i) the average number of seed per pod ( $F(4, 7) = 1.709$ ,  $p=0.246$  for year 1 and  $F(7, 4) = 1.251$ ,  $p=0.360$  for year 2), (ii) the average weight of seed ( $F(4, 7) = 1.198$ ,  $p=0.392$  for year 1 and  $F(7, 4) = 1.333$ ,  $p=0.349$  for year 2), and (iii) the viability of seed (germination rates) ( $F(4, 7) = 1.881$ ,  $p=0.208$  for year 1 and  $F(7, 4) = 1.403$ ,  $p=0.290$  for year 2).



**Figure 7.6. The effect of pollen source on the number of seed produced per fruit on *A. ligulata* plants within two recipient stands in Kinchega National Park over two consecutive years:** The average number of seed per fruit pod produced as a result of the application of manual pollen addition and natural pollinators in a) 2010 & b) 2011. \*Error bars represent variation (SE) between plants within recipient stands.



**Figure 7.7. The effect of pollen source on the average weight of fruit and seed produced on *A. ligulata* plants within two recipient stands in Kinchega National Park over two consecutive years:** The average weight of seed produced as a result of the application of manual pollen addition and natural pollinators in a) 2010 & b) 2011. \*Error bars represent variation (SE) among plants within recipient stands.

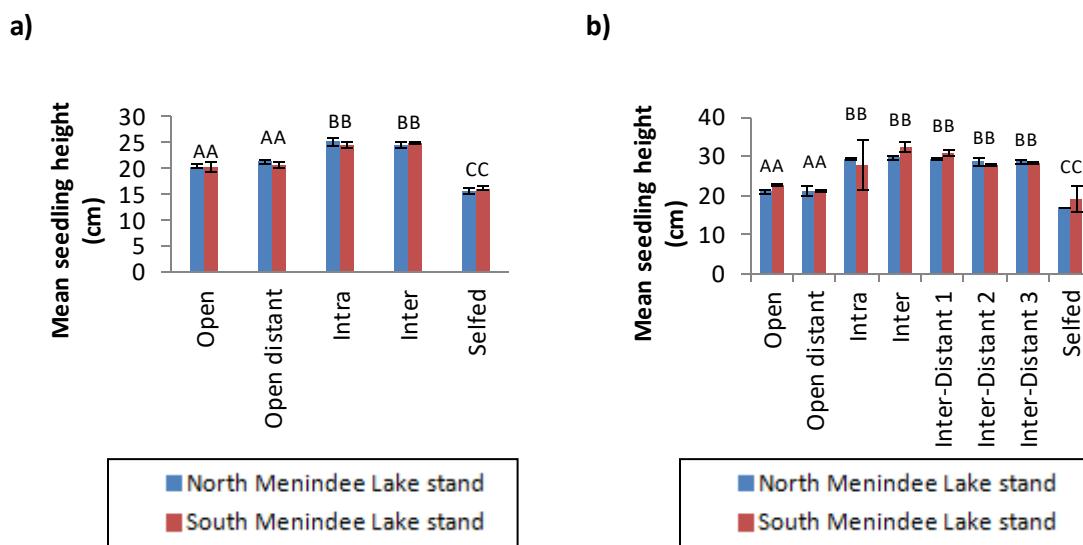


**Figure 7.8. The effect of pollen source on the viability of seed produced on *A. ligulata* plants within two recipient stands in Kinchega National Park over two consecutive years:** The average proportion (%) of seed produced as a result of the application of manual pollen addition and natural pollinators that germinated in a) 2010 & b) 2011. \*Error bars represent variation (SE) among plants within recipient stands.

(iv) *Fitness of seedlings*

*Average growth rate (cm/time)*

Pollen treatment had a significant effect on the growth of *A. ligulata* seedlings in each year the experiment was run ( $F(4, 7) = 109.758$ ,  $p < 0.001$  and  $F(7, 4) = 96.255$ ,  $p < 0.001$  for years 1 and 2 respectively). Post Hoc analysis using Tukey's tests revealed that *A. ligulata* seedlings produced by experimental addition of outcrossed pollen were significantly taller than those resulting from natural pollination ('open pollination' treatments), after a year of growth. In contrast, seedlings produced by addition of self pollen were significantly shorter at two years of age than either of the treatments where flowers were left to pollinators to service ('open' and 'open distant' treatments) in both years the experiment was run (Figure 7.9 a & b).

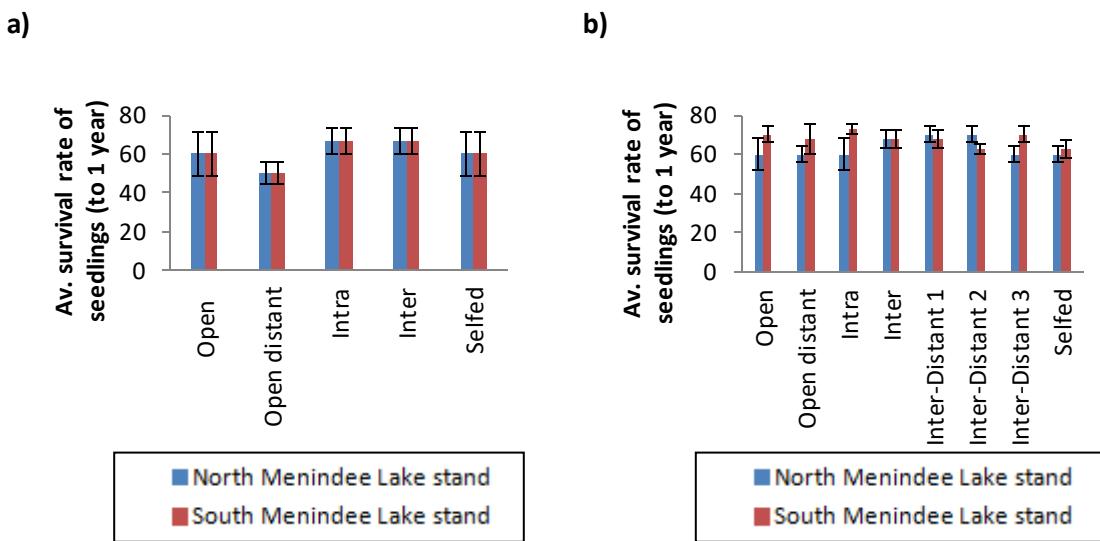


**Figure 7.9. The effect of pollen source on the growth rates of seedlings produced by *A. ligulata* plants within two recipient stands in Kinchega National Park over two consecutive years:** a) & b) The average above ground height of potted seedlings grown under coastal conditions produced as a result of exposure to manual pollen addition and natural pollinators after a year of growth in 2010 & 2011 respectively. \*Error bars represent variation (SE) among plants within recipient stands.

*Seedling survival (%)*

In contrast to seedling growth, pollen source (treatment) was found to make no noticeable difference to the two year survival rates of the seedlings produced in either year

the experiment was run. Indeed, analysis of variance found no effect of treatment on the two year survival rates of these seedlings ( $F(4, 7) = 2.500$ ,  $p=0.146$  and  $F(7, 4) = 0.511$ ,  $p=0.810$  for years 1 and 2 respectively) (Figure 7.10 a & b).



**Figure 7.10. The effect of pollen source on the survival rates of seedlings produced by *A. ligulata* plants within two recipient stands in Kinchega National Park over two consecutive years:** a) & b) The average proportion of potted seedlings produced by manual pollen addition and natural pollinators that survived under coastal conditions to one year of age in 2010 & 2011, respectively. \*Error bars represent variation (SE) between plants within recipient stands.

## 7.5 Discussion

### Overview of significant results

Although manual pollination experiments revealed little about the state of *A. carneorum*'s current mating system, other than to highlight the naturally low levels of fecundity in this species, the finding that naturally set seed on *A. ligulata* plants was on average outperformed at the growth stage by all seed set through manual outcrossing, was a surprising discovery. This result suggests that managers concerned with the future persistence of species considered officially threatened should also consider the long term threats to species that may be considered thriving but whose mating systems may have been altered nonetheless.

### ***A. carneorum***

For plants that are capable of both sexual and asexual modes of reproduction, sexual reproduction is often rare (Harper, 1977; Stearns, 1987; van Kleunen et al., 2001; Zobel, 2008). Given that fecundity levels were observed to be naturally very low for *A. carneorum* plants that do set seed, it is likely that the number of flowers I was able to manually pollinate meant that I had a low level of power to detect any effects of different treatments, or to expect any seed set at all. As such I was unable to determine conclusively whether historic reproductive success and failure in *A. carneorum* stands has been a result of self incompatibility, incompatibility with surrounding genets, or simply a natural lack of sexual capacity in the majority of stands. As such, definitive conclusions about reproductive health in *A. carneorum* stands are impossible to make with certainty. Whilst manual pollination of more flowers may produce seed, given the number of flowers required to be pollinated with pollen of an appropriate source to expect enough seed to compare between treatments, such an effort may be impractical. Moreover, such an experiment may only be possible when another rare large scale rain event occurs, which could be decades away. In the absence of such an event, or the capacity to perform such a labour intensive experiment, the best alternative would be to genotype these seed and carry out a paternity analysis.

### ***A. ligulata***

In stark contrast to my ineffectiveness in experimentally initiating fruit set on *A. carneorum* plants, the large numbers of seed produced both manually and naturally on *A. ligulata* plants allowed for a robust investigation into the effect of pollen source on fecundity, offspring fitness and hence the state of the current mating system. My finding that *A. ligulata* seed generated through selfing grew on average more slowly than outcrossed seed was a novel finding for this species, despite the preference for outcrossed pollen in terms of seed set levels previously reported (Whitney, 2005) and confirmed again here. My finding that naturally produced seed also grew significantly slower on average compared with seed produced by manual outcrossings should be of particular interest to managers. Finding that flowers left to be pollinated naturally set proportionally less fruit, which on average grew slower than those produced through manual pollinations, likely reflects the fact that most of the seed set by these plants was a result of selfing rather than outcrossing. This is understandable given that pollination is dominated by honeybees with local foraging

behaviour (Chapter 6, thesis). As discussed previously, honeybees have been found to increase levels of inbreeding in many native plants (Taylor and Whelan, 1988; Ramsey, 1988; Vaughton, 1996; and England et al., 2001).

Nevertheless, I found that many seedlings from naturally set seed grew as quickly as those from outcrossed seed, whilst the growth rates of the others resembled that of manually selfed seed. This likely reflects the mixed pollen load applied by native pollinators, which would likely include some pollen outcrossed pollen as along with the large amounts of self pollen predicted to be deposited by previous pollinator observations (Chapter 6, thesis). Paternity analysis of naturally produced seed should be performed in the future to demonstrate that this is the case.

Given that evolutionary theory predicts that historic levels of gene flow will produce the fittest offspring on average (Kolreuter, 1761; Darwin, 1868, 1876; East & Jones, 1919), my findings that manually applied outcrossed pollen produced more and on average better performing offspring than natural pollinations, indicates that the mating system currently operating in these *A. ligulata* stands may not be optimal. Without a better understanding of the way in which this species has evolved to reproduce however, it is impossible to know whether the realised mating system currently operating simply reflects reproduction that falls happily within a broad range that leads to a healthy set of seedlings in numbers that maintain a steady population. The large numbers of seed produced naturally on *A. ligulata* plants suggests that while selfed pollen only rarely leads to fruit set, the efficiency of honeybees pollinating the vast majority of flowers compensates for this, leading to still substantial levels of fruit set.

The findings of this study provide some evidence that the ‘hijacking’ of pollination by introduced honeybees might be cause for future concern for the health of local *A. ligulata* stands, as has been found for many other native plant species (Taylor and Whelan, 1988; Paton and Turner, 1985; Vaughton, 1992; and England et al., 2001). Managers may need to consider strategies such as genetic rescue to counteract the loss of genetic diversity within these stands moving forward. My findings of a lack of a noticeable preference between the outcrossed pollen delivered to plants from different sources, whether from the same stand or from a distant stand, likely reflects the high levels of genetic diversity that still exist within and between populations. This suggests that if genetic rescue of *A. ligulata* stands is deemed to be required in the future, that multiple local sources of pollen could be used to generate

outcrossed seed without issue. It must be considered though that crosses between stands too divergent have resulted in outbreeding depression in other species, which is often masked in the first generation but apparent in subsequent generations (Lynch, 1991).

### **Conservation considerations**

At the very least it would be advisable for managers to survey the pollinators servicing populations across the region to determine whether *Apis mellifera* are visiting *A. ligulata* plants universally or are restricted to certain stands. Given that honeybees require access to a permanent source of water, it is possible the high numbers of honeybee visits to *A. ligulata* plants surveyed on Kinchega National park (Gilpin et al., 2014; Chapter 6, thesis) is only possible because of their proximity to Menindee lakes as well as these surveys being conducted after a large scale rain event. Broad region wide genetic surveys of *A. ligulata* populations would also aid in understanding the state of *A. ligulata* stands and to determine whether loss of genetic diversity is associated with proximity to water.

## **Chapter 8: Genetic estimation of mating systems within rare fruiting populations of *A. carneorum*.**

### **8.1 Abstract**

A prolonged lack of seed set in the majority of stands of the highly threatened and iconic *A. carneorum* across western NSW has been hypothesised to be a consequence of their highly fragmented states. Previous studies provide no evidence this is a result of the condition of plants, or pollen limitation. Here I use a genetic approach to determine how the plants in an unusually fecund *A. carneorum* stand are mating. I genotyped 100 seed collected from five plants within the only *A. carneorum* stand in or around Kinchega National Park fecund enough to provide large numbers of seed, using a set of eight microsatellite loci. I also genotyped all the plants within the stand and those in two neighbouring stands approximately 1 and 4 km away, that may have also fathered the seed. I used Cervus 3.0 to assign paternity to the seed. I found that all plants within all three of the *A. carneorum* stands surveyed were monoclonal with each stand displaying a unique multilocus genotype. With 99% confidence, paternity analysis assigned 73% of the genotyped seed to either the maternal plant (selfing) or any one of the other 120 plants within the same clonal stand, and the other 27% to the clone represented by 15 plants within the nearest neighbouring stand 1 km away. Given previous findings that the native insect pollinators of *A. carneorum* tend to forage locally and it would be expected that most pollen transported to flowers would be self or local pollen, this finding indicates that these plants are preferentially outcrossing. Moreover, whilst outcrossing over approximately 1 km has been detected, outcrossing over more than 6 km was not. While we cannot determine whether seed set failure in most *A. carneorum* stands in the region is a consequence of self incompatibility or sterility, for those stands that can reproduce sexually, isolation clearly favours inbreeding. Given the pressures climate change is predicted to have on arid species, maintaining some adaptive capacity through the ability to produce genetically diverse seed is likely to become of great importance for this species in the future.

## 8.2 Introduction

Fragmented plant populations that reproduce sexually can suffer reduced reproduction or, in the most acute cases, total reproductive failure as a result of their isolation (Schemske, 1983; Lamont et al., 1993; Aizen & Feinsinger, 1994). For stands of the threatened species *A. carneorum* across western NSW, high levels of flowering, as well as high levels of rainfall, have not resulted in seed set for decades in the majority of these stands (Chapter 3, thesis). Given my previous findings that these plants do not seem to be pollen limited (Chapter 6, thesis), this lack of seed set might be best explained by the deposition of incompatible pollen on flowers, resource limitation, or simply maternal sterility. Previous attempts to determine whether this was the result of plants receiving incompatible pollen by manually pollinating plants with a range of pollen from different sources, were inadequate to draw definitive conclusions, other than that pollen incompatibility cannot be the sole cause of reproductive failure in these stands (Chapter 7, thesis). Failure to produce seed set through hand pollination could reflect genetic incompatibility or other factors that ensure low seed set regardless of pollen source.

My previous study of pollinator foraging behaviour suggests that flowers are likely to be receiving mostly self and local pollen (Chapter 6, thesis). Previous population genetic surveys by my group have revealed that remaining *A. carneorum* stands across the region represent groups of clonal plants that vary in genotype between each stand (O'Brien, 2014; Roberts et al., 2016). Whilst this finding suggests that these stands are likely to have arisen through asexual means (presumably suckering though parthenogenesis has not been ruled out), no genetic analysis of *A. carneorum* seed has been undertaken. Without such information it could be hypothesised that the lack of seed set in most stands is a result of an incompatibility with the selfed and local pollen from neighbouring clone mates being overwhelmingly supplied to plants by locally foraging insects. Whilst I have no evidence as to these plants being resource limited, different populations of a single plant species can also vary in their reproductive strategies and hence their capacity to sexually reproduce (Douglas, 1981; Richards, 1997; Sartor et al., 2011; Hardion et al., 2015). It is perfectly possible that fruit setting stands and those that don't set fruit just vary in their reproductive strategies naturally.

Genetic analysis of naturally produced seed provides the opportunity to determine whether, at least within stands that are setting fruit, whether plants are setting both selfed and outcross seed, as well as allowing the possibility of assigning paternity to plants within neighbouring stands. Indeed by comparing the genotypes of maternal parents and their offspring it may be

possible to determine if seed was produced parthenogenically, through selfing or through outcrossing with plants in other stands (Brown et al., 1986).

To determine whether seed are generated parthenogenically, through selfing or outcrossing, I determine the genotypes of 100 seed from five maternal plants from one stand with a history of setting seed, as well as all adult plants within 7km of these maternal plants. I also use paternity analysis to determine the geographic location of sires of outcross seed.

## **8.3 Methods**

### **8.3.1 Selection of plants and stands**

The only *A. carneorum* stand close to Kinchega National Park that produced large enough numbers of seed to give robust estimates of how likely inbreeding and outbreeding occurs in this species, was used as the focal *A. carneorum* stand for this study (Middle Camp stand). This stand is located approximately 25 km from the southern border of Kinchega National Park, just on the edge of Lake Tandou and consists of 120 old plants. The closest neighbouring stand (Mallee stand) to this fecund focal stand is located approximately 1km away towards Kinchega National Park and consists of 15 senescent plants. Comprehensive searches of the area by 4WD and with the use of binoculars, revealed that the next two closest *A. carneorum* stands (Tandou 1 and Tandou 2 stands) are located approximately 6 and 7 km from the focal Middle Camp stand. These stands each consisted of only two remaining senescent plants in each stand. The next closest stands to the Middle Camp stand are located over 10 km away within Kinchega National Park.

### **8.3.2 Experimental design / sample collection**

I collected young leaves of all of the adult plants within all four *A. carneorum* stands mentioned above for genetic analysis, along with 20 mature fruit from each of five randomly selected plants within the Middle Camp fruiting site (Figure 8.1). I randomly sampled a single mature seed from each fruit pod for genetic analysis.



Figure 8.1. *A. carneorum* seed on plants in the Middle Camp stand.

### 8.3.3 Genetic analysis of leaf and seed

Genetic material for analysis was obtained from the leaves of mature plants and from the embryo of seed that did not germinate after scarification and exposure to water, or from the leaf of seedlings that did germinate from this seed. Both leaf and embryos were processed using a pulveriser before genomic DNA was extracted using the CTAB method described in Doyle and Doyle in 1987. I used eight microsatellite primers developed by Roberts et al. (2013) and PCR protocol described in (Hayden et al., 2008) to obtain multilocus genotypes for these samples.

### 8.3.4 Assigning paternity to seed

Cervus 3.0 (Kalinowski et al., 2007) a likelihood-based parentage analysis software package for co-dominant loci was used to assign paternity of seed at a specified significance

threshold. To run simulations, I used the following parameters: 10,000 simulated mating events; minimum number of matching loci equals eight; allele frequencies were estimated using all genotyped trees to compare the genotype of the seed simultaneously to the genotypes of putative mothers and fathers. For Cervus's likelihood calculations, I allowed a typing error rate of 0.01 and used a strict confidence level of 99% significance threshold to ensure valid assignment. To assign paternity, I used the maternal plants as a known parent and included all other genotyped adults present in the stand, as well as all other plants in the nearby Mallee, and distant Tandou 1 & 2 stands as candidate paternal plants. All offspring genotypes were manually checked against their maternal and assigned paternal genotypes to ensure that Cervus was assigning paternity feasibly. Results of the paternity analysis were used to determine the proportion of seed that came about through selfing and outcrossing as well as the origin of the paternal plants contributing to outcrossed seed. These results were then used to determine a) whether these plants are self compatible and b) the distance that pollen is being moved to produce these matings.

### **8.3.5 Viability of rare *A. carneorum* seed**

The viability (germination rate) of the 73 selfed/ inbred and 27 outcrossed *A. carneorum* seed collected from the Middle Camp was determined by scarifying these seed (using sandpaper), placing them in Petri dishes in lots of 20 on top of moistened filter paper. The dishes were placed in an incubator with a day/night regime of 28 to 14 °C. Seed were checked each day for three weeks for germination. Germinated seed were then grown in 10cm by 10cm by 20 cm deep plastic punnets in coastal soils inoculated with soil from where the seed were collected, grown under greenhouse conditions, watered every second day and their growth rates and their mortality / survival rates were compared.

## **8.4 Results**

### **8.4.1 Genetic analysis of leaf**

All plants in all four stands were found to be diploid. The Middle Camp, Mallee, Tandou 1 and Tandou 2 stands were all found to be monoclonal stands, with each stand representing a single distinct multilocus genotype (Appendix 8.6.1). Across eight nuclear microsatellite markers, 12 different alleles were found in the clonal adult plants comprising the Middle Camp

stand (1.5 alleles / locus) and 14 in the Mallee, Tandou 1 and Tandou 2 stands (1.75 alleles / locus). Adult plants in the Middle Camp stand contained six alleles not found in the Mallee, Tandou 1 and Tandou 2 stands. Conversely, the Mallee, Tandou 1 and Tandou 2 stands contained 7 alleles each not shared by any of the other stands. Average  $H_e$  per locus was notably lower in the adult plants of the Middle Camp site ( $0.250 \text{ SE} \pm 0.094$ ) than in the Mallee, Tandou 1 and Tandou 2 stands stand ( $0.375 \text{ SE} \pm 0.082$ ).

#### 8.4.2 Genetic analysis of seed

A total of 19 alleles were found in the 100 seed sampled from five maternal plants in the Middle Camp stand across the eight loci surveyed (2 alleles / locus), all of which were found in either the adult plants in the Middle Camp stand or plants in the neighbouring Mallee stand.

While the plants within both the Middle Camp and the Mallee stands were each monoclonal, in sharp contrast, genetic diversity was found within the seed from the Middle Camp stand. Moreover, this seed includes alleles unseen in the maternal adult plants within that stand. I found 16 distinct multilocus genotypes among the 100 Middle camp seed (Appendix 8.6.1). Of these distinct genotypes, 11 displayed alleles found only in the maternal plant's genotype, with the remaining five containing at least one allele not found in the maternal genotype, but which existed in the genotype of mature plants in the neighbouring Mallee stand. The majority of seed (73%) was found to have multilocus genotypes with alleles all common to the maternal clonal genotype at all 8 loci, while the remaining 27% of the seed were found to contain at least one or more alleles not present in the maternal genotype, but present in the clonal genotype of adult plants in the Mallee stand. Only 24 of the 100 seed had the exact same multilocus genotype as the maternal plant, whilst the remaining 49 seed that contained only alleles shared with the maternal genotype were recombined at at least one of the four loci heterozygous in the maternal genotype. Of these 73 seed, the frequency of heterozygotes and homozygotes found at the four loci that were heterozygous in the maternal genotype was roughly in line with the 1:2:1 ratios of homozygotes and heterozygotes predicted for Mendelian inheritance and true selfing (Table 8.1). Nevertheless, the large number of seed (24) with an identical multilocus genotype to the maternal plants also suggest parthenogenesis, given we might only expect to see a single seed with this exact genotype under true selfing.

**Table 8.1. Observed and expected ratios of heterozygosity of 73 *A. carneorum* seed suspected to have been a product of selfing / local inbreeding, at four loci that were found to be heterozygous for the maternal genotype:** Seed were sourced from five plants within a single monoclonal stand (Middle Camp), just outside Kinchega National Park in far western NSW.

		Genotype of seed						
		Observed						
Locus (heterozygous in maternal genotype)	Maternal Genotype	#	%	#	%	#	%	
1	211/223	211/211		211/223		223/223		
		20	27	35	48	18	25	
2	230/232	230/230		230/232		232/232		
		11	15	42	58	16	22	
3	210/216	210/210		210/216		216/216		
		14	19	44	60	15	21	
4	225/226	225/225		225/226		226/226		
		14	19	41	56	17	23	
Expected								
#		#	%	#	%	#	%	
18.25		36.5	25	50	18.25	25		

#### 8.4.3 Assigning paternity to seed

For the 100 seed collected from five maternal plants in the Middle Camp stand, paternity was assigned, with 99% confidence, to the maternal plant's clone (73 seeds), or to the clone within the neighbouring Mallee stand (27 seeds). None were attributed to matings with plants in either the Tandou 1 or Tandou 2 stands located 6 and 7 km away. Matings with the next closest stand of plants located over 10 km away were therefore deemed very unlikely and were not considered in the analysis.

#### 8.4.4 Viability of *A. carneorum* seed

Levels of seed viability (germination rate), growth rates and two year survival rates of potted *A. carneorum* seedlings were roughly equivalent between the 73 inbred and 27 outcrossed seed and the resulting 50 and 18 seedlings respectively (Figure 8.2).

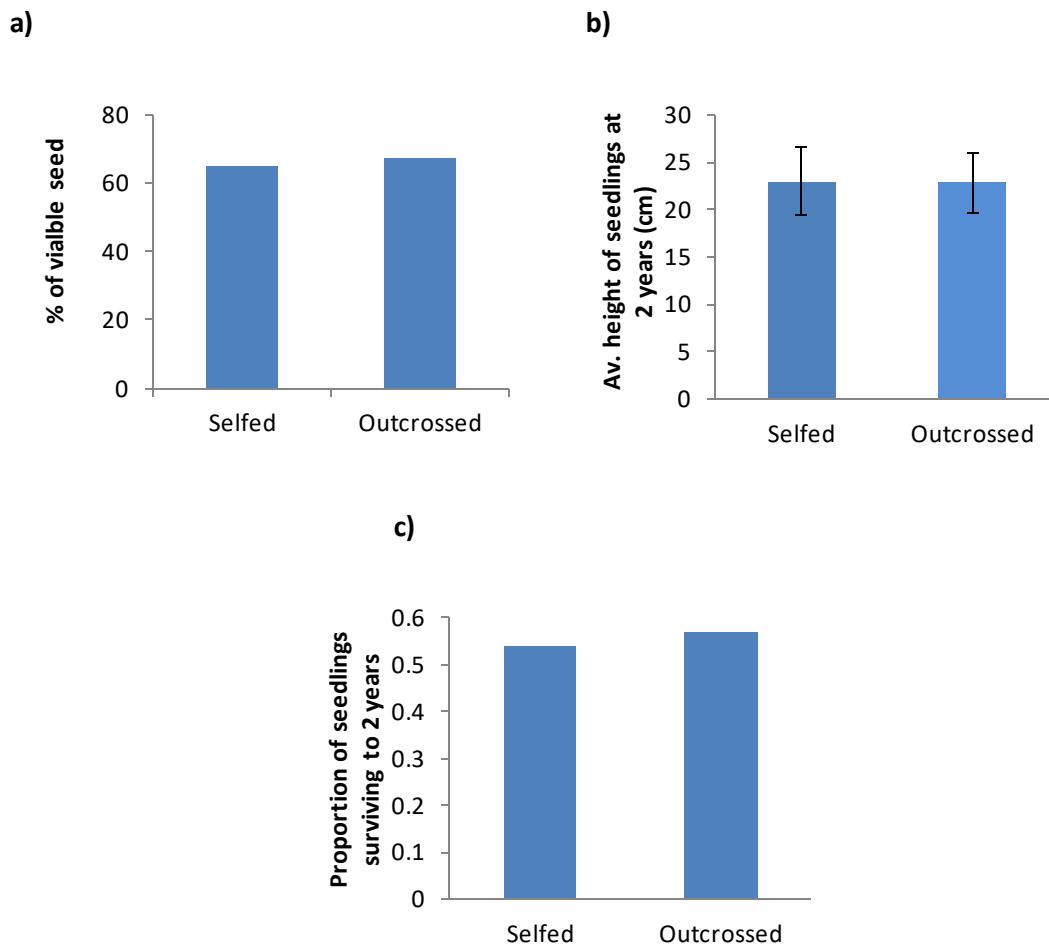


Figure 8.2. Tests of the fitness of *A. carneorum* seed produced through outcrossing and selfing on 10 plants from within the Mallee stand measured as: a) the percentage of 27 outcrossed and 73 selfed seed that were found to be viable, b) the average height of 18 outcrossed and 50 selfed seed that germinated at 24 months after germination. \*Error bars (SE) represent the variance between the ten plants seed were sampled from, c) Proportion of seedlings generated through outcrossing and selfing that survived to two years of age.

#### 8.5 Discussion

##### Major findings

Genetic analysis of seed from Middle Camp provides the first indication of the *A. carneorum* mating system and provides intriguing insights into the reproductive failure of

other populations. Seed was a mix of self and outcrossed, with outcrossing indicating that at least for one population, self incompatibility is not a barrier to seed set, and that outcrossing can occur among neighbouring stands over at least 1 km. Further to this, by genotyping every individual within these four stands, I confirmed previous studies which genotyped a random sample of plants within this and other *A. carneorum* stands, that *A. carneorum* stands are truly monoclonal (Roberts et al., *in press*). Indeed, the latter finding made it relatively straightforward to detect selfed/inbred and outcrossed seed, and assign them to the Middle camp and Mallee clones respectively as the sole sires. My finding that 73% of seed likely came by way of selfing, inbreeding and parthenogenesis is novel information for this species and suggests a flexible reproductive strategy. Almost all of the seed produced were germinable after scarification, with selfed and outcrossed seed displaying similar vigour.

### **Inferring current mating system dynamics**

Given that my previous pollinator observations imply that an overwhelming majority of *A. carneorum* flowers are likely to be pollinated by selfed and local pollen (Chapter 6, thesis), the finding that approximately one third of the seed collected from both stands was derived through outcrossing suggests a strong preference for outcrossed pollen. These findings indicate the presence of a maternal mate choice mechanism preferentially selecting outcrossed pollen over self pollen and pollen from neighbouring clone mates. Given all the outcrossed seed sampled could be assigned back to paternal plants from the closest neighbouring stand, crosses between the next closest stands approximately 6 km away are unlikely to occur, or are at best extremely rare. As limited dispersal distances are not unusual in insect pollinated species, this finding gives us a benchmark for the degree of isolation required to effectively cut off connectivity between *A. carneorum* stands in their altered environment. With recent surveys finding that the majority of stands are separated from one another by several kilometres, it is possible many or most stands are currently disconnected from others across the region.

As insects may have foraged more frequently over larger distances between *A. carneorum* stands when the landscape was more connected, these findings allow us to broadly predict which stands are likely to be still connected now. It should be considered however, that I may not have sampled enough fruit to detect any seed fathered by plants in stands further away, and such crosses might happen from time to time. Indeed crosses over much larger spatial scales have been recorded in both insect pollinated Grevillea (Roberts et al.,

2007) and Eucalypt species (Field et al., 2010). Locating and looking at outcrossing distances in several other fruit setting *A. carneorum* populations might provide a more general picture of pollen dispersal limits for this species.

Even extremely rare outcrossing events within largely clonal species could be significant given the long life expectancy of iteroparous plants, with many flowering seasons in which these rare crosses might occur and many chances for any resulting seed to recruit. Indeed it could also be that suitable conditions for the recruitment of seedlings naturally occur incredibly infrequently for *A. carneorum*. Clearly intervals between sexual recruitment must be sufficient for demographic processes to ensure that extant stands contain only a single clone. Even if such additions of genetic diversity occurred very rarely within a stand, such events would have a larger consequence in subsequent generations when their genetic characteristics were then spread throughout stands via more frequent local matings. Although somatic mutation should provide some level of diversity between stands over time (Whitham & Slobodchikoff, 1981), occasional recruitment of outcrossed seedlings would give clones the capacity to change their genotype over time and either, or both, may explain the fact that every *A. carneorum* clone in the region has a distinct genotype (O'Brian et al., 2014; Roberts et al., unpublished work).

The presence of self incompatibility and / or a strong mate choice mechanism in plants provides insight into the types of matings that have occurred for an extended period historically and which produce the most and /or fittest offspring through selection (Wilson and Burley, 1983; Seavey & Bawa 1986; Uyenoyama, 1986; Holsinger, 1988). The finding of a strong preference for outcrossed pollen in *A. carneorum* stands that set seed, provides the first indication that this species has been an outcrossing species historically, despite retaining a degree of self compatibility. It is yet to be determined, given that outcrossing with only one other stand was detected, whether the source of outcrossed pollen matters to the quantity and quality of offspring produced. I found that this was seemingly not the case for co-occurring *A. ligulata* stands (Chapter 7, thesis), however *A. ligulata* is an obligately sexual species and unlikely to be a good comparison.

### Possible explanations for the presence and absence of seed set among stands

Differences in reproductive capacities between populations / stands of a species can be either a result of natural or unnatural disturbance in certain stands, or natural variance

between stands. Irrespective of whether all, some or no *A. carneorum* requires sexual recruitment for healthy long term persistence or not, the contrast between the stands capable and incapable of setting fruit begs explanation. Previous studies ruled out differences in plant age, plant and stand condition, local rain levels, local temperatures and flowering effort as causal factors for this inter stand variability in seed set. It is still possible however, that plants in the majority of stands that did not set fruit differ from those that did, by being both self incompatible and incompatible with the genotypes of plants in the neighbouring stands with which they are connected. Differences in the mating systems of plants in different stands has been found for other species, including differences in self compatibility (Wyatt, 1986; Rathcke & Real, 1993; Fishman & Wyatt, 1999) and differences in sexual reproductive ability (Sartor et al., 2011; Hajrudinović et al., 2015; Hardion et al., 2015). This can often reflect differences in environmental conditions across a broad geographical distribution. Recent genetic surveys of *A. loderi* stands that co-occur with *A. carneorum* have found increased clonality of similarly old stands by way of suckering in the western most distribution of its range, with stands in the eastern parts of its distribution displaying high levels of genetic diversity as a result of sexual recruitment (Roberts et al., 2016). In many cases where this is found, such intra species variation in mating systems is driven by differences in ploidy, with certain stands being diploid and others being polyploid (Richards, 1997; Sartor et al., 2011; Husband et al., 2012; Hardion et al., 2015). In other cases, hybridization and polyploidy are associated with changes in the mating system, from reproducing sexually to asexually (Ozias-Akins & van Dijk, 2007; Potter et al., 2007; Talent & Dickinson, 2007; Cosendai & Hörandl, 2010; Hojsgaard et al., 2014). Previous genetic surveys of *A. carneorum* stands found that both the majority of stands that set seed and those that did not were both seemingly diploid (Roberts et al., *in review*), suggesting another mechanism is responsible for this difference in reproductive capacity.

While it is possible that only certain clones of this species ever reproduce sexually, the contrasting reproductive capacities between some *A. carneorum* stands might be explained by a larger scale evolutionary shift from sexual to obligately asexual reproduction. If this were the case, the minority of sexually capable stands would represent the tail end of a switch to an exclusively asexual pathway as has occurred in many other species (Kearney, 2003; Honnay & Jacquemyn, 2008; Vallejo-Marín et al., 2010), rather than a variant within a steady state reproductive strategy. Indeed the lack of genetic diversity within the stands that produced seed is puzzling as we might have expected some genetic diversity in these stands as a result of the occasional recruitment of an outcrossed seed, or even recombined selfed seed. Moreover, carbon 14 dating of recently deceased plants within a subset of the same *A. carneorum* stands

dates the largest plants at approximately 200 years of age or older (Auld & Denham, 2001; Chapter 9, thesis) meaning a lack of sexual recruitment in these stands likely predates anthropogenic disturbance in the region. This means that leading theories that hypothesise that the lack of genetic diversity found in *A. carneorum* stands (O'Brian et al., 2014; Roberts et al., unpublished) is a consequence of overgrazing by introduced grazers (rabbits and goats) fall short, despite the undoubtedly impact they have on recruitment in general (Auld 1993, 1995, Porteniers, 2001). In combination, this genetic and carbon dating data lends significant weight to the hypothesis that the low levels of sexual reproduction / recruitment are indeed natural rather than a product of suboptimal mating systems induced by unnatural stand augmentation and loss of connectivity (fragmentation).

### Conservation concerns

Plants with the capacity to self fertilize, or that have pollinators that can bridge gaps between populations in a fragmented landscape, should theoretically be more resilient to unnatural isolation compared to species that are obligate outcrossers and possess less mobile pollinators (Templeton & Levin 1979; Dick et al., 2003; Aguilar et al. 2006; Ottewell et al., 2009). For the minority of *A. carneorum* stands that are setting seed, the combination of their self compatibility and their pollinators capacity to facilitate a significant amount of outcrossing between nearby stands, suggests that while these stands have undoubtedly been impacted by the fragmented landscape they exist within, their mating systems display some level of tolerance to such conditions. Moreover, the strong preference for outcrossing in this species, uncovered here, is also generally considered to be a favourable attribute for plants that prefer the production of diverse offspring. Despite the clear preference for outcrossed pollen, given that pollinators are almost certainly providing mostly local and self pollen to flowers (Chapter 6, thesis) from genetic clones within the same stand, increased inbreeding as a result of their fragmented states is likely to result in lower levels of genetic diversity in seed cohorts than would be expected if stands were more connected.

If sexual recruitment still plays a role in the healthy functioning of *A. carneorum* stands in the region, and is not simply the relic of a past reproductive strategy in transition, any loss of connectivity to other stands of divergent genetic make up should be viewed as a conservation issue. While I found no evidence of fitness tradeoffs in selfed *A. carneorum* seed compared to outcrossed seed when germinated and grown, losing the capacity to introduce some genetic diversity into stands may reduce the capacity of stands to adapt to changing local conditions.

Moreover, we must be cautious drawing conclusions about the fitness of the seed / offspring produced in these stands, other than that selfed seed are fit enough to be viable. These results may simply reflect inadequacies in my methodology for testing offspring fitness, and / or the temporal scale of tests. Looking at later stages of development and longer term survival, especially under drier more physiologically stressful natural conditions, could highlight deficiencies in fitness which might have gone undetected in the relatively benign coastal conditions where these seedlings were grown. Furthermore, whilst paternity analysis of this seed can tell us about the types of matings occurring currently (realised mating system), such an approach cannot tell us anything about the mating system that we might observe given a diverse range of possible mates (preferred mating system).

With climate change is predicted to impact arid and semi arid environments particularly harshly (IPCC, 2001) and species in these regions already existing on a climatic knife edge, maintaining genetic diversity within populations is expected to be paramount to their continued persistence (Huntley, 1991; Easterling et al., 2000; Hughes, 2003; Godfree, 2013). As such, the presence of genetically diverse and viable seed produced in at least some *A. carneorum* stands opens up an important conservation option given that this seed likely represents the last feasible source of material managers could use to restore severely contracting stands.

## 8.6 Appendix

**Appendix 8.6.1: Genetic data used to assign paternity to *A. carneorum* seed collected from plants within the Middle Camp population in western NSW:**  
 Clonal multilocus genotype of the five maternal plants and 120 potential paternal plants in the Middle Camp stand, the 129 and three potential paternal plants in the Mallee stand and Tandou stands respectively, and the multilocus genotypes of the 100 seed collected from five Middle Camp maternal plants.

Sample	Loci / Alleles																
	CDRBZ_- a	CDRBZ_- a	CO3P6_- a	CO3P6_- b	AQBUV_- a	AQBUV_- b	ARU19_- a	ARU19_- b	COF94_- a	COF94_- b	EEH17_- a	EEH17_- b	EFO3V_- a	EFO3V_- b	CTFGS_- a	CTFGS_- b	
<b>Middle Camp clones: 5 maternal plants and an additional 115 possible paternal plants</b>																	
Middle Camp (x120)	258	258	128	128	211	223	230	232	210	216	204	204	225	226	242	242	
<b>Other potential paternal clones: Mallee 15 plants, Tandou 1 3 plants, Tandou 2 2 plants</b>																	
Mallee (x15)	256	258	128	130	215	223	230	242	208	212	190	190	225	226	242	242	
Tandou 1 (x3)	254	258	128	132	209	223	230	240	206	218	196	196	225	226	242	242	
Tandou 2 (x2)	250	258	128	126	207	223	230	236	214	220	200	200	225	226	242	242	
<b>Seed (offspring) collected from Middle Camp maternal plants</b>																	
1	256	258	128	128	211	215	232	242	208	210	190	204	226	226	242	242	
2	258	258	128	128	211	223	230	232	210	212	190	204	226	226	242	242	
3	258	258	128	128	223	223	232	242	208	216	190	204	226	226	242	242	
4	256	258	128	128	215	223	230	232	212	216	190	204	225	226	242	242	
5	258	258	128	128	211	223	230	232	210	210	204	204	225	226	242	242	
6	258	258	128	128	211	223	232	232	210	210	204	204	225	226	242	242	
7	258	258	128	128	211	223	230	232	216	216	204	204	225	226	242	242	
8	258	258	128	128	223	223	230	232	216	216	204	204	225	226	242	242	
9	258	258	128	128	211	211	232	232	210	210	204	204	225	225	242	242	
10	258	258	128	128	211	211	230	232	210	216	204	204	225	226	242	242	

11	258	258	128	128	211	223	230	232	210	216	204	204	225	226	242	242
12	258	258	128	128	211	223	230	232	210	210	204	204	225	225	242	242
13	256	258	128	128	211	215	232	242	208	210	190	204	225	225	242	242
14	258	258	128	128	211	223	230	232	210	212	190	204	225	226	242	242
15	258	258	128	128	223	223	232	242	208	216	190	204	225	226	242	242
16	256	258	128	128	215	223	230	232	212	216	190	204	225	225	242	242
17	258	258	128	128	211	223	230	230	210	216	204	204	226	226	242	242
18	258	258	128	128	211	223	230	230	210	216	204	204	226	226	242	242
19	258	258	128	128	211	211	230	232	210	210	204	204	226	226	242	242
20	258	258	128	128	211	223	230	232	210	210	204	204	225	226	242	242
21	258	258	128	128	223	223	232	242	208	216	190	204	225	226	242	242
22	256	258	128	128	215	223	230	232	212	216	190	204	225	226	242	242
23	258	258	128	128	211	223	230	232	216	216	204	204	225	226	242	242
24	258	258	128	128	223	223	230	232	216	216	204	204	225	226	242	242
25	258	258	128	128	211	211	232	232	210	216	204	204	225	225	242	242
26	258	258	128	128	211	211	230	232	210	216	204	204	225	226	242	242
27	258	258	128	128	211	223	230	232	210	216	204	204	225	226	242	242
28	258	258	128	128	211	223	230	232	210	210	204	204	225	225	242	242
29	256	258	128	128	211	215	232	242	208	210	190	204	225	225	242	242
30	258	258	128	128	211	223	230	232	210	212	190	204	225	226	242	242
31	258	258	128	128	223	223	230	232	216	216	204	204	225	226	242	242
32	256	258	128	128	215	223	230	232	212	216	190	204	225	225	242	242
33	258	258	128	128	211	223	230	230	210	216	204	204	226	226	242	242
34	258	258	128	128	211	223	230	230	210	216	204	204	226	226	242	242
35	258	258	128	128	211	211	230	232	210	216	204	204	226	226	242	242
36	258	258	128	128	211	223	230	232	210	216	204	204	225	226	242	242
37	258	258	128	128	211	223	230	232	210	216	204	204	225	226	242	242
38	258	258	128	128	223	223	232	232	210	216	204	204	225	226	242	242

39	258	258	128	128	211	223	230	232	216	216	204	204	225	226	242	242
40	258	258	128	128	223	223	230	232	216	216	204	204	225	226	242	242
41	258	258	128	128	211	211	232	232	210	216	204	204	225	225	242	242
42	258	258	128	128	211	211	230	232	210	216	204	204	225	226	242	242
43	258	258	128	128	223	223	230	232	210	216	204	204	225	226	242	242
44	256	258	128	128	211	215	232	242	208	210	190	204	225	225	242	242
45	258	258	128	128	211	223	230	230	210	216	204	204	226	226	242	242
46	258	258	128	128	211	223	230	232	210	212	190	204	225	226	242	242
47	258	258	128	128	223	223	232	242	208	216	190	204	225	226	242	242
48	256	258	128	128	215	223	230	232	212	216	190	204	225	225	242	242
49	258	258	128	128	211	223	230	230	210	216	204	204	226	226	242	242
50	258	258	128	128	211	223	230	230	210	216	204	204	226	226	242	242
51	258	258	128	128	211	211	230	232	210	216	204	204	226	226	242	242
52	258	258	128	128	211	223	230	232	210	216	204	204	225	226	242	242
53	258	258	128	128	211	223	230	232	210	216	204	204	225	226	242	242
54	258	258	128	128	223	223	232	232	210	216	204	204	225	226	242	242
55	258	258	128	128	211	223	230	232	216	216	204	204	225	226	242	242
56	258	258	128	128	223	223	230	232	216	216	204	204	225	226	242	242
57	258	258	128	128	211	211	232	232	210	210	204	204	225	225	242	242
58	258	258	128	128	211	211	230	232	210	210	204	204	225	226	242	242
59	258	258	128	128	223	223	230	232	210	210	204	204	225	226	242	242
60	256	258	128	128	215	223	230	232	212	216	190	204	225	225	242	242
61	258	258	128	128	211	223	230	230	210	216	204	204	226	226	242	242
62	258	258	128	128	211	223	230	230	210	216	204	204	226	226	242	242
63	258	258	128	128	211	211	230	232	210	216	204	204	226	226	242	242
64	258	258	128	128	223	223	230	232	210	216	204	204	225	226	242	242
65	256	258	128	128	211	215	232	242	208	210	190	204	225	225	242	242
66	258	258	128	128	211	223	232	232	210	210	204	204	225	226	242	242

67	258	258	128	128	211	223	230	232	216	216	204	204	225	226	242	242
68	258	258	128	128	211	223	230	232	216	216	204	204	225	226	242	242
69	258	258	128	128	211	211	232	232	210	210	204	204	225	225	242	242
70	258	258	128	128	211	211	230	232	210	216	204	204	225	226	242	242
71	258	258	128	128	223	223	230	232	210	216	204	204	225	226	242	242
72	258	258	128	128	223	223	230	232	210	216	204	204	225	225	242	242
73	256	258	128	128	211	215	232	242	208	210	190	204	225	225	242	242
74	258	258	128	128	211	223	230	232	210	212	190	204	225	226	242	242
75	258	258	128	128	223	223	232	242	208	216	190	204	225	226	242	242
81	258	258	128	128	211	223	230	230	210	216	204	204	226	226	242	242
82	258	258	128	128	211	223	230	230	210	216	204	204	226	226	242	242
83	258	258	128	128	211	211	230	232	210	216	204	204	226	226	242	242
84	258	258	128	128	211	223	230	232	210	216	204	204	225	226	242	242
85	258	258	128	128	223	223	230	232	210	216	204	204	225	226	242	242
76	256	258	128	128	215	223	230	232	212	216	190	204	225	225	242	242
77	256	258	128	128	211	215	232	242	208	210	190	204	225	225	242	242
78	258	258	128	128	211	223	230	232	210	212	190	204	225	226	242	242
79	258	258	128	128	223	223	232	242	208	216	190	204	225	226	242	242
80	256	258	128	128	215	223	230	232	212	216	190	204	225	225	242	242
86	258	258	128	128	223	223	232	232	210	210	204	204	225	226	242	242
87	258	258	128	128	211	223	230	232	216	216	204	204	225	226	242	242
88	258	258	128	128	223	223	230	232	216	216	204	204	225	226	242	242
89	258	258	128	128	211	211	232	232	210	216	204	204	225	225	242	242
90	258	258	128	128	211	211	230	232	210	216	204	204	225	226	242	242
91	258	258	128	128	223	223	230	232	210	216	204	204	225	226	242	242
92	258	258	128	128	211	223	230	232	210	216	204	204	225	225	242	242
93	258	258	128	128	223	223	230	232	216	216	204	204	225	226	242	242
94	258	258	128	128	211	211	232	232	210	210	204	204	225	225	242	242

95	258	258	128	128	211	211	232	232	210	216	204	204	225	225	242	242
96	258	258	128	128	211	211	232	232	210	216	204	204	225	225	242	242
97	258	258	128	128	211	211	232	232	210	216	204	204	225	225	242	242
98	258	258	128	128	223	223	232	232	210	216	204	204	225	226	242	242
99	258	258	128	128	211	223	230	232	216	216	204	204	225	226	242	242
100	258	258	128	128	223	223	230	232	216	216	204	204	225	226	242	242

## **Chapter 9: Carbon dating of recently deceased *A. carneorum* trees in Kinchega National Park**

### **9.1 Abstract**

I estimated the age of five recently deceased *A. carneorum* trees sampled from five different stands on Kinchega National Park in far western NSW, using bomb-pulse  $^{14}\text{C}$  techniques to test the hypothesis that the monoclonal structure of these stands could have been the result of introduced grazers (rabbits and goats) removing seedlings and promoting clonality. The age of these trees ranged from 26 +/- 2 and 133 +/- 63 years old for the two smaller plants with trunk radius from pith to bark of 40 and 46 mm respectively, to 191 +/- 69, 188 +/- 63 and 184 +/- 105 years old for the two larger and one medium sized tree with trunk radius measurements of 125, 143 and 75 mm respectively. It is likely that the oldest three trees, and possibly the fourth, recruited prior to the impacts of rabbit colonization of the area somewhere between 1860 and 1890. The monoclonal nature of most of the *A. carneorum* stands in the region therefore likely predates the unnaturally intense grazing regimes they persist under today.

## 9.2 Introduction

Genetic surveys of threatened *A. carneorum* stands across western NSW have found that almost all stands are monoclonal and likely to be the product of asexual reproduction (suckering) (O'Brian et al., 2013; Roberts et al., *in review*), although some stands are known to produce seed (Chapter 3, thesis). New *A. carneorum* recruits (seedlings and suckers) are voraciously consumed by feral grazers, namely rabbits and goats (Auld, 1993). It has therefore been hypothesised that the current monoclonal structure of these stands may have been established as a function of these unnatural grazing pressures (Auld, 1993). Alternatively, the lack of genetic diversity within these stands may simply represent a natural lack of sexual reproduction found in most, and may be the long term product of a natural shift from sexual to asexual reproduction as has occurred in many arid species (Silander, 1985; Caraco & Kelly 1991; Stuefer et al., 1996; Piquot et al. 1998; Honnay & Bossuyt 2005). Moreover, temporal gaps between years with successful sexual recruitment have been found to be highly variable in length in clonal plants, ranging from zero to thousands of years (Eriksson, 1989). Unfortunately, there is little reliable data on genet longevity and genet turnover rates in such plants, because these are difficult to measure (Dietz & Schweingruber, 2002).

One way to determine whether the genetic structure of these stands might be a consequence of European modification of the region and the introduction of feral grazers would be to determine whether the plants comprising these stands predate European settlement. If the plants comprising stands do not predate European influence / introduction of feral grazers then we will not be able to distinguish whether the genetic structure of stands we currently see is natural or a product of anthropogenic disturbance. If however, they do predate European influence on the landscape we can rule out the impacts post European settlement as a driving force for their structure and conclude that the structure of stands that we see is to some extent natural. Previous estimates of the age of five *A. carneorum* plants on Kinchega national park using Carbon dating techniques placed them at between 120 and 330 years old (Auld & Denham 2001). If the oldest of these plants are representative of the majority of plants now making up the bulk of remaining stands, there is a good chance these plants recruited into these stands prior to the worst effects of rabbit colonization of the region, if not prior to their arrival altogether. Given the homogenous demographics of these *A. carneorum* stands, which generally consisted of only old and senescent plants (Auld 1993; Chapter 2, thesis), it is likely that attaining the age of even a single plant within each stand is a good predictor of the approximate age of the other plants within the stand.

An AINSE (Australian Institute of Nuclear Science and Engineering) grant of \$30,000 funded the use of the Radio Carbon dating and ITRAX facilities at ANSTO (Australian Nuclear Science and Technology Organisation) to date the ages of five *A. carneorum* trees that had recently died on Kinchega National Park in far western NSW. In this study, I carbon date multiple recently deceased *A. carneorum* trees to get an estimate of the age of stands. Specifically, I test the hypothesis that these plants predate European influence in the region and therefore could not be monoclonal as a consequence of introduced grazing regimes.

## 9.3 Methods

### 9.3.1 Sample collection

Five fully mature and recently deceased *A. carneorum* trees, from different stands within Kinchega National Park in far western NSW were cut down and a cross section of their trunks (disks) was collected for further processing at 30 cm up from the base of the tree (Figure 9.1). I purposefully selected one small, one medium and two of the largest trees in these stands so as to look for any relationships between tree size and age. The trees were classed into one of three size categories based on the relative diameter of their trunks (large, medium and small) (see Table 9.1)). From each disk a 1 cm wide strip was cut extending from the bark to the pith (core). Depending on the size of the tree from which this core was sampled between four and six 2-3 mm squares were then cut at even intervals along the core from just inside the bark to the pith (Table 9.1). Multiple samples across the core are taken to attempt to match the levels of  $^{14}\text{C}$  in each sample on the time line of known calibration curves and verify the accuracy of the value found at the centre of the core. The year of death was known for all these plants (Table 9.1).



**Figure 9.1. National Parks ranger cutting down recently deceased *A. carneorum* trees in Kinchega National Park to attain material to carbon date their age.**

Table 9.1. ***A. carneorum* samples for carbon dating:** Plant Ids, size, core sample measurements and date of death for five trees from five different stands in Kinchega National Park.

Sample number	Sample name	GPS coordinate	ITRAX number	Size	Trunk Radius (Distance from core to edge (bark) of sample)	No. of samples taken for dating from core	Distance between each sample	Width of each sample	Date of death:
1	LOG 1 SBD	S32 31.912 E142 10.074	0033	LARGE	12.5cm	6	21mm	3mm	11/2/1989
2	BD6 lower	S32 31.912 E142 10.073	0128	LARGE	14.3cm	6	2.3cm	3mm	between 2011 and 2012
3	KNP Fruiting 3	S32 31.907 E142 09.060	0125	MEDIUM	7.5cm	5	1.6cm	2mm	between 2011-2012
4	SWA	S32 34.396 E142 07.685	0133	SMALL	4.6cm	4	1.1cm	2mm	1991
5	SD 76	S32 32.999 E142 09.983	0124	SMALL	4cm	4	1cm	2mm	between 2008-2010

### **9.3.2 Estimating the age of plants (bomb-pulse $^{14}\text{C}$ dating)**

I dated the 2-3mm square samples of wood taken from each core using bomb-pulse  $^{14}\text{C}$  techniques to estimate the growth history of each stem in much the same way described in Santini et al. (2013). Firstly I pre-treated these samples to extract alpha-cellulose using the method described in (Hua et al., 2004). Alpha-cellulose was then combusted to  $\text{CO}_2$  and reduced to graphite (Hua et al., 2001) for  $^{14}\text{C}$  analysis using the STAR accelerator mass spectrometry (AMS) facility at the Australian Nuclear Science and Technology Organisation (Fink et al., 2004). With the assistance of collaborator Quan Hua, measured  $^{14}\text{C}$  values were converted to calendar ages using the “Simple Sequence” deposition model of the OxCal calibration program (Ramsey, 2008), and a calibration data set for the last 350 years for the Southern Hemisphere (SH), made up of the updated SH bomb radiocarbon data (Hua Q & Barbetti, 2004) and the SHCal04 data for the pre-bomb period (McCormac et al., 2004).

### **9.3.3 Analysis of bomb pulse $^{14}\text{C}$ dating data**

I employed the Bayesian-based OxCal program with a chronological constraint (outer samples are younger than inner samples) to model the calibrated (cal) ages for each tree.

*\*All five models were found to be acceptable as their overall indices are higher than the accepted value of 60% (Appendix 9.6.1).*

## **9.4 Results**

The two large trees (0033) and (0128) were found to have similar ages at the time of their death (191 +/- 69 and 188 +/- 63 years old, respectively). The medium sized tree (0125) was also of a similar age (184 +/- 105 years old). The two smaller trees (0124) and (0133) were found to have significantly different ages (26 +/- 2 for 0124 and 133 +/- 63 years old) (Appendix 9.6.1 for calibrated versus modelled ages).

## **9.5 Discussion**

My findings that *A. carneorum* plants are capable of living for centuries confirms previous estimates of the longevity of these species by Auld & Denham (2001) and is in line

with many other studies around the world that have found various clonally replicating plant species that are very long lived (Wherry, 1972; Lynch et al., 1998; Brundu et al., 2008).

Importantly, these findings suggest that three and possibly four of the *A. carneorum* plants dated were likely to have been established before feral rabbits were present in significant numbers in the semi arid regions of NSW. This suggests that the current genetic structure of *A. carneorum* stands may not be far from the natural structure they possessed prior to European arrival. While it might be argued that some genetic diversity could have been lost in these stands since the arrival of introduced feral grazers, this data suggests this is probably not the case.

Whilst many studies use size and annual size increments of a plants and genets to measure age (e.g. Vasek, 1980; Steinger et al., 1996; Reusch et al., 1998; Wesche et al., 2005), size and age are not always linearly correlated and relationships can vary from site to site (Lucienne & Jürg Stöcklin, 2010). Whilst no clear relationship between the radius of these five *A. carneorum* trunks and their age was evident here, this was largely due to the big difference in age between the two smaller trees. This difference may reflect differences in the quality of the two different areas that these two plants were taken from, such as soil quality, or a slowing down of growth after plants reach a certain size. It is possible that suckers grow at a fast pace in the early stages to mid stages of their life and then slow down dramatically after they detached their underground lateral root connections with parental plants. It is unknown at what age this occurs in *A. carneorum* plants and little to no information is available for other long lived arid plants. It may be that after a certain age, there is a long period of steady growth until senescence / death that might be modelled by a linear relationship between size and age. Further research in this field is clearly needed for this species.

Given that the relationship between plant size and age cannot always be relied on for accurate dating and carbon dating of many individual stems can be prohibitively expensive, it would be preferable to be able to accurately date the age of *A. carneorum* plants / stands by other means. One of the most inexpensive and common methods is to use annual growth rings to date the age of trees (dendrochronology) (Ehrlén & Lehtilä, 2002). This is not always possible for plants that do not produce annual growth rings however, as is the case for many long lived arid species that grow sporadically and in conjunction with rare and unpredictable rain events (Büsgen & Münch, 1929; Fenner, 1998; Letnic & Dickman, 2006; Wardle et al., 2013). There is considerable effort being put into finding alternative methods to estimate the age / longevity of plants / genets that are not based on their size. For example, molecular

divergence based on somatic mutations and cell-growth estimates (Ally et al., 2008) or the proportion of ramets to genets (variation due to somatic mutation versus recombination (Mock et al., 2008) are alternate methods being used. Stage-based population or transition-matrix models are now also being used to investigate life history, dynamics and individual longevity (Ehrlén & Lehtilä, 2002).

While Carbon dating is a useful tool to estimate the age of plants, it is very expensive and time consuming. Dendrochronology, or the dating of plants using growth rings, can provide an inexpensive and quick way to ascertain the age of trees. As part of the AINSE grant I received, I was given access to the newly installed ITRAX facility at ANSTO to scan the same *A. carneorum* tree samples as well as many other cores from deceased trees in the region, to look for any evidence of annual tree rings, or growth patterns that might be able to be correlated with major rain events in the region. ITRAX scanners, which are usually used to date sediment cores (Rothwell & Rack, 2006), are increasingly being used to date plant cores. They combine radiographic x-ray imaging (XRF), optical imaging and magnetic susceptibility measurement, to determine all chemical elements along a core (<http://www.coxsys.se/>). The resolution is high enough to accurately see the width of year rings and the maximum density of the sample and it is possible to test for the presence of elements from magnesium and heavier (<http://treeringsusv.weebly.com/itrax-multiscanner.html>). So far I have not found consistent patterns that would indicate annual growth rings, however my work into matching growth patterns with past rain records to date trees is ongoing.

## 9.6 Appendix

**Appendix 9.6.1. Table of results for the bomb pulse  $^{14}\text{C}$  dating data obtained for five recently deceased A. carneorum plants sampled from five different stands on Kinchega National Park:** Calibrated un-modelled and modelled ages for samples from the pith to the Bark of each tree core. Calibrated ages of modelled samples at the pith of each tree core are shaded in grey. \* Bayesian-based OxCal program with a chronological constraint (outer samples are younger than inner samples) to model the cal ages for each tree. \*\*Modelled ages of plants are highlighted.

Radiocarbon Results - AINSE Grant 11/099

Tree ID	Lab ID	Sample ID	Distance from bark (mm)	$\delta^{13}\text{C} (\text{\textperthousand})$		pMC		Calibrated ages (AD)						Modelled ages (AD)						Index		
				Mean	1 $\sigma$	Mean	1 $\sigma$	1 $\sigma$ range		2 $\sigma$ range		Mean	1 $\sigma$	Median	1 $\sigma$ range		2 $\sigma$ range		Mean	1 $\sigma$	Median	
0033 - Large	OZQ257	0033-B1	126	-20.3	0.1	97.61	0.36	1669	1954	1660	1955	1785	84	1770	1757	1937	1668	1942	1818	69	1829	Amodel 88.9% Aoverall 89.6%
	OZQ258	0033-B2	105	-18.7	0.1	97.88	0.31	1677	1951	1671	1955	1812	89	1827	1799	1938	1685	1947	1851	57	1861	
	OZQ259	0033-B3	84	-19.8	0.2	98.42	0.33	1701	1925	1693	1955	1844	76	1858	1849	1946	1810	1948	1883	45	1892	
	OZQ260	0033-B4	63	-19.6	0.2	97.96	0.32	1681	1949	1674	1955	1820	87	1839	1888	1951	1839	1952	1911	38	1925	
	OZQ261	0033-B5	42	-19.1	0.1	97.55	0.35	1668	1954	1654	1954	1778	81	1765	1936	1955	1867	1955	1937	26	1947	
	OZQ262	0033-B6	21	-20.7	0.1	112.36	0.39	1994	1996	1958	1997	1990	12	1995	1994	1996	1958	1997	1992	10	1995	
		0033-Bark	0					2009	2009	2009	2009	2009	0	2009	2009	2009	2009	2009	2009	0	2009	
0128 - Large	OZQ549	0128-1	142	-21.9	0.2	97.91	0.29	1679	1951	1672	1955	1815	89	1835	1798	1894	1676	1919	1824	63	1837	Amodel 77.2% Aoverall 79%
	OZQ550	0128-2	118	-22.8	0.1	98.75	0.33	1711	1955	1698	1955	1854	68	1876	1811	1915	1700	1923	1853	54	1859	
	OZQ551	0128-3	94	-21.9	0.1	98.56	0.36	1708	1925	1695	1955	1847	74	1862	1820	1926	1714	1936	1873	49	1889	
	OZQ552	0128-4	70	-21.9	0.1	97.92	0.31	1679	1950	1672	1955	1815	88	1835	1838	1941	1828	1949	1893	45	1911	
	OZQ553	0128-5	46	-22.1	0.1	97.56	0.35	1668	1954	1655	1954	1779	82	1765	1869	1952	1839	1954	1911	38	1929	
	OZQ554	0128-6	23	-21.6	0.1	98.37	0.3	1700	1925	1693	1955	1843	77	1858	1929	1955	1881	1955	1931	24	1940	
		0126-Bark	0					2012	2012	2012	2012	2012	0	2012	2012	2012	2012	2012	2012	0	2012	
0125 -	OZQ544	0125-1	80	-20.3	0.1	97.34	0.36	1666	1953	1647	1955	1760	72	1758	1671	1953	1649	1954	1828	105	1791	Amodel

Chapter 9: Carbon dating of recently deceased *A. carneorum* trees in Kinchega National Park.

Medium	OZQ545	0125-2	64	-22.8	0.1	97.45	0.34	1667	1953	1650	1955	1767	75	1760	1754	1954	1671	1954	1855	92	1863	78.2%
	OZQ546	0125-3	48	-19.2	0.2	97.43	0.3	1668	1953	1651	1955	1762	71	1758	1774	1955	1741	1955	1892	77	1948	Aoverall 74.2%
	OZQ547	0125-4	32	-20.6	0.2	147.51	0.41	1972	1972	1963	1973	1971	2	1972	1972	1973	1963	1973	1971	3	1972	
	OZQ548	0125-5	16	-22.4	0.1	112.49	0.34	1994	1996	1958	1996	1990	12	1994	1994	1995	1993	1996	1995	1	1995	
		0125-Bark	0					2012	2012	2012	2012	2012	0	2012	2012	2012	2012	2012	2012	0	2012	
0124 - Small	OZQ540	0124-1	39	-20.1	0.1	122.09	0.34	1983	1985	1961	1985	1980	8	1984	1984	1985	1983	1985	1984	2	1984	Amodel 107.1% Aoverall 107.2%
	OZQ541	0124-2	29	-21.2	0.1	116.34	0.36	1989	1991	1959	1992	1986	10	1990	1989	1991	1988	1992	1990	1	1990	
	OZQ542	0124-3	19	-21.8	0.2	113.62	0.36	1993	1994	1958	1995	1989	12	1993	1993	1994	1992	1995	1993	1	1993	
	OZQ543	0124-4	9	-19.6	0.2	110.61	0.38	1997	1999	1958	2000	1997	8	1998	1997	1999	1996	2000	1998	1	1998	
		0124-Bark	0					2010	2010	2010	2010	2010	0	2010	2010	2010	2010	2010	2010	0	2010	
0133 - Small	OZQ555	0133-1	45	-20.9	0.2	97.98	0.34	1682	1949	1673	1955	1820	87	1839	1805	1938	1679	1945	1858	63	1871	Amodel 80.4% Aoverall 81.6%
	OZQ556	0133-2	34	-20.7	0.1	98.46	0.3	1708	1925	1696	1955	1847	74	1861	1881	1944	1810	1948	1889	48	1900	
	OZQ557	0133-3	23	-18.7	0.1	98.24	0.34	1698	1946	1684	1950	1836	81	1853	1912	1950	1836	1954	1915	39	1926	
	OZQ558	0133-4	12	-18.7	0.1	97.29	0.34	1666	1953	1646	1955	1755	68	1757	1947	1954	1870	1955	1942	28	1951	
		0133-Bark	0					1991	1991	1991	1991	1991	0	1991	1991	1991	1991	1991	1991	0	1991	



## Chapter 10: General Discussion

### 10.1 Overview

The primary aim of this thesis was to investigate the effects of fragmentation on the reproductive health of long lived plants, using a suite of long lived semi arid overstory *Acacia* existing as unnaturally isolated stands within a highly fragmented landscape in western NSW. Whilst many studies have looked at the effects of fragmentation on the reproductive health of annuals and shorter lived perennials (Young & Clarke, 2000), relatively few studies have focused on long lived species with temporally irregular reproduction.

I used a multifaceted, comparative approach to quantify the state of the remaining stands of several threatened and one co-occurring *Acacia* species thought to be thriving. I used these data to answer six major questions about these species current condition, reproductive strategies and mating systems. This investigation adds to previous assessment of threatened overstory *Acacia* in this region and has filled a significant gap in our understanding about the effects of fragmentation on the reproduction and recruitment dynamics of these long lived plants. Whilst practical constraints meant I could not answer every one of these six questions robustly for every one of the five model *Acacia* species chosen, I was able to address all of these questions for at least one of the four threatened species. Taken together these investigations have provided a comprehensive assessment of the current condition of these *Acacia* species and their reproductive health, as well as providing the grounds for making predictions about their future fate and recommendations for their management.

Below I provide answers to the six major questions I set out to answer in this thesis and discuss the broader implications of these findings for our understanding of these semi arid *Acacia* and their conservation. I also make recommendations for the future management of these threatened *Acacia* stands and discuss the broader implications of my findings for predicting how long lived plants will fare within fragmented landscapes.

### 10.2 Are stands old and senescing across their whole range in western NSW?

My characterization of the demographic structure of stands of several overstory *Acacia* species across the region (Chapter 3) highlighted a huge imbalance in the age structure of remaining stands of *A. melvillei*, *A. loderi* and *A. carneorum* favouring older and often

senescent plants. The finding that *Acacia melvillei*, *A. loderi* and *A. carneorum* stands are typically isolated and are comprised overwhelmingly of older plants, with little sign of either recent sexual or asexual recruitment, implies that even when or if sexual recruitment has occurred throughout the long life of the currently mature and senescent trees, recruitment has still not followed. While no baseline information exists for these species on the numbers or proportion of recruits that have historically come by way of sexual and asexual reproduction, it has been suggested that asexual reproduction (suckering) is likely to be naturally low in *A. melvillei* stands (Batty & Parsons, 1992). It might be assumed that the same is true for closely related *A. homalophylla* and *A. loderi*, although this has not been researched. Moreover, genetic surveys and preliminary genetic investigations of mature plants of these three threatened *Acacia* species have revealed that many stands are genetically diverse and have likely come by way of sexual reproduction and recruitment alone (Forrest et al., 2015; Forrest et al., unpublished work & Chapter 5). This is not always the case though, with monoclonal stands of these species also detected (Roberts et al., 2016; Forrest et al., 2015; Forrest et al., unpublished work).

Assessments of the natural contributions of sexual versus asexual reproduction in plant populations within anthropogenically disturbed landscapes are complicated by the fact that suckering is promoted by anthropogenic disturbance to plants' roots (Batty & Parsons, 1992). As such, we would expect more suckers to have been initiated in western NSW, post European settlement than previous to it, owing to the introduction of agricultural and feral grazers, disturbance from farming and clearing for roads. Indeed this most likely explains the relatively high levels of suckering found within *A. homalophylla* stands, given their more eastern distributions on farm land and along road side verges where human activity is higher than further west. Regular clearing of road side vegetation by councils likely disturbs the roots of these remaining trees promoting unnaturally dense suckering, and livestock are often encouraged to graze intensely on road side verges when feed levels in adjacent paddocks are low. Ironically, this disturbance regime has meant that stands that might otherwise now only consist of senescent adult plants, as found for *A. melvillei*, *A. loderi* and *A. carneorum*, contain plants of a wide range of age classes.

Whilst the proliferation of suckers within small and senescent stands may indeed serve to prolong the life of a stand, long term negative effects may ensue if genetic diversity is reduced as a result. Given that *A. melvillei*, *A. homalophylla* and *A. loderi* have naturally formed genetically diverse stands in the past (Roberts et al., 2013; Forrest et al., 2015; Roberts

et al., 2016) we must expect this diversity plays, or at least has played, an important role in their health and survival. Moreover, with current predictions of human induced temperature rises in Australia (IPCC 2001) a reserve of genetic diversity within stands is likely to become important for resilience by allowing species to adapt to the new conditions (Hughes & Westoby, 1994; Hughes, 2003). Whilst asexual recruitment that has almost certainly been unnaturally induced in many *A. homalophylla* stands surveyed, this may represent a better situation for this species in the region compared with the other threatened species in the short to medium term. In the long term however, any genetic homogenization of *A. homalophylla* stands could become problematic given the capacity to adapt to climate change will be reduced. On a positive note, the longevity of *A. melvillei*, *A. homalophylla* and *A. loderi* plants means that while genetic diversity is likely to have been lost through genetic drift, the older remaining plants would likely still be sources of close to natural levels of genetic diversity if they were recruited prior to landscape modification. Any attempts to restock stands of these species should be done using seed produced by these older plants before they senesce or die and these genotypes are lost.

While the finding of large numbers of recently recruited suckers in most *A. homalophylla* stands represented the most striking demographic difference between the four threatened species, the detection of subtler heterogeneity in the demographic structures of the other three species is also likely to be important information to managers prioritizing conservation of these species. Of particular note, I found that the vast majority of *A. melvillei* plants were fully mature but not senescing, in contrast to vast majority of *A. loderi* and *A. carneorum* plants. The distinctions are useful for predicting a rough time frame for the opportunity for rescue before these stands will be lost, as well as helping managers prioritise which stands most urgently require limited conservation resources. My findings confirm that stands of *A. carneorum* are most often senescing and consequently in most urgent need of conservation, but it may also be prudent to give *A. loderi* conservation priority over *A. melvillei*, as the window for persistence of *A. melvillei* appears to be relatively longer. It seems the longevity of remaining *A. homalophylla* stands may have been extended by the unnaturally high levels of anthropogenically induced suckering.

*A. ligulata* stands in the region were more evenly structured demographically with a larger number of younger plants in all stands surveyed, indicating relatively continuous sexual recruitment, and supporting thinking to date that this species is fairing relatively better in the region, than those listed as threatened.

The limitations of using biometric measurements to estimate the relative age of plants must be conceded. Natural variance will mean, that plants with similar values for trunk width / height and canopy cover will not die at exactly the same time so we must be careful estimating the lifespan of plants based on those I observed to die during this study. Observed deaths may also be due to other stresses limited to those particular trees rather than simply the morphological / age classes we assigned to them. This is worth considering for *A. melvillei* as the majority of trees observed to die during this survey were based in only two stands within Mungo National Park. Conversely, plants of the other four species that were observed to die came from multiple stands in multiple regions of their distribution and therefore would be less likely to reflect site specific stresses. In any case, recently deceased trees of all five species give us some idea about the physical properties plants of each species are likely to possess at the end of their life. Estimates of plant age could of course be made vastly more accurate with the use of dendrochronology, carbon dating or a combination of both. Prohibitively expensive costs could be dealt with by only carbon dating samples of each species and ground truthing dendrochronology methods to assess the bulk of the plants to be dated. Taking core samples for dendrochronology work from threatened plants that are old and weak however, might arguably be too much of a risk. At the very least my results serve as a rough relative guide to predict how long stands have left, given continued reproductive and recruitment failure.

### **10.3 Are stands highly fragmented and in poor condition, or is there important heterogeneity between them?**

My characterization of the physical condition of the extant stands of the four threatened species (Chapter 4, thesis) confirmed previous reports that they are often small stands consisting of only a handful of plants disconnected within a fragmented landscape (Benson, 1988; Batty & Parsons, 1992; Cunningham, 2000; Auld & Denham, 2001; Porteniers, 2001). Nevertheless, I found great variation between stands of the same species for all five species studied here across the region, ranging from lone plants and stands with a handful of senescent plants, to stands with hundreds of plants ranging in age (Chapter 4, thesis). This finding gives us a better indication of the true condition of stands in the region than we have had to date, their varied states across the region and therefore the level of pressure they face. As such, these findings should be used to prioritise the conservation of particular stands over others.

It could be argued that the smallest and most disconnected stands should be prioritised for conservation given that these stands are most likely to suffer pressures on their mating systems, have a higher risk of extinction and may contain locally adapted alleles that may be lost for good. Alternatively, it may be more practical to conserve bigger stands first, if smaller stands are beyond rescue, or else the resources required to recover these stands are unavailable. It may be preferable to focus on maintaining the size and health of larger more robust stands that are likely to contain more genetic diversity and have healthier mating systems (Crow & Kimura, 1970; Oostermeijer et al., 1995; Goverde et al., 2002; Aguilar et al., 2006; Kapos, 1989; Matlack, 1993, 1994b, Malcom, 1998; Jules & Rathcke, 1999; Meiners & Pickett, 1999).

Despite a great variance in the structure of stands of these Acacia species across the region, my finding that the plants comprising these different stands were all of a similar condition highlights that these plants have a level of physiological tolerance to even the harshest of local conditions within the fragmented landscape. While this physiological tolerance is clearly associated with the older well-established plants with long tap roots that largely remain in these stands, such resilience however, should not necessarily be supposed for younger plants of the same species. Indeed, if larger numbers of younger plants were present in these stands, it is likely that the condition of these younger plants would be worse, given that we would expect less established plants to be less resilient than mature plants. Observations of the way in which younger / smaller plants reproduce would be useful in gaining a more holistic picture of these species resilience to prevailing conditions.

## **10.4 Are stands still capable of sexual reproduction and recruitment after a large scale rain event?**

### *(i) Fruit / Seed set*

My findings of generally high levels of fecundity in the majority of *A. melvillei*, *A. homalophylla* and *A. loderi* stands after a large scale La Niña rain event beginning in 2010 (Chapter 3), as well as recruitment of seedlings in many stands (Chapter 5) disprove previous hypotheses that these plants were potentially incapable of sexual reproduction and recruitment in their current state. Mass seed set and recruitment of seedlings in some stands

of these three *Acacia* species, for the first time in decades implies that sexual reproduction in these species is naturally rare and seemingly coincides with large cyclical rain events. This ‘boom and bust’ reproductive dynamic is seen in many other arid species (Büsgen & Münch, 1929; Davies 1976; Norton & Kelly, 1988; Fenner, 1998; Letnic & Dickman, 2006; Wardle et al., 2013). It also reinforces the need to assess reproductive success of long lived species over longer periods, especially in arid environments where rain is infrequent and obviously crucial to reproduction (Letnic & Dickman, 2006; Wardle et al., 2013).

The reproductive resilience displayed by these *Acacia* to what is likely harsher abiotic conditions than would exist in un-fragmented stands, may again just simply reflect the physiological resilience of these established mature plants. As mentioned previously, whilst we might expect the mature established plants with big tap roots that comprise these stands to be resilient to surface conditions, if younger reproductively mature plants of smaller stature had been present in these stands, years of drought stress may have exhausted their capacity to set fruit when the rains arrived (Zhu, 2002; Yamaguchi-Shinozaki and Shinozaki, 2006). Nevertheless, the mating systems of these mature plants have also displayed a level of resilience to their acute isolation, irrespective of their stature. My findings of large numbers of hermaphroditic flowers (Chapter 3, thesis), and diverse and robust pollinator services (Chapter 6, thesis), in combination with the capacity to produce viable progeny through self fertilization (Chapter 7; Forrest et al., unpublished work), confers a level of resilience to an isolated state that an obligately seeding species with a less diverse, or more vulnerable pollinator service would lack.

My finding of a total lack of fruit in a minority of *A. melvillei*, *A. homalophylla* and *A. loderi* stands, was noteworthy especially given it could not be explained by the structure or condition of these stands. Similarly, the lack of fruit set in the majority of *A. carneorum* stands, despite a minority setting fruit requires explanation, given the abundance of water in the environment. For these stands it suggests that lack of water was historically not the only factor limiting sexual reproduction, as it appears to have been for the majority of stands.

Several possible explanations could account for plants which flower readily and are not pollen limited, but fail to produce fruit. Firstly, plants can fail to produce fruit if they do not receive compatible pollen as a result of being disconnected from compatible mates (Jennersten, 1988; Lande, 1995; Steffan-Dewenter & Tscharntke, 1999). Secondly, it is possible that the reproductive strategy of plants in these stands differs from plants in the majority of stands, by being obligately asexual. Indeed differences in the reproductive strategies between

populations of the same species are not uncommon and can be either evolutionary responses to differing environmental conditions across the species range (Richards, 2007; Sartor et al., 2011; Hardion et al., 2015), plastic responses (Abrahamson, 1975; Douglas, 1981; Bierzychudek and Eckhart, 1988; Cheplick, 1995; van Kleunen et al., 2002; Vallejo-Marín et al., 2010), or the start or tail end of a general evolutionary shift in the reproductive strategy of a species (Silander, 1985; Caraco & Kelly 1991; Stuefer et al., 1996; Piquot et al., 1998; Honnay & Bossuyt, 2005).

An evolutionary shift from sexual to asexual reproduction might best explain the lack of sexual reproduction across the majority of *A. carneorum* stands, despite still flowering. Indeed, the few sexually reproductive stands remaining may represent the tail end of this shift rather than part of a steady state reproductive strategy, the result of fortunate positioning near compatible mates, or as a function of being relatively healthier than the majority of surrounding stands. Previous genetic analysis (O'Brian et al., 2013; Roberts et al., *in review*) and carbon dating (Auld & Denham et al., 2001; Chapter 9) of *A. carneorum* plants in the same stands surveyed here supports the idea that all stands have recruited solely via asexual means at a period prior to any major disturbance by European settlement. As such, a lack of sexual function is unlikely to be the reason for the contraction of *A. carneorum* stands currently being seen, but rather the intense / unnatural consumption of any new suckers since the introduction of feral grazers as has been previously reported (Auld, 1993). Determining whether differences in the reproductive response between different stands is the product of evolutionary differences between plants, or plastic responses to prevailing conditions would require the use of reciprocal transplant experiments (Kawecki & Ebert, 2004; Leimu & Fischer, 2008; Hereford, 2009).

### (ii) Recruitment

My finding of a high level of variance in recruitment rates of seedlings between stands of all four *Acacia* species that set fruit after the La Niña rain event, irrespective of the number of seed produced by plants (Chapter 5, thesis), suggests significant local variance in the integrity of the environment or the health of seed produced. While some level of variance in recruitment rates between stands of the same species is expected in any natural system, in stands where I found only a handful of *Acacia* recruits despite high levels of fecundity, it might be safe to assume that this is suboptimal recruitment. Moreover any recruits that do emerge are unlikely to survive to maturity given the harsh conditions that will inevitably return after the region dries out (Boyd & Brum, 1982; Baskin & Baskin, 1998; Fenner, 2000; De La Cruz et

al., 2008). Indeed, even stands that might recruit optimal numbers of seedlings would still be unlikely to produce an adequate number of recruits to avoid substantial decline given the unnaturally intense grazing pressures in the region (Auld 1993, 1995; Cohn & Bradstock, 2000; Auld & Denham, 2001).

Determining what levels of recruitment to expect within healthy and fecund stands is difficult without some historic baseline information for comparison with current observations. As we do not know the natural rate of sexual and asexual recruitment of these *Acacia* prior to European colonization, it is difficult to assess whether we would have seen more *A. melvillei*, *A. loderi* and *A. carneorum* seedlings and suckers under pre European grazing regimes or not. Although I recorded mortality rates of seedlings over the first three years of their lives, I did not get the chance to observe their level of resilience under the harsher, drier conditions they will inevitably face, which would provide us with a true indicator of these stands prospects of future persistence. Whether ample numbers of recruits survive to reproductive age following this recruitment event or not, the level of fecundity remaining in senescent *A. melvillei*, *A. homalophylla* and *A. loderi* stands, provides some hope for restoration. Managers can be confident of at least having access to large amounts of seed for active manual restoration strategies, which are likely to be necessary in the future, so long as adult plants remain and climatic conditions are suitable for reproduction occur.

## **10.5 Is reproductive success and failure determined by the age or condition of stands?**

### *(i) Fruit /seed set*

I found no discernible differences in the apparent age structure (morphological characteristics) or the physical condition of plants and stands of *A. melvillei*, *A. homalophylla*, *A. loderi* and *A. carneorum* plants that set fruit and those that did not (Chapter 4). Moreover, I found no relationship between the fecundity of plants that did set fruit and their age or condition (3 & 4), showing that historic failure of some plants in the minority of *A. melvillei*, *A. homalophylla*, *A. loderi* and a majority of *A. carneorum* stands was almost certainly not explained by senescence, poor local environmental / climatic conditions or physiological weakness of old plants.

My finding that all the *A. loderi* stands surveyed that failed to set seed were always comprised of a single genet, along with preliminary data suggesting the same for *A. melvillei* and *A. homalophylla* stands (Forrest et al., unpublished work) indicates a genetic component to reproductive capacity. I also found monoclonal stands of each species that set equally large numbers of fruit per plant as the most fecund and genetically diverse stands, ruling out clonality alone as a reliable predictor of reproductive failure. Nevertheless, the finding provides the first indication that the sexual capacity of these *Acacia* stands may be linked to the genetics of plants rather than their physical condition.

A lack of genetic diversity in all *A. carneorum* stands surveyed, irrespective of their capacity to set seed or physical / demographic condition, suggests that maternal capacity in this species is also likely to be determined by their specific genotype. To determine whether these seemingly sterile monoclonal stands do indeed represent truly maternally sterile genotypes, rather than plants that just so happen to be disconnected from compatible mates, pollen from a range of near and distant sources should be manually provided to these plants to attempt to initiate seed set (Waser & Price, 1989). While I did this for *A. carneorum* without effect (Chapter 7), it was clear that I did not pollinate enough flowers to be confident that seemingly sterile stands were not simply less fecund than those with a history of setting seed naturally.

Understanding whether seemingly maternally sterile *Acacia* clones exist as a result of anthropogenic disturbance, or have established naturally, is clearly of great importance in making sensible management decisions. Namely, if these stands represent naturally asexual stands, then conservation should only extend so far as to protect any naturally produced suckers from being lost through grazing by feral animals. If these stands are instead a result of loss of genetic diversity in certain areas, through reductions in stand size (fragmentation) and genetic drift, more drastic conservation measures may be required to conserve the remaining genotype, as well as return genetic diversity and sexual function.

The fact that many of the remaining stands of these *Acacia* species, which were comprised of only senescent plants in some of the most acutely fragmented stands in the region, were observed to set copious amounts of fruit and recruit seedlings, suggests that it is unlikely that more subtle assessments of their health would have revealed undetected correlations between their condition and reproductive capacity. Nevertheless, I acknowledge that my assessments of stand and plant condition may have been insufficient in detecting more subtle yet important signs of strength and weakness in plants that could otherwise

correlate with reproductive success and failure. For example, diseases with cryptic symptoms would have remained undetected by my assessments of leaf condition as a proxy for plant health. Although assessments of plant age could be improved with the use of dendrochronological techniques or carbon dating of plants as previously discussed, the health of plants could be more accurately assessed with the use of various spectrometry techniques, to measure the chlorophyll content of leaves, which is known to be a strong correlate of plant health (Cui et al., 2009; Johnstone et al., 2013; Cortazar et al., 2015).

### *(ii) Seedling recruitment*

I found positive correlations between the number of recruits and stand size for *A. melvillei* and *A. loderi*, number of suckers and recruits for *A. homalophylla*, as well as a negative correlation with the distance between *A. ligulata* stands and recruits. Vast differences in seedling recruitment levels, between relatively closely situated stands of all four *Acacia* species that recruited seedlings however, could not be reliably explained by differences in the structure and condition of stands and plants, nor could it be explained by differences in the fecundity or the fitness of seed produced in different stands. This implies a more cryptic cause for this variance. While some variation in recruitment rates between stands of the same species is expected naturally, especially if they are at opposite ends of the geographic range of the species where climatic and ground conditions may vary significantly, my finding of wildly differing recruitment rates in neighbouring stands of the same species implies there are differences in a much more local scale driving this variance.

I found no evidence that the vast differences in recruitment rates between stands was a result of differences in the number or fitness of seed produced in different stands. It should be noted however that there may be cryptic differences in the fitness of these seed undetected by our lab germination and growth experiments under benign coastal conditions. Any small differences in seed fitness are likely to be amplified and become more obvious under high stress conditions, such as those experienced during the dry summer period in their natural arid environments. These findings suggest that under conditions during and soon after a La Niña rain event when water is ample in the environment and temperatures cooler, we are unlikely to notice such differences in germination levels, growth rates or even mortality if they do exist. In any case, any small differences in fitness between seed cohorts from different stands may be inconsequential compared to the difference in the quality of the highly modified local environments during periods of plenty.

It could be argued that the great variance in recruitment rates between these *Acacia* stands is likely to be best explained by differences in the seed's ability to penetrate (lodge) the soil and take root within each site (Dardel et al., 2014). The result of our manual seed planting experiment in the field (Chapter 5) also supports this theory given the proportionally higher level of initial recruitment that was found when seeds were manually assured proper burial / lodging than naturally recruited within stands (Chapter 5). Given this, the lack of a correlation between the level of understory and recruitment was particularly unexpected, given that in many systems a healthy understory both prevents the loss of seeds through run off and provides them with more suitable ground conditions for germination and survival (Dardel et al., 2014). One reason for the lack of this correlation may be that none of the understories of any of these remaining stands is likely to resemble a natural assemblage, or natural densities. Indeed it may reflect that in fragmented stands we tend to find too much or too little understory. In these altered stands, a complete lack of understory as a result of heavy grazing, or an unnaturally dense one as a result of a thinned canopy providing access to more light as well as the introduction of many weeds coming in from adjacent farm land, has likely created two habitats, either too hot and harsh or with too much competition for resources and space to expect natural levels of recruitment (Kearns et al., 1998; Wiser et al., 1998; White et al., 2002; Yates et al., 2004). Where unnatural ground conditions prevail, other local factors not considered in this study, might begin to play a larger role in providing suitable ground conditions for recruitment, such as the type and quality of the soil and the micro topographical features of the land. These physical attributes of the local topography should be crucial in determining whether seeds remain and become buried locally during periods of great run off (Harper et al., 1961, 1965). Indeed when I looked closer at these stands I found mass recruitment in patches that were associated with grooves and divets where water and seed might be expected to pool.

My finding that nurse plants positively affect the three year survival rates of seedlings of these *Acacia* species by reducing grazing damage implies that an understory is likely of great importance to these species. With the onset of drier conditions and the inevitable dying off of ephemeral plants, the protection of nurse plants should become even more important for seedlings as grazing pressure inevitably increases (Auld & Denham, 2001). Moreover, in particularly isolated stands in areas characterized by few overstory plants, we might expect grazing pressures on new recruits to be especially high, as grazers are disproportionately attracted to these stands in order to seek shade when temperatures rise and water availability falls. In arid environments where evaporation rates are high, the presence of a canopy and

dense understory can have a drastic effect on retaining water after a rain event for far longer than adjacent bare soil (Callaway et al., 1996; Castro et al., 2002; Armas & Pugnaire, 2005). This protection must be balanced against the capacity for dense understory vegetation to compete with seedlings for resources however (Bush & Van Auken, 1990; Callaway & Walker, 1997).

While canopy shade serves to buffer seedlings from the most extreme temperatures, a general trade off between this protection from desiccation and grazing, with growth, is well established (Alexander & Maggs, 1970; Piper, 1986; Popma & Bongers, 1988; Minore, 1988; Pierson et al., 1990; Turner, 1990; Bush & Van Auken, 1990; Seiwa, 2007). My finding of noticeably slower growth rates of *Acacia* seedlings located under the protection of nurse plants is a likely consequence of such a trade off. While at first the growth rate of seedlings may not seem as important as their mortality rate, when considering their long term survival in this environment, it may be that growing a long tap root more quickly may be more advantageous in the long run when surface water quickly dries up after a rain event. Indeed in many plant species, faster germination rates and growth rates have been linked to higher chances of survival and is ultimately selected for these exact reasons (King et al., 1986). My findings of higher long term survival rates of seedlings that originated from the soil seed bank, and seemingly germinated at the start of the La Niña event, approximately one year before seed produced in the canopy had reached the ground, is also testament to this. While historically a bet hedging strategy was likely to be optimal to insure against particularly hot spells soon after recruitment or peaks in local grazing pressures, under the unnaturally high grazing regime of the past 150 years, this balance may become skewed towards favouring faster growth, putting evolutionary pressure on these plants to select for genotypes that grow faster.

Despite finding recruits in several stands of *A. melvillei*, *A. loderi* and *A. homalophylla* that survived to at least three years of age this does not guarantee their survival to adulthood. The capacity for seedlings to survive drier times would be greatly reduced, particularly considering the condition of remaining stands is unlikely to represent anywhere near a natural pre settlement quality. Even a small deterioration in local abiotic conditions through lesser understory due to lesser canopy shade may be enough to significantly increase seedling mortality in a species already living on a physiological knife edge. The few seedlings that find themselves in local refugia and survive the general increase in local temperature are likely to be targeted and consumed by feral goats, rabbits and livestock as vegetation in the region

becomes more scarce (Auld, 1993, 1995; Cohn & Bradstock, 2000; Auld & Denham, 2001). Indeed past recruitment events have clearly not resulted in substantial numbers of recruits, given the lack of plants found in the mid range age structure in remaining stands (Chapter 2). While tracking the fate of these seedlings up to the three year mark (Chapter 5) provides us with a better estimate of their likelihood to persist than initial observations of recruitment can, it is still only a relatively small window of time in the recruits journey to adulthood and does not tell us whether recruits are fit enough to survive long term. Given we only captured a snapshot of the performance of these recruits under relatively benign conditions compared to what they will face for the majority of their juvenile period, any predictions of effective recruitment to the adult stage based on these observations are likely to be an overestimate.

## **10.6 Are mating systems of these species currently suboptimal or particularly susceptible to any increased levels of fragmentation?**

My findings that none of the five *Acacia* species studied here were pollen limited (Chapter 6), and are self compatible but preferentially outcrossing (Chapter 7, 8 & Forrest et al., unpublished work) suggests that unnatural and increasing levels of isolation should lead to increasing levels of inbreeding and loss of genetic diversity in stands of both species over time. While I found no obvious fitness consequences of inbreeding for the fitness of *A. carneorum* offspring (Chapter 8), my finding of reduced growth rates of selfed *A. ligulata* offspring compared with outcrossed offspring, as well as reduced fitness of naturally set seed compared to manually set seed (Chapter 7), indicates that *A. ligulata* stands are susceptible to and may be already suffering from inbreeding depression. While plans to attain this same information for *A. melvillei*, *A. homalophylla*, *A. loderi* were hindered by flood, genetic analysis of *A. loderi* stands (Chapter 4), as well as genetic analysis of some *A. melvillei* stands (Forrest et al., 2015; Forrest et al., unpublished work), suggests that these species are also preferentially outcrossing. They are therefore likely to be at least susceptible to loss of genetic diversity with increasing fragmentation, if not inbreeding depression also.

Whilst the level of self compatibility discovered in *A. carneorum* and *A. ligulata*, as well as indicated by my preliminary paternity analysis data for the other three *Acacia* species, provides these species with a level of reproductive resilience against total reproductive failure under the conditions of restricted gene flow that my pollinator observations imply, concerns that this type of local foraging has lead to undesirable levels of inbreeding is warranted given

their clear preference for outcrossed pollen. The fact that mate choice / self incompatibility mechanisms have evolved to maximise the number of outcrossed seed matured on these plants, implies that genetic diversity within offspring is, or at least has been, of evolutionary value to this *Acacia* species. Levels of gene flow are likely to already be suboptimal in many *A. ligulata* populations, and any further isolation of stands through further land clearing in the region would only be expected to intensify these effects. Understanding the way in which gene flow is affected by fragmentation is therefore likely to become crucial information in deciding how these stands should be managed, such as how much connectivity should be maintained between populations / stands.

The detection of significant levels of outcrossing between two *A. carneorum* stands separated by one kilometre but not over four or six kilometres (Chapter 8), gives us some idea for the first time of the distances over which *A. carneorum* stands remain connected. While the distance over which outcrossing currently occurs is unlikely to represent the distance achieved by pollinators acting within an unaltered landscape, this result provides important information to managers looking to restore connectivity in the region. If I had analysed more seed or studied more sexually reproducing stands it is possible that I may have detected movements of pollen between more distant stands. However, given not one of the 100 seed I analysed was fathered by a plant further than approximately one kilometre away, I can safely say these movements would be rare at best.

*A. ligulata* pollination has been found to have been hijacked by introduced honeybees and is likely to be having a large impact on the level of outcrossing occurring in these stands (Gilpin et al., 2014). Whilst paternity analysis of naturally produced *A. ligulata* seed should be performed in the future to determine the distances over which matings are occurring, the finding that *A. ligulata*'s pollinator services are now dominated by *Apis mellifera* means that gene flow has likely been restricted beyond that which would otherwise be occurring. This change in pollinator ecology is almost certainly responsible for the reduction in offspring quality inferred from my manual pollination experiments, and subsequent decreases in genetic diversity and consequent losses of adaptive capacity of these stands will also make them more vulnerable in the long term. There is much literature on the increase in inbreeding caused by the relatively restricted foraging behaviours of honeybees compared with native pollinators of Australian plants, especially in bird pollinated systems (Paton & Turner, 1985; Taylor & Whelan, 1988; Paton, 1993; Richardson et al., 2000). Whilst this occurs as a result of the restricted distances over which honeybee forage (Taylor and Whelan, 1988; Ramsey, 1988;

Vaughton, 1996; England et al., 2001), they can also exhaust pollen loads depriving any native pollinators, that might facilitate outcrossing over larger distances, of pollen (Vaughton, 1992; Vaughton, 1996; England et al., 2001). Caution must be taken however, basing assumptions about pollination of all *A. ligulata* populations across the region on these and previous studies conducted within Kinchega National Park (Gilpin et al., 2014). Given that honeybee colonization is heavily dependent on access to an ample water supply (Seeley, 1985; Heinrich, 1996) and these studies were based very close to a large source of standing water (Lake Menindee), findings may not represent the majority of populations that are located further from a water source. Moreover, the large amounts of water across the whole region after the La Niña rain event might allow honeybees to at least temporarily move to areas they might be excluded from in drier times. It is possible therefore that honeybees play a significant part in the pollination of a far broader cross section of stands after a rain event than they would otherwise. If so, we would expect lower levels of inbreeding and selfing to occur in most years of reproduction. However, given that a significantly higher number of recruits are likely to survive during a large scale rain event, the contribution of matings facilitated by honeybees to the genetic structure of stands might be significant over the long term, as their contribution would be realised in mass recruitment pulses. Studies into pollinator assemblages in stands further from a water source would help determine this.

While observations of pollinator foraging behaviour can be useful for predicting what types of matings are likely to be occurring and which insects are likely to be responsible for inbreeding and outbreeding, this method can risk underestimating the amount of outcrossed pollen delivered to flowers. Although the majority of pollen supplied to flowers of both *A. carneorum* and *A. ligulata* plants would undoubtedly be selfed and local pollen, the capacity of these pollinators to supply plants with a mix of outcrossed pollen is hard to determine through observation. Indeed, it would not be surprising if even the most sedentary of pollinators make their way across a much larger area during a foraging bout than we might expect, based on the snap shot of time I observed them. We might expect longer range movements to be more common in *A. ligulata* stands than *A. carneorum* stands in the same area, given the higher numbers of flowering and ephemeral plants in the landscape during spring could provide stepping stones between isolated stands. On the other hand, during the harsher summer conditions when *A. carneorum* plants flower, fewer flowering plants of other species may in fact encourage interstand movements between isolated *A. carneorum* stands that might not otherwise occur if more resources were available (Mustajärvi et al., 2001; Goverde et al., 2002; Andrieu et al., 2009; González-Varo et al., 2010). At the very least, pollinator observations

cannot tell us anything about important pollinators that may have been lost as a result of anthropogenic disturbances in a region. Without information prior to disturbance, we cannot know whether key pollinator species, crucial for outcrossing in these *Acacia* species, may have been lost.

## 10.7 What conservation strategies should managers adopt to conserve these stands?

With the current predictions of climate change expecting temperature rises between one and five degrees Celsius within 60 years (by 2070) in Australia (IPCC 2001), and flora expected to be particularly vulnerable (Preston & Jones, 2006), the fate of these stands is sure to worsen (Hughes & Westoby, 1994; Hughes, 2003; Bell & Gonzalez, 2009, Bell, 2013, Gonzalez & Bell, 2013). Moreover, it has been found that fragmentation of natural environments can exacerbate the stresses imposed on species by climate change (Eckert et al., 2010; Hoffmann et al., 2011) and inbreeding depression may even become stronger as a result of climate change as conditions become harsher (Hauser & Loeschcke, 1996; Armbruster & Reed; 2005; Leimu et al., 2010). Whilst some stands may tolerate climatic changes via phenotypic plasticity (Crispo, 2008; Chevin et al., 2010; Reed et al., 2011), adapt to the new conditions (Anderson et al., 2012; O'Connor et al., 2012; Gonzalez & Bell., 2013), or migrate to track their climatic niches across the landscape (Parmesan, 2006; Loarie et al., 2009), it is expected that many will fail to respond. This is especially the case in small stands (Aitken et al., 2008; Anderson et al., 2012; O'Connor et al., 2012). A reserve of genetic diversity within populations is likely to become important for resilience by allowing species to adapt to new conditions (Huntley, 1991; Easterling et al., 2000; Hughes, 2003; Godfree, 2013). For plants in semi arid and arid regions already living on a physiological knife edge, managers may need to consider increasing the level of genetic diversity within stands lacking it to confer as much adaptive capacity and resilience so as to buffer them against future climate change (Huntley, 1991; Easterling et al., 2000; Hughes, 2003; Godfree, 2013). This will be of particular concern for monoclonal *A. carneorum* stands where loss of sexual reproduction in many monoclonal stands is thought to constrain local adaptation and evolution across its distribution (Eckert, 2002; Dorken et al., 2004).

Conservation strategies for these *Acacia* species that have so far focused on encouraging recruitment by reducing grazing pressures through culling feral grazers and fencing stands, will

almost certainly prove inadequate. Uncertainty about the time until natural recruitment occurs again, even if seedlings are protected, and concerns about restricted gene flow coupled with the predicted effects of climate change in the region mean such strategies are almost certain to fall short of long term success. The novel findings of this thesis that; a) the majority of *A. carneorum* and *A. loderi* plants will soon die; b) evidence that insect mediated pollination under fragmented conditions is increasing inbreeding and loss of genetic diversity; c) the discovery that sufficient seed lodging and recruitment rates are likely to be suboptimal given unnatural ground conditions within remaining stands; d) understory ‘nurse plants’ may play a role in increasing survivorship of new recruits; and e) fruit are unlikely to be set until the next large scale rain event which could be years too late for the majority of senescenting plants, suggests active rather than passive conservation is required. It is therefore apparent that any conservation strategy aimed at reversing the contraction of threatened semi arid *Acacia* stands and halting local extinction, would need a multifaceted approach taking into account; a) the temporal urgency of the situation, considering the late age of most plants; b) the vulnerability of new recruits to harsh conditions and grazing pressure; c) the continued loss of local genotypes (genetic diversity); and d) the threat of climate change and the need for adaptive capacity.

Conservation efforts in the form of restocking are likely to be required to restore contracting stands of all four threatened *Acacia* species. Such efforts would need to be conducted right after a large scale rain event, such as the one observed during this study, to maximise the chances that new plants introduced into stands survived long term. Any restocking should also aim to introduce as much genetic diversity into deficient stands as possible, especially those stands which are clonal. Indeed, it might even be wise to bolster those that already contain relatively high levels of genetic diversity to provide them with the best possible chance of adapting to future climate change (Tallmon et al., 2004, Byrne et al., 2011, Miller et al., 2012; Whalley et al., 2013). Collecting genetically diverse seed and growing seedlings in vitro to be used as stock to replenish depauperate stands is likely to be the most effective method of doing this. For *A. carneorum* this might mean utilizing the seed produced by sexually capable stands to boost the diversity and adaptive capacity of all stands, regardless of their sexual capacity, in preparation for changing climatic conditions.

Any efforts to manually restock stands would also need to be accompanied with a strategy to ameliorate feral grazing pressures from around these plants, to ensure any transplanted seedlings survive long term. Ideally new seedlings should be protected by fences

that exclude grazers as has been done in select stands of *A. carneorum* (Auld, 1995), however, this is very time consuming and expensive. At the very least encouraging the establishment of native understory plants within remaining stands so as to provide ample nurse plants for seedlings would be advised. Invasive weeds within stands should be discouraged given the risk that they may compete for space and resources with new recruits (Kearns et al., 1998; Wiser et al., 1998; White et al., 2002; Yates et al., 2004).

In the worst case scenario where seed were not available, or not available in time to restock particularly threatened *Acacia* stands, material for restocking might have to be produced via asexual propagation. These techniques are time consuming and expensive, and *Acacia* are also known to be notoriously hard to strike from cuttings. Optimizing a method for achieving this must be thought of as a high conservation priority. I spent months at Mt Annan Botanic gardens attempting to generate new plants from cuttings under the guidance of professional horticulturalists. This was the first attempt to do this with any of these semi arid *Acacia* species (Figure 10.1). Whilst I exhausted a range of standard protocols recommended to grow plants from 500 fresh cuttings of *A. melvillei*, *A. loderi* and *A. ligulata* each taken from four different stands of each species (all requiring rooting hormones and growth within a greenhouse with a controlled irrigation system), I was only able to get 90 *A. ligulata* seedlings to take. Whilst none of the *A. melvillei* or *A. loderi* cuttings took, there were some signs of early stage root development in some of the *A. melvillei* and *A. loderi* cuttings providing some hope that with younger / fresher material, some cuttings may take. If all else fails, the encouragement of suckering through disturbance to the roots of plants, as has obviously occurred in the majority of remaining *A. homalophylla* stands, may be a radical way of prolonging the persistence of stands.



**Figure 10.1. Attempts to propagate *A. melvillei*, *A. loderi* and *A. ligulata* plants from cuttings at Mt Annan Botanic Gardens:** (clockwise from top left) Pruning cuttings, dividing *A. melvillei* cuttings up into treatment groups, cuttings in treatment groups ready for planting, cuttings stored in a greenhouse with automatic irrigation system.

Strategies designed to combat the loss of genetic diversity and offspring fitness resulting from unnaturally high levels of honeybee driven inbreeding in *A. ligulata* stands might take the form of directly controlling bee populations in the vicinity of stands. Where *A. ligulata* stands are adjacent to farm land which relies on honeybees for pollination of commercial crops, or if control of feral hives is unrealistic, more direct genetic rescue strategies may need to be employed. This action would maintain optimally healthy stands in the short term, as well as maintaining natural levels of genetic diversity likely to become crucially important to facilitate adaptation in the face of future climate change. This could be achieved by the periodic introduction of plants from neighbouring or distant stands that can maintain diversity for long periods of time through natural pollination.

Understanding the risks that each conservation strategy brings to threatened stands of plants that one is attempting to conserve is crucial to increasing the chances of success.

Fencing stands to exclude grazers is unlikely to have any negative impact on threatened stands of Acacia. However, if it is not effective, the next step of introducing genetic material into stands lacking diversity may have come with bigger risks. Indeed, when introducing foreign genotypes into a population, care should be taken to maintain local provenance, if possible, to protect against the introduction of locally maladapted genotypes into stands (Brown & Kodric-Brown, 1977; Fischer & Matthies, 1997; Edmands, 2007; Whalle, et al., 2013), as well as potential effects of outbreeding depression (Oostermeijer et al., 1996 and Peck et al., 1998; Frankham et al., 2011). Concerns about maintaining such local genetic provenance may however be ultimately outweighed by the need for extra genetic diversity in these stands to optimise their adaptive capabilities and chances of survival (Tallmon et al., 2004, Byrne et al., 2011, Miller et al., 2012; Whalley et al., 2013). Indeed, arguments have even been made for the bolstering of genetic diversity of populations that are not necessarily lacking natural levels of genetic diversity to inoculate them further against future effects of climate change (Aitken & Whitlock, 2013). The use of soil stored seed banks to boost genetic diversity of above ground populations has been discussed as a possible way of getting around inefficiencies associated with transplanting and avoiding the negative impacts of outbreeding depression (Ottewell et al., 2011). It must be cautioned though, that if seed banks have become homogenised over time by excessive inbreeding from altered mating systems, they will be less useful sources of material for genetic rescue. At the very least, trialling what types of crosses produce the fittest offspring (optimal outcrossing distance experiments) has become the recommended prerequisite to investing in costly genetic rescue efforts. If genetic rescue is deemed to be needed, knowing the optimal outcrossing distance will then allow us to know the distances over which we should manually move plant material (pollen, seed, and plants) to restore optimal fitness levels to the next generation (Waser and Price, 1983, 1989, 1991; Waser et al., 1987; Forrest et al., 2011). The abundance of these studies across many plant species has strengthened evidence for the idea of an intermediate optimal outcrossing distance for preferentially outcrossing species, which avoids both the effects of inbreeding depression and outbreeding depression (Appendix 10.1).

## **10.8 Final conclusions about the effects of fragmentation on the persistence of long lived semi arid *Acacia* existing within severely fragmented landscapes of far western NSW**

My investigations into the way in which reproduction is effected in long lived, semi arid overstory plant species in highly fragmented landscapes, has highlighted that an understanding of the life history, reproductive strategy and species specific mating system parameters are essential when predicting the speed and manner in which fragmented stands will be affected in the short and long terms.

Given the longer period of time long lived plants have to successfully reproduce and recruit, such plants may have the luxury of being able to see through environmentally harsh times, when local conditions are particularly bad for recruitment, and take advantage of more favourable conditions when they arise (Steinger *et al.*, 1996; Eriksson, 2000; Körner, 2003; García *et al.*, 2008; Morris *et al.*, 2008). During such periods of reproductive torpor, contractions of stands size might not be as much cause for alarm as would be the case for annual species, so long as contractions are not too severe, and are not accelerated by extra pressures such as acute grazing. My findings that *A. melvillei*, *A. homalophylla* and *A. loderi* reproduce and recruit in synchronicity with rare large scale rain events, while the much shorter lived *A. ligulata* reproduces annually, exemplifies two contrasting reproductive strategies driven by the differing life histories of these species. This difference is likely to have consequences for their persistence within their highly modified environment.

In a natural undisturbed environment, the two contrasting reproductive strategies employed by the threatened *Acacia* species studied here and *A. ligulata* were likely to have been similarly successful over the long term however the relative success of *A. ligulata* plants in the region suggests that annual turnover is a more successful recruitment strategy under current conditions. Whilst my observations suggest that both reproductive strategies are successfully setting large amounts of viable seed, it may be that continual annual recruitment, even if minimal during drought years, may be preferable to irregular reproduction coinciding with rain under the unnaturally intense grazing regime that has followed the introduction of cattle, rabbits and goats in the region. The period when seedlings in semi arid environments are likely to be most susceptible to being grazed is likely to be directly after the end of a large scale rain event when the region dries up, ephemeral vegetation becomes scarce, and the large numbers of feral grazers built up during favourable conditions in the region are left to

target seedlings that persist (Auld, 1993, 1995; Cohn & Bradstock, 2000; Auld & Denham, 2001; Hein, 2006). Given long lived *Acacia* like *A. melvillei*, *A. homalophylla* and *A. loderi* set seed and recruit all at once after such rain events, seedlings are at their most vulnerable and palatable stage when this period of intense grazing occurs. It is clear that unlike these long lived *Acacia* species, many *A. ligulata* recruits have survived to maturity, most likely as they were produced in relatively drier years when the region could not support as many grazers, making grazing pressures lower. While *A. ligulata* seedlings are likely to be voraciously consumed in the dry period directly after a large scale rain event, seedlings recruited in earlier years would be larger and more resilient to grazing damage at this point. As such, the more frequent, potentially annual recruitment of *A. ligulata* plants during drier times may give recruits the opportunity to become established resilient plants before the worst of these grazing effects happen.

For sexually reproducing plants that maintain genetic diversity within stands, the advantages of long life extend beyond their capacity to reproduce multiple times over a long period to take advantage of changing conditions. Their capacity for long life means that they can remain a source of genetic variation over longer periods of time which is especially important during periods when pollen dispersal levels might have been unnaturally restricted, as is often the case in fragmented stands. In shorter lived plants with higher turnover, genetic diversity is expected to be lost relatively quickly under conditions of intense inbreeding, even in stands with initially high levels of genetic diversity (Kolreuter, 1761; Crow & Kimura, 1970; Lande & Schemske, 1985; Charlesworth & Charlesworth, 1990). This process is slowed down if long lived plants serve to maintain historic levels of genetic diversity within subsequent generations despite increased inbreeding (Hamrick et al., 1992). *A. melvillei*, *A. homalophylla* and *A. loderi* consist of genetically diverse older plants which may well be the product of a mating system operating prior to anthropogenic fragmentation in the region (Chapter 9). As such, most stands would still be expected to be contributing to genetically diverse seed cohorts given the reservoir of genetic diversity still remaining in the older plants. Moreover, with the exception of *A. homalophylla* stands which have recruited many suckers through unnatural levels of root disturbance (Chapter 2), and expected losses through genetic drift, the lack of recruitment in these stands for many years means that their genetic structure has been virtually unchanged by any restrictions to outcrossing rates. For plants that have continually recruited such as *A. ligulata*, the same cannot be expected since stands became drastically fragmented, and inbreeding levels have almost certainly increased.

The finding of potentially very different reproductive strategies even between *A. carneorum* and the other threatened *Acacia* species studied here, highlights the importance of understanding such differences when predicting how long plants will persist within fragmented landscapes. The reliance of *A. melvillei*, *A. homalophylla* and *A. loderi* plants on sexual reproduction to maintain population numbers is almost certainly a liability under current conditions. Firstly, seedling mortality is expected to be far higher than mortality of asexual recruits such as suckers, given the support they receive from maternal plants (Piquot et al., 1998; Honnay & Bossuyt, 2005). Secondly, under conditions of intense inbreeding, plants may also suffer the effects of inbreeding depression, such as may be being felt within at least some *A. ligulata* stands. Even though the discovery of self compatibility affords the mating systems of *A. melvillei*, *A. homalophylla* and *A. loderi* some natural resilience to the restrictions in gene flow, genetic diversity and adaptive capacity will eventually be lost over time, in a manner that might not occur if these species were pollinated by birds or mammals capable of maintaining connectivity over larger distances rather than insects that generally forage much more locally (Kolreuter, 1761; Fischer & Matthies, 1998; Ghazoul, 2005; Leimu et al., 2006; Bowman et al., 2008; Schleuning et al., 2009). For species such as *A. carneorum* that seem to rely heavily, and in many cases seemingly only on asexual reproduction, concerns over reproduction within fragmented stands should focus on the harshened local abiotic and grazing pressures on the physiological health of parental plants and suckers (MacGarvin et al., 1986; Warren, 1987; Kapos, 1989; Matlack, 1993, 1994). Clearly distinctions between the mating systems of different long lived plant species must also be considered when determining how they will ultimately respond to being unnaturally fragmented.



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**Appendix 1.1. Examples of inbreeding depression detected in offspring of plants:** Inbreeding depression found in a variety of plant species with the stage of offspring development where reductions in fitness were detected specified as either: 'Early stage'= seed set, seed size, seed weight, pollen viability or 'Late stage'= rosette size, number of leaves, surviving plants, seedling size, growth rate, flowering, height at year one, seed production of progeny, age at first flowering, and root development.

Species	Early stage	Late stage	Author
<i>Geranium caespitosum</i>	x		Hessing, 1988
<i>Ipomopsis aggregata</i>	x	x	Waser & Price, 1989
<i>Datisca glomerata</i>	x	x	Rieseberg, 1993
<i>Aquilegia caerulea</i>	x	x	Montalvo & Arlee, 1994
<i>Lythrum salicaria</i>		x	O'Neil, 1994
<i>Salvia pratensis</i>	x	x	Ouborg & Van Treuren, 1994
<i>Scabiosa columbaria</i>	x	x	Van Treuren et al., 1994
<i>Schiedea lydgatei &amp; S. salicaria</i>	x	x	Norman et al., 1995
<i>Gentiana pneumonanthe</i>	x	x	Oostermeijer et al., 1995
<i>Agave schottii</i>	x		Trame et al., 1995
<i>Lupinus texensis</i>		x	Heinenrm & Schaal, 1996
<i>Gentianella germanica</i>	x	x	Fischer & Matthies , 1997
<i>Epilobium angustifolium</i>	x	x	Husband, 1997
<i>Yucca filamentosa</i>	x	x	Pellmyr et al., 1997
<i>E. resinorum</i>	x	x	Byers, 1998
<i>Phacelia dubia</i>	x		del Castillo & Rafael , 1998
<i>Cakile edentula</i> var. <i>lacustris</i>	x		Donohue, 1998
<i>Dombeya acutangula</i>	x		Gigord et al., 1998
<i>Plantago coronopus</i>	x	x	Koelwijin, 1998
<i>Silene vulgaris</i>	x	x	Emery & McCauley, 2002
<i>Arnica montana</i>	x	x	Luijten et al., 2002
<i>Cochlearia bavarica</i>	x	x	Paschke et al., 2002
<i>Syzygium rubicundum &amp; Shorea cordifolia</i>	x	x	Stacy , 2001
<i>Knautia arvensis</i>		x	Vange, 2002
<i>Platanthera leucophaea</i>	x		Wallace, 2003
<i>Swertia perennans</i>	x		Lienert & Fischer, 2003
<i>Cucurbita pepo</i>		x	Stephenson et al., 2004
<i>Silene caryophyllaceae</i>		x	Kephart, 2004
<i>Cucurbita pepo</i>	x	x	Hayes et al., 2004
<i>Mimulus guttatus</i>		x	Ivey et al., 2004
<i>Nigella degenerii</i>	x	x	Andersson, 2004
<i>Senecio squalidus</i>		x	Brennan, et al., 2005
<i>Akebia quinata</i>	x		Kawagoe & Suzuki, 2005
<i>Silene vulgaris</i>	x	x	Glaettli & Goudet, 2006
<i>Hypochoeris radicata</i>	x	x	Becker et al., 2006
<i>Silene acaulis</i>	x	x	Keller & Schwaegerle, 2006
<i>Banksia marginata</i>	x	x	Vaughton & Ramsey,2006
<i>Panax quinquefolius</i> (American ginseng)		x	Mooney & Mcgraw, 2007
<i>Ceratodon purpureus</i>	x		Taylor et al., 2007

<i>Geranium maculatum</i>	x		Chang, 2007
<i>Succisa pratensis</i>	x	x	Pico et al., 2007
<i>Scalesia affinis</i>	x	x	Nielsen et al., 2007
<i>Populus nigra L</i>		x	Benetka et al., 2008
<i>Digitalis purpurea</i>		x	Grindeland, 2008
<i>Harrisia portoricensis</i>	x	x	Rojas-Sandoval & Melendez-Ackerman, 2009
<i>Silene latifolia</i>		x	Teixeira, et al., 2009
<i>Mercurialis annua</i>	x	x	Eppley & Pannell, 2009
<i>Aster amellus</i>	x	x	Raabova et al., 2009
<i>Rhododendron brachycarpum</i>	x	x	Hirao, 2010
<i>Solanum carolinense</i>		x	Kariyat, Scanlon, Mescher, de Moraes & Stephenson, 2011
<i>Astragalus exscapus</i>	x		Becker, Voss & Durka, 2011
<i>Comastoma pulmonarium</i>	x	x	Zhang, Irwin, Wang, Yang & Duan, 2011
<i>Polemonium vanbruntiae</i>		x	Birmingham & Brody, 2011
<i>Mimulus laciniatus</i>		x	Sexton, Strauss & Rice, 2011
<i>Eugenia dysenterica DC</i>		x	Chaves, Vencovsky, Silva, Zucchi & Coelho, 2011
<i>Datura stramonium</i>		x	Bello-Bedoy & Nunez-Farfan, 2011
<i>Broughtonia lindenii</i>	x		Vale, Rojas, Alvarez & Navarro, 2011
<i>Heliconia metallica</i>	x	x	Schleuning, Templin, Huaman, Fischer & Matthies, 2011
<i>Muscari tenuiflorum</i> ( <i>Hyacinthaceae</i> )	x		Hornemann, Weiss & Durka, 2012
<i>Gymnadenia conopsea</i>	x	x	Sletvold, Grindeland, Zu & Agren, 2012
<i>Lolium multiflorum</i>		x	Firestone & Jasieniuk, 2012
<i>Saxifraga granulata</i>	x	x	Walisch, Colling, Poncelet & Matthies, 2012
<i>Ostrya rehderiana</i>		x	Li, Guan, Yang, Luo & Chen, 2012
<i>Robinia pseudoacacia</i>	x	x	Yuan, Li, Want, Gu & Zhou, 2013
<i>Wild radish</i>	x	x	Pierson, Swain & Young, 2013
<i>Arabidopsis lyrata ssp. Petraea</i>	x	x	Sletvold, Mousset, Hagenblac, Hansson & Agren, 2013
<i>Vaccinium angustifolium</i> ( <i>lowbush blueberry</i> )	x		Bobiwash, Schultz & Schoen, 2013
<i>Agrostemma githago</i> ( <i>Caryophyllaceae</i> )		x	Goodrich, Beans & Roach, 2013
<i>Wild senna</i> ( <i>Senna hebecarpa</i> )		x	Berry, Wheeler & Darnowski, 2013
<i>Brassica nigra</i>		x	Prill, Bullock, van Dam & Leimu, 2014
<i>Eucalyptus globulus</i>		x	Costa E Silva, Potts & Lopez, 2014
<i>Phaseolus coccineus L.</i>	x	x	Gonzalez, De Ron, Lores & Santalla, 2014
<i>Arabidopsis lyrata</i>		x	Joschinski, van Kleunen & Stift, 2015
<i>Gymnadenia conopsea</i>			Gijbels, Ceulemans, van Den Ende & Honnay, 2015

*Appendix*

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<i>Centaurea cyanus</i>	x	x	Bellanger, Guillemin, Touzeau & Darmency, 2015
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**Appendix 1.2. Evidence of outbreeding depression in plants:** Examples of plant species where outbreeding depression has been detected, the specific effects detected and the generation effects detected.

Species	Specific effects detected	Generation	Reference
<i>Epilobium angustifolium &amp; Epilobium ciliatum</i>	seed number, percent germination, and mature biomass	F1, F2	Parker et al., 1995
<i>Gentianella germanica</i>	Germination rate, number of leaves, rosette size	F1	Oostermeijer et al., 1995; Fischer & Mathies, 1997
<i>Agave schotti</i>	Seed set	F1	Trame et al., 1995
<i>Asplenium ruta-muraria</i>	Spore lengths	F1	Schneller, 1996
<i>Gentianella germanica (Gentianaceae)</i>	Germination, number of leaves, rosette size	F1	Fischer & Matthies, 1997
<i>Eucalyptus globulus</i>	Seedling growth	F1	Hardner et al., 1998
<i>Hypoxis decumbens</i>	Fruit set	F1	Raimundez & Ramirez, 1998
<i>Ipomopsis aggregata</i>	Seelings, survival, flowering	F1	Waser et al., 2000
<i>Silene alba</i>	Above-ground dry matter after one growing season	F1	Keller et al., 2000
<i>Papaver rhoeas</i>	Above-ground dry matter after one growing season	F1	Keller et al., 2000
<i>Agrostemma githago</i>	Above-ground dry matter after one growing season	F1	Keller et al., 2000
<i>Chamaecrista fasciculata</i>	germination, survivorship to flowering, and total fruit production	F3	Fenster & Galloway, 2000
<i>Arabian oryx</i>	Juvenile survival	F1	Marshall & Spalton, 2000
<i>Agrostemma githago, Papaver rhoeas, Silene alba</i>	Seed mass, seedling growth, survival	F1, F2	Keller et al., 2000
<i>lotus scapus</i>	Seed set per flower, germination rates, seedling survival	F1	Montavlo & Ellstrand, 2001
<i>Auchusa crispa</i>	Number of cymes, fewer seeds, survival	F1, F2	Quilichini et al., 2001
<i>Eucalyptus camaldulensis</i>	Seedling growth rates	F1	Butcher & Williams, 2002
<i>Hypochoeris radicata</i>	Seed production, germination	F1	Becker et al., 2006
<i>Calylophus serrulatus</i>	Seed set, germination, growth, survival	F1	Heiser & Shaw, 2006
<i>Geum urbanum</i>	Growth and survival	F1	Vandepitte et al., 2011
<i>Grevillea mucronulata</i>	Seed set, seed size, germination and seedling growth	F1	Forrest et al., 2011

<i>Hordeum spontaneum</i>	Lower seed viability, hererosis in F1, depression in F2	F1, F2, but disappeared in F3	Volis et al., 2011
<i>Gymnadenia conopsea</i>	Outbreeding depression for seed production, germination and female fitness	F1	Sletvold et al., 2012
<i>Saxifraga granulata</i>	Survival under stress	F1 but lost in F2	Walisch et al., 2012
<i>Pyrrocoma racemosa var. racemosa</i>	Seed set	F1	Severns, 2013
<i>Arabidopsis thaliana</i>	Number of fruits produced per plant, number of seeds per fruit per plant, germination rate, seedling mortality	F1, F2	Oakley et al., 2015

Appendix 1.3. **Examples of plant species affected by fragmentation:** Measured as reductions in SS=Seed Size, SLS= Seedling Size, GD= Genetic Diversity, R= Number of Rosettes, G=Germination, Sur=Survival, SST=seed set, and other.

Vegetation studied	Location (if specific)	SS	SLS	GD	R	G	Sur	SST	Other	Author
Perennial caryophyllaceous herb, <i>Dianthus 6ustralis</i> L.	Southwest Sweden							x		Jennersten, 1988
Perennial prairie plant, <i>Silene regia</i> (Royal catchfly)	Multiple locations in USA					x				Menges, 1991
<i>Banksia goodii</i>	Australia							x		Lamont et al., 1993
Three species, <i>Prosopis nigra</i> (Mimosoideae), <i>Cercidium 6ustral</i> (Caesalpinoideae), and <i>Atamisquea emarginata</i> (Capparaceae)	Argentina							x		Aizen & Feinsinger, 1994
Rare perennial plant species <i>Gentiana pneumonanthe</i> (Gentianaceae).	Netherlands	x	x				x			Oostermeijer et al., 1994
Three species, <i>Corydalis ambigua</i> , <i>Polygonatum odoratum</i> var. Maximowiczii and <i>Aconitum yesoense</i>	Hokaido, Japan							x		Yasaka et al., 1994
Perennial <i>Ipomopsis aggregata</i>	Arizona, USA					x			Seed mass Resistance to herbivory	Heschel & Paige, 1995
Threatened perennial, <i>Salvia pratensis</i>	Netherlands	x	x						Offspring growth rate	Ouborg & Van Treuren, 1995
Perennial, <i>Salvia pratensis</i>	Netherlands			x						Ouborg & Van Treuren, 1995

Self-incompatible herb, <i>Lythrum salicaria</i>	Northern Sweden					x		Agren, 1996
Endangered biennial, <i>Gentianella germanica</i>	Central Europe			x	x		Number of leaves of progeny Rosette size of progeny	Fischer & Matthies, 1997
Neotropical populations of <i>Spondias mombin</i> (Anacardiaceae) and five monoecious species of <i>Ficus</i> (Moraceae)	Lowland Amazon, Bazil and Malaysian rainforest.		x		x			Nason & Hamrick, 1997
Rare plant, <i>Gentianella germanica</i>	Chalk grasslands Zurich, Switzerland				X but no seed mass!!!	x		Fischer & Matthies, 1998
prairie species, <i>Silene Eregia</i>	USA					x		Menges, 1991; Menges & Dolan, 1998
<i>Trillium ovatum</i>	Western North American conifer forests		x	x	x			Jules & Rathcke, 1999
Endangered <i>Rutidosis leptorrhynchoides</i> (Asteraceae)	Temporate grasslands, South-eastern Australia			NO!		x		Morgan, 1999
Mustard ( <i>Sinapis arvensis</i> ) and radish ( <i>Raphanus sativus</i> ).	Calcareous grasslands in central Europe				x			Steffan-Dewenter & Tscharntke, 1999
<i>Acacia brachybotrya</i> , <i>Senna artemisioides</i> , <i>Eremophila glabra</i> , and <i>Dianella revolute</i> .	Mallee woodlands of central New South Wales, Australia				x			Cunningham, 2000

Clonal plant, <i>Ranunculus reptans</i>	Zurich, Switzerland		x	x					Fischer et al., 2000
<i>Clarkia concinna concinna</i> (Onagraceae),	California, USA							Plant height Fecundity Above-ground biomass	Groom & Preuninger, 2000
<i>Arnica montana</i>	Netherlands	x		NO	x	x	x	Number of flowering stems and flowerheads	Luijten et al., 2000
Dipterocarp tree, <i>Shorea siamensis</i>	Thailand and forest fragmentation in Costa Rica						x		Ghazoul & McLeish, 2001
<i>Clarkia concinna concinna</i>	Northern California, USA							Seed initiation	Groom, 2001
Oak trees	UK						x		Knapp et al., 2001
Perennial herb, <i>Oenothera macrocarpa</i>	Missouri, USA							Seed initiation	Moody-Weis & Heywood, 2001
<i>Heliconia acuminata</i>	Amazon, Brazil				x				Bruna, 2002
Seven perennial plant species	Renosterveld shrublands in South Africa						x		Donaldson et al., 2002
Endangered grassland herb, <i>Gentianella campestris</i>	Sweden						x		Lennartsson, 2002
Understory perennial, <i>Trillium camschatcense</i>	Hokkaido, Japan						x		Tomimatsu & Ohara, 2002
Mown fen meadows, <i>Carex davalliana</i>	Switzerland							Biomass Fewer tillers	Hooftman et al., 2003

							Fewer flowering tillers	
Distylous fen plant, <i>Primula farinosa</i>	North-east Switzerland					x		Lienert & Fischer, 2003
<i>Zostera marina</i> (eelgrass)	South-western Baltic Sea					x		Reusch, 2003
Upland prairie	Western Oregon, USA					x		Severns, 2003
Threatened species <i>Lupinus sulphureus</i> , kincaidii (Kincaid's lupine)	Willamette Valley upland prairie in western Oregon, USA					x		Severns, 2003
<i>Lapageria rosea</i>	Maulino forest in central Chile	x		x				Henriquez, 2004
Clonal plant, <i>Ranunculus reptans</i>	Central Europe		x					Willi et al., 2005
Endangered herb, <i>Kirengeshoma palmata</i>	Eastern Asia					x		Chang et al., 2007
<i>Crataegus monogyna</i>	Cantabrian range, Spain					x	Pollen tubes	Garcia & Chacoff, 2007
Declining perennial herb, <i>Lychnis flos-cuculi</i>	Switzerland		x		x		Adaptation to survive in new location	Bowman et al., 2008
<i>Acacia dealbata</i>	Fragmented landscapes across New South Wales				x	x		Broadhurst et al., 2008
Isolated populations of the clonal plant <i>Ranunculus reptans</i>	Zurich, Switzerland		x					Fischer et al., 2000
<i>Aconitum napellus</i> , <i>lusitanicum</i>	Northern France			x	x	x		Le Cadre et al., 2008
<i>Acacia dealbata</i>	NSW, Australia					x		Broadhurst et

									al., 2008
<i>Astrocaryum mexicanum</i>	Mexico						Diversity and abundance		Aguirre & Dirzo, 2008
<i>Euphorbia palustris, Pseudolysimachion longifolium</i>	In the Weser and Elbe river systems in North-western Germany.			x			Seed mass, soil quality		Winter et al., 2008
<i>Trillium grandiflorum</i>	eastern North America					x			Schmucki & De Blois, 2009
<i>Trifolium montanum</i>	Germany					x	Seed production of offspring		Schleuning et al., 2009
<i>Polylepis australis</i>	Argentina			x		x	Pollen germination, pollen tube growth		Seltmann et al., 2009
<i>Rutidosis leptorrhynchoides</i>	South-Eastern Australia		x			x			Young & Pickup, 2010
<i>Derris ovalifolia, Ixora pavetta</i>	Pondicherry region of Southern India						Fruit set		Nayak & Davidar, 2010
<i>Allocasuarina verticillata</i>	South-western Victoria, Australia		x						Broadhurst, 2011
<i>Lupinus oreganus</i>	Oregon, USA					x			Severns et al., 2011
<i>Acacia caven, Celtis ehrenbergiana, Croton lachnostachyus, Rivina humilis, Schinus fasciculatus</i>	Chaco Serrano Forest			x			Seed mass		Ashworth & Marti, 2011
<i>Muscari tenuiflorum</i>	near Halle and Naumburg, Germany		x						Hornemann et al., 2012

<i>Gymnadenia conopsea</i>	Norway				x		x		Sletvold et al., 2012
<i>Silene otites</i>	northeastern Germany		x				x		Lauterbach et al., 2012
<i>Eucalyptus socialis</i>	Southern Australia							Reduced pollen diversity	Breed et al., 2012
<i>Gentianella bohemica</i>	Bavaria, Czech Republic, Austria							Reduced genetic connectivity	Koniger et al., 2012
<i>Dysoxylum malabaricum</i>	Kodagu region of the Western Ghats biodiversity hotspot, Sri Lanka			x					Ismail et al., 2012
<i>Prosopis caldenia</i>	Central Argentina							Frowth rate & mortality	Aguilar et al., 2012
<i>Ostrya rehderiana</i>	Northwestern Zhejiang Province, China			x					Li et al., 2012
<i>Banksia sphaerocarpa var. Caesia</i>	Australia	x	x			x			Llorens et al., 2013
<i>Convolvulus lineatus L.</i>	France							Fruit set	Berjano et al., 2013
<i>Magnolia stellata</i>	Japan						x	Selfing rate, male reproductive success	Setsuko et al., 2013
<i>Phyteuma spicatum</i>	north-western Germany							Seed mass & Flowering duration	Weber & Kolb, 2014

<i>Prunus virginiana</i>	North America						Fruit set & pollen limitation	Suarez-Gonzalez & Good, 2014
<i>Ligustrum lucidum</i>	36 km north of Córdoba city, central Argentina					x	Seedling number, Water retention in soil, total nitrogen in soil, organic matter in soil, carbon in soil	Aguirre-Acosta et al., 2014
<i>Centaurea hyssopifolia, Lepidium subulatum, Helianthemum squamatum</i>	Tajo River Basin, near Chinchón, central Spain					x	Seed mass & seed predation	Matesanz et al., 2015
<i>Bruguiera gymnorhiza (lam.)</i>	Singapore						Pollinator visitation rates, fruit set	Wee et al., 2015

**Appendix 1.4. Factors that impact on population viability and persistence of fragmented populations:** a) Deterministic factors directly affected by habitat fragmentation(generally harshened or reduced), b) Stochastic factors whose affects are exacerbated by fragmentation, c) Factors that may be influenced by habitat fragmentation and which may reduce population viability, d) Factors that influence the susceptibility and tolerance of fragmented populations.

a) Deterministic factors directly affected by habitat fragmentation (generally harshened or reduced)			b) Stochastic factors whose affects are exacerbated by fragmentation	c) Factors that may be influenced by habitat fragmentation and which may reduce population viability		d) Factors that influence the susceptibility and tolerance of fragmented populations
Abiotic effects	Population factors	Biotic effects	Environmental stochastic events	Demographic factors	Genetic factors	Dependant factors
Humidity	Size	Growth rates	Floods	Allee (density threshold) effects	Effective population size	Physical structure
Vapour pressure	Abundance	Mortality rates	Wildfires	Number of available mates	Inbreeding depression	Fragment geometry
Air temperature	Connectivity	Species turnover	Storms	Mortality	Outbreeding depression	Fragment quality
Soil temperature	Isolation	Species richness	Drought	Fecundity	Genetic drift	Landscape around fragments
Light levels	Population density	Species composition	Landslides	Recruitment	Gene flow	Reproductive strategy
Short wave radiation	Fragment shape	Competitors		Progeny fitness	Genetic diversity within popns	Species specific
Soil moisture	Diversity	Pathogens		Individual fitness	Genetic diversity among popns	Level of self compatibility
Precipitation	Mortality	Parasites		Dispersal distances	Genetic divergence	Mating system

					among populations	
Agrochemical intrusion	Sex ratio	Predators			Genetic bottlenecks	Dispersal mode/ capability
Deposition of pollutants		Mutualists			Founder effects	Population size
Nutrients					Adaptive capacity	Sex ratio
Gap formation						Demographics of population
Wind						
Edge effects						
Water						
Space						
Physical disturbance						
Chemical and nutrient pollutants						
Susceptibility to natural disturbances						

**Appendix 1.5. Studies to date that have found reductions to pollinator services as a result of fragmentation:** Measured as pollinator deficits (PD), reduced diversity of pollinator assemblages (PA) and restrictions to pollinator foraging behaviour (RFB).

Vegetation Type	Locality information	PD	PA	RFB	RD	other	Author
Perennial caryophyllaceous herb, <i>Dianthus deltoids</i> .	Southwest Sweden	x	x				Jennersten, 1988
<i>Prosopis nigra</i> (Mimosoideae), <i>Cercidium australe</i> (Caesalpinoideae), <i>Atamisquea emarginata</i> (Capparaceae).	Dry subtropical forest in north-western Argentina	x					Aizen & Feinsinger, 1994
perennial herbs <i>Corydalis ambigua</i> , <i>Polygonatum odoratum</i> var. <i>maximowiczii</i> and <i>Aconitum yesoense</i>	14 forest islands, 5 of which were situated in residential areas and 9 in cultivated land.	x					Yasaka et al., 1994
<i>Ipomopsis aggregate</i> (scarlet gilia)	Arizona, USA						Heschel & Paige, 1995
Mustard ( <i>Sinapis arvensis</i> ) and Radish ( <i>Raphanus sativus</i> )	Calcareous grasslands in central Europe	x	x				Steffan-Dewenter & Tscharntke, 1999
<i>Shorea siamensis</i>	Thailand and forest fragmentation in Costa Rica			x			Ghazoul & McLeish, 2001
<i>Clarkia concinna</i>	California, USA	x					Groom, 2001
Endangered grassland herb <i>Gentianella campestris</i>	Sweden	x					Lennartsson, 2002
Renosterveld Shrublands	South Africa		x				Donaldson et al., 2002
Calcareous grasslands <i>Betonica officinalis</i> L. (Lamiaceae)	Movelier and Nenzlingen in the north-western Swiss Jura mountains	x		x			Goverde et al., 2002

<i>Ceiba grandiflora</i>	central Pacific coast of Mexico	x					Quesada et al., 2003
<i>Lupinus sulphureus</i> ssp. <i>kincaidii</i> (Kincaid's lupine), a threatened species	Willamette Valley upland prairie in western Oregon, USA	x					Severns, 2003
Perennial herb <i>Oenothera macrocarpa</i>	Missouri, USA	x					Moody-Weis, 2001
<i>Lapageria rosea</i>	Temperate forests of Chile	X	x				Valdivia et al., 2006
<i>Hippocratea comosa</i>	Central Europe	X		NO		Pollinator abundance, seed set	Meyer B et al., 2007
<i>Dipteryx panamensis</i>	Costa Rica					Pollinator dispersal distances and outcrossing rates	Hanson et al., 2008
<i>Phyteuma spicatum</i>	between Bremen and Hamburg in northwestern Germany					Pollinator visitation rate, seed set, herbivory levels	Kolb, 2008
<i>Astrocaryum mexicanum</i>	Mexican tropical forest	X					Aguirre & Dirzo, 2008
<i>Crepis sancta</i> (Asteraceae),	Southern France	X					Andrieu et al., 2009
<i>Amorpha canescens</i> (Fabaceae),	Prairie remnants in Iowa and Minnesota	x	x				Slagle & Hendrix, 2009
<i>Verbascum nigrum</i>	eastern Estonia	x				Florivory intensity	Sober et al., 2009
<i>Trollius europaeus</i>	northeast Switzerland	x					Klank et al., 2010
10 different plant species	Five European countries	x					Dauber et al., 2010
<i>Betonica officinalis</i>	Northern Swiss Jura mountains			x			Rusterholz & Baur, 2010
<i>Copaifera langsdorffii</i>	Brazil					Genetic diversity & seedlings	Sebbenn et al., 2011

<i>Vaccinium uliginosum</i>	Belgium	x		x			Mayer et al., 2012
<i>Lotus corniculatus</i>	Paris, France	x	x				Pellissier et al., 2013
<i>Kniphofia linearifolia</i> Baker	Pietermaritzburg, South Africa	x				Fruit set, seed set	Duffy et al., 2013
<i>Eucalyptus</i>	Australia					Selfing rate	Breed et al., 2013
<i>Avicennia marina</i>	Australia	x				Pollen deposition rates, pollinator foraging time	Hermansen et al., 2014
<i>Heliconia tortuosa</i>	Coto Brus Canton in southern Costa Rica	x					Hadley et al., 2014
<i>Cherry trees (Prunus avium L.)</i>	Swiss plateau	x					Schuepp et al., 2014
<i>Bruguiera gymnorhiza</i>	Singapore	x					Wee et al., 2015
<i>Avicennia marina</i>	Australia	x		x			Hermansen et al., 2015
<i>Acer mono</i>	Japan					Long distance pollinator movements inferred between lone trees	Kikuchi et al., 2015

**Appendix 1.6. Studies to date that have found seed dispersal affected as a result of fragmentation:** Measured as a) Reduced seed removal, b) Reduced frugivore abundance, c) Reduced distance of seed dispersal, and d) Reduced diversity of frugivores.

Vegetation study	Location	a)Reduced Seed Removal	b)Reduced Frugivore Abundance	c)Reduced Distance of Seed Dispersal	d)Reduced Diversity of Frugivores	Author
Neotropical tree, <i>Cabralea canjerana</i> (Meliaceae)	Forested areas in southeastern Brazil		x			Pizo, 1997
Spanish juniper, <i>Juniperus thurifera</i>	Central Spain		x			Santos et al., 1999
Endemic tree <i>Leptonychia usambarensis</i> (Sterculiaceae)	Rainforest in the East Usambara Mountains of Tanzania		x	x		Cordeiro & Howe, 2003
<i>Duckeodendron cestroides</i>	Central Amazon, Brazil	x		x		Cramer et al., 2007
Hawthorn tree ( <i>Crataegus monogyna</i> Jacq.)	Cantabrian range, Spain		x			Garcia & Chacoff, 2007
Tropical cloud forest	Costa Rica's Talamanca Mountains		x			Gomes et al., 2008
<i>Ficus thonningii</i> trees	Kakamega Forest, western Kenya		x		x	Kirika et al., 2008
<i>Leptonychia usambarensis</i>	Tanzania			x		Cordeiro et al., 2009
Mountain cloud forest	Kenya	x				Lehouck et al., 2009
Multiple fragmented rainforest species	Subtropical Australia		x			Moran et al., 2009
Understory herb, <i>Heliconia acuminata</i>	Amazon, Brazil		x			Uriarte et al., 2010
<i>Crataegus monogyna</i> , <i>Ilex</i>	Northern Spain		x			Herrera & Garcia, 2010

aquifolium						
<i>Copaifera langsdorffii</i> Desf	Brazil			x		Sebbenn et al., 2011
<i>Brosimum alicastrum</i> , <i>Dialium guianense</i> , <i>Manilkara zapota</i> , <i>Nectandra ambigens</i>	Northern Mexico			x		Anzures- Dadda et al., 2011
<i>Attalea humilis</i>	Brazil		x	x		Andreazzi et al., 2012
<i>Myrtus communis</i>	Mediterranean		x			Gonzalez-Varo et al., 2012
<i>Castanopsis sclerophylla</i>	Southeastern China			x		Want et al., 2012
<i>Tristerix corymbosus</i>	Chiloé Island, Chile	x				Magrach et al., 2013
Oak	Mediterranean			x		Moran-Lopez et al., 2015

**Appendix 1.7: Studies to date involving *Acacia* of the semi arid regions of western NSW and their major findings.**

Vegetation studied	Study	Findings	Author
<i>A. aneura</i> (Mulga)	Studies into the requirements for flowering and seed set in <i>Acacia aneura</i> populations.	Flowering was heavier, and seeding more successful, when additional water or good rains reduced the normally severe water stress. Vegetative regeneration is apparently common in Queensland, following the “pulling” or lopping of Mulga for stock feed during drought, however such regeneration is unusual in western New South Wales	Preece, 1971
<i>A. aneura</i> (Mulga)	Studies into the recruitment dynamics (germination) of <i>Acacia aneura</i> populations.	Regeneration of the species is episodic and it has been estimated that in far western New South Wales, conditions suitable for Mulga germination are likely to occur only once in 9 years.	Preece, 1971
Multiple <i>Acacia</i> species	Rabbit grazing and the failure of regeneration in Australian arid zone acacia.	Experiments demonstrated that even with the lowered post-myxomatosis population densities, rabbit grazing pressure would significantly affect recruitment in arid zone <i>Acacia</i> populations in the absence of stock.	Lange & Graham, 1983
Multiple <i>Eucalyptus populnea</i> woodland species	The diet of sheep was recorded by direct observation in a shrub-dominated <i>Eucalyptus populnea</i> woodland between Nyngan and Bourke, NSW.	As perennial grass matured, sheep ate firstly increasing quantities of perennial forbs and then mulga ( <i>Acacia aneura</i> ). When these foods were depleted, sheep subsisted on dead perennial grass, tree litter and broad leaf hopbush ( <i>Dodonaea viscosa</i> ). All other shrub species were unacceptable. Goats demonstrated a potential for overgrazing in dry times	Harrington, 1986
Multiple species within <i>Acacia</i> shrublands	Establishing priorities for the conservation of rare and threatened plants and plant associations in New South Wales	The future of <i>Acacia</i> Shrublands in grazing areas is tenuous as they are considered to be one of the most threatened and poorly conserved vegetation communities in New South.	Benson, 1989

<i>A. carneorum</i>	Regeneration in populations of the arid zone plants <i>Acacia carnie</i> and <i>A. oswaldii</i>	Of the seven arid zone Acacia species discussed only for <i>A. Oswaldii</i> is there any chance that recruitment is sufficient to maintain current population densities.	Auld, 1990
<i>A. melvillei</i>	A review of the threatened status of <i>Acacia melvillei</i>	<i>Acacia melvillei</i> is also considered to be vulnerable in Victoria but its extent of reservation is uncertain there	Gullan et al., 1990
<i>A. aneura</i> (Mulga lands)	Vegetation and soil patterns across a 200 ha semi-arid site 40 km north-west of Lough, NSW, are described using plant cover data from line transects and soils data from points, sampled systematically (50 m intervals) across the site.	This paper demonstrates that patterning in mulga lands is more extensive geographically, and has a wider climatic range, than previously reported.	Tongway & Ludwig, 1990
<i>A. aneura, Cassiane mophila, Dodonaea viscosa</i>	Influence of fire and edaphic factors on germination of the arid zone shrubs <i>Acacia aneura</i> , <i>Cassia nemophila</i> and <i>Dodonaea viscosa</i>	Controlled experiments have shown that the germination of Mulga seeds was enhanced after they were subjected to fires of particular intensities	Hodgkinson & Oxley, 1990
The vegetation of Mallee Cliffs National Park	Report on the plant communities and conditions	Many plant communities are in poor condition –fragmented (including Acacia species)	Morcom & Westbrooke , 1990
<i>A. melvillei</i>	The regeneration of the tree <i>Acacia melvillei</i> (yarran) was studied in a semi-arid area within 100 km of Balranald, south-west New South Wales	Reported an almost total failure of <i>Acacia melvillei</i> regeneration in pastoral areas studied nearby at Balranald. The transplants showed that seedling establishment can be strongly limited by low soil moisture in spring and summer and by rabbit browsing	Batty & Parsons, 1992
Semi-arid Mallee shrublands	Soil temperatures were measured during 11 experimental fires in semi-arid mallee shrublands in central NSW.	Temperatures between 60 and 120-degrees-C were recorded to 5 cm depth under Eucalyptus fuels while putative lethal temperatures for seeds occurred occasionally at 0-2 cm depth. The results indicated greatest potential for stimulation of	Bradstock et al., 1992

		germination and death of buried seeds under Eucalyptus fuels, although the level of variability of temperature was highest under Eucalyptus fuels.	
<i>A. aneura</i> (Mulga)	Survey on condition and human impacts and fragmentation and inappropriate fire regimes in the past may have contributed to the demise of Mulga in the area.	Mulga is susceptible to fire and burning and has been used in the past as a management tool to thin out dense populations in the region leading to the demise of many populations in the region.	Cunningham et al., 1992
The natural vegetation of the Balranald-Swan Hill area ( <i>Acacia</i> species included)	survey and report on condition of local vegetation (including local threatened <i>Acacia</i> species)	Generally poor condition of many <i>Acacia</i> populations was reported.	Scott, 1992
<i>A. carneorum</i>	The impact of grazing on regeneration of the shrub <i>Acacia carnei</i> in arid Australia	Rabbit grazing was primarily responsible for the observed lack of recent regeneration of <i>Acacia carnie</i> .	Auld, 1993
<i>A. ligulata</i> , <i>A. Loderi</i> , <i>A. oswaldii</i> & <i>C. pauper</i>	The seedbanks of four arid trees from Australia were examined using periodic recovery of seeds buried in nylon mesh bags in the field	Both <i>C. pauper</i> and <i>A. oswaldii</i> must rely on the production of annual seed-crops to maintain a seedbank through time and hence, take advantage of infrequent and irregular rains promoting germination and establishment. For <i>A. ligulata</i> and <i>A. loderi</i> , occasional seed production is all that is required to ensure the presence of a soil seedbank	Auld, 1995
<i>A. oswaldii</i>	Seedling survival under grazing in the arid perennial <i>Acacia oswaldii</i>	Recruitment of seedlings into a population of <i>Acacia oswaldii</i> is largely prevented by rabbits within Kinchega National Park in western NSW, Australia. The limited seedling recruitment that is occurring is frequently associated with seedlings that avoid grazing by growing inside the canopy of unpalatable shrubs such as <i>Maireana pyramidata</i> .	Auld, 1995
<i>A. oswaldii</i> , <i>A.</i>	Soil seedbank patterns of four trees and shrubs	Whilst <i>A. oswaldii</i> and <i>A. loderi</i> has a relatively short lived soil	Auld , 1995

<i>ligulata</i> & <i>A. loderi</i> .	from arid Australia	stroed seed banks (1-5 years maximum), for <i>A. ligulata</i> the seedbank were relatively persistent, although this did vary between different populations.	
<i>A. carneorum</i>	The impact of herbivores on regeneration in four trees from arid Australia	Experimental studies in Kinchega National Park have shown that intensive and vigilant protection from rabbits (ripping of burrows and poisoning) promoted the production and enhanced the survival of vegetative suckers	Auld, 1995
Multiple species of Mungo National Park (incl. <i>Acacia</i> species).	Vegetation of Mungo National Park, western New South Wales was surveyed.	The poor state of many plant communities of Mungo National Park, western New South Wales was reported (namely <i>Acacia melvillei</i> and <i>A. Loderi</i> ).	Westbrooke,& Miller, 1995
<i>A. loderi</i> & <i>A. melvillei</i>	Studied the phylogeny of <i>Acacia loderi</i> and <i>Acacia melvillei</i>	<i>Acacia loderi</i> is a more distinctive taxon, however the species is reputed to hybridise with <i>Acacia melvillei</i> .	Entwistle et al., 1996
Multiple species (incl. <i>Acacia</i> ) around the Pooncarie region	A survey of the natural vegetation of the Pooncarie	Report on the plant communities and conditions—many in poor condition fragmented (including local <i>Acacia</i> species)	Porteners et al., 1997
Multiple species Tarawi Nature reserve.	Report on the state of plant communities and their threatened nature in Tarawi Nature reserve.	Reported the poor condition of several species in the reserve to the NSW National Parks and Wildlife Service	Porteners, 1998
<i>A. ligulata</i>	A study of bet-hedging and germination in the Australian arid zone shrub <i>Acacia ligulata</i> .	Heating increased the mortality of <i>A. ligulata</i> seeds and ingestion of seeds by birds may break seed dormancy and hence enable some seeds to germinate soon after dispersal. Alterneeds not eaten by birds are likely to remain dormant until sufficiently scarified by soil or stimulated by fire.	Letnic et al., 2000
Multiple species	Flora conservation issues at Kinchega National	grazing and desiccation reduce recruitment in small stands which	Auld & Denham,

(incl. <i>Acacia</i> )	Park, western NSW.	have also been destroyed by flooding from the over-filling of Lake Menindee.	2001
Multiple species (incl. <i>Acacia</i> )	Report on the types and condition of vegetation including local <i>Acacia</i>	Report on the plant communities and conditions—many in poor condition fragmented (including local <i>Acacia</i> species)	Westbrooke et al., 2001
<i>A. melvillei</i>	Draft Report for the NSW Scientific Committee; Identification and review of <i>Acacia melvillei</i> specimens in New South Wales.	Reported on the decline of <i>Acacia melvillei</i> populations in New South Wales.	Kodela, 2001
<i>A. ligulata</i>	Study of the seed dispersal distance of <i>Acacia ligulata</i> by meat ants ( <i>Iridomyrmex viridiaeneus</i> ).	<i>Iridomyrmex viridiaeneus</i> moved <i>A. ligulata</i> seeds over distances of 7- 180 m (mean 93.9 m) from the source trees to their nests.	Whitney, 2002
<i>A. ligulata</i>	Study of the survival and recruitment of seedlings and suckers of trees and shrubs of the Australian arid zone following habitat management and the outbreak of Rabbit Calicivirus Disease (RCD)	Seedling survival was particularly low regardless of the level of herbivore exclusion, largely due to desiccation. Reduction of grazing impacts may only allow recruitment into populations of species reliant on seedlings under more favourable climatic circumstances than experienced in this study.	Denham & Auld, 2004
<i>A. ligulata</i>	A study providing experimental evidence that both parties benefit in a facultative plant-spider mutualism.	As spider colonies occupy only a fraction of a plant's volume, average benefits ranged from 0.4 to 6% increases in whole-plant seed production. These benefits were strongest in years of low seed production, suggesting that spiders may buffer plants against female reproductive failure	Whitney, 2004
<i>A. ligulata</i>	A study concerning fruit colour polymorphism in <i>Acacia ligulata</i> : seed and seedling performance, clinal patterns, and chemical variation	Patterns indicate that both abiotic and biotic factors may contribute to selection on the <i>A. ligulata</i> polymorphism.	Whitney & Lister, 2004
<i>A. ligulata</i>	A study on insect seed predators as novel agents of selection on fruit colour.	Found that the most obvious selective agents (that is, seed dispersers) may not always be the most important.	Whitney & Stanton, 2004

<i>A. carneorum</i>	A new species of gall-inducing thrips, <i>Oncothrips kinchega</i> , is described and its biology on the host plant <i>Acacia carneorum</i> was investigated.	The study showed that a single foundress initiates a gall.	Wills et al., 2004
<i>A. ligulata</i>	Genetic control of a fruit-colour polymorphism in <i>Acacia ligulata</i> was studied.	Evidence for simple genetic control of a fruit-colour polymorphism in <i>Acacia ligulata</i> which should aid in linking ecological processes such as frugivory and seed dispersal to the evolutionary trajectories of plant populations.	Whitney, 2005
<i>A. ligulata</i>	A study linking frugivores to the dynamics of a fruit colour polymorphism	Consumer biases produced spatiotemporal variability in the relative fitness of <i>A. ligulata</i> color morphs.	Whitney, 2005
Multiple species (incl. <i>A. ligulata</i> & <i>A. aneura</i> )	The effects of browsing by feral and re-introduced native herbivores on seedling survivorship in the Australian rangelands	Excluding rabbits and stock may benefit the germination and survival of mulga, silver cassia and sandhill wattle.	Munro et al., 2009
Multiple species	An examination of the impact of increased temperatures on the longevity and dynamics of the persistent soil seed banks of eight ephemeral species from arid Australia.	Showed that the risk spreading mechanism provided by persistent seed banks could be compromised by the mechanistic impact of forecast temperature increases in arid habitats.	Ooi et al., 2009
<i>A. melvillei</i>	A survey and report of the condition of Yarran populations in NSW.	Yarran is not uncommon in the general region, however remaining populations are highly disturbed and in decline, due to factors preventing regeneration such as grazing and population senescence.	Porteniers et al., 1997
<i>A. ligulata</i>	To investigate the benefits of providing a dispersal structure attractive to ants and birds.	Ingestion of seeds by birds may break seed dormancy and hence enable some seeds to germinate soon after dispersal. Alternatively, seeds not eaten by birds are likely to remain dormant until sufficiently scarified by soil or stimulated by fire.	Letnic et al., 2000
<i>A. melvillei</i>	Vegetation survey of potential addition to	Seedling establishment of <i>Acacia melvillei</i> is strongly limited by	Sundstrom,

	Mungo National Park of <i>Acacia melvillei</i> .	rabbit browsing and stock grazing. A review of the conservation status of this community should be considered a priority, as its sole representation within a conservation reserve is in Mungo National Park. Several other authors have raised concerns for the community in south-western New South Wales.	2000 & Kodela, 2001
<i>A. loderi</i>	A survey and report of the condition of Nelia populations in NSW.	While very uncommon in the region, Nelia has been recorded sporadically during several vegetation surveys in south-western New South Wales).	Westbrooke et al., 2001
<i>A. carneorum</i>	To assess the impact of rabbit control recruitment of seedlings or vegetative suckers.	Survival of suckers was slightly greater than prior to rabbit control, but in general, a pattern of little or nil recruitment is likely in suckers exposed to rabbits or to all grazing mammals.	Denham & Auld, 2004

**Appendix 1.8. Studies that detected optimal outcrossing distances in plant species:** Plant species, distance range pollen was moved across, the distance pollen was transferred between mates that produced the fittest offspring and the stage of offspring development where fitness consequences were detected (pre and post germination).

Species	Distance range	Result	Offspring fitness component tested		reference
			Pre germination	Post germination	
<i>Picea abies</i>	0-32000m	Intermediate distances by 49% (seed size) & 16% (offspring fitness)	%good seed, size at 3.5 months	none	Coles and Flower, 1976
<i>Castilleja miniata</i>	0-30m	Intermediate distances by 41%	Seed set	none	Lertzman, 1981
<i>Mimulus guttatus</i>	0-4500m	Intermediate distances by 31%	Seed set	none	Waser and Price, 1983
<i>Costus allenii</i>	0-300m	Intermediate distances by 8.5% (seed set) & 15% (offspring fitness)	Seed set	Germination x 5 month biomass	Schemske and Pautler, 1984
<i>Calochortus leichtlinii</i>	0-400m	Intermediate distances by 7% (seed set) & 27% (offspring fitness)	Seed set, seed mass	none	Holtsford, 1984
<i>Phlox drummondii</i>	0-200m	Longer distances by 15%	Seed abortion	none	Levin, 1984
<i>Inga brenesii</i>	0-300m	Longer distances by 70%	Fruit set	none	Koptur, 1984
<i>Inga punctata</i>	0-3000m	Longer distances by 73%	Fruit set	none	Koptur, 1984
<i>Clintonia borealis</i>	0-200m	Longer distances by 65%	%seed set	none	Galen et al., 1985

<i>Erythronium americanum</i>	0-80m	Longer outcrossing distances out perform shorter by 20%	%seed set	none	Harder et al., 1985
<i>Erythronium grandiflorum</i>	0-100m	Selfing optimal	#pollentubes reaching ovary	none	Stratton et al., 1985
<i>Delphinium nelsonii</i>	1-100m	10m= optimal distance	#pollen tubes reaching ovary	none	Waser et al, 1987; Waser and Price 1991; Waser and Price unpubl.
<i>Mimulus guttatus</i>	0-500m	shorter outcrossing distances outperform longer by 10%	none	Seed maturation F1 fitness in growth chamber	Ritland and Ganders, 1987
<i>Delphinium nelsonii</i>	0-1000m	Intermediate outcrossing distance outperforms shorter and longer distances by an average of 16.5%	Seed set	Overall fitness to year 7-11*	Waser, et al., 1987; Waser and Price 1991; Waser and Price, unpubl.
<i>Espeletia schultzii</i>	1-500m	Intermediate distances by 19.5%	%filled achenes	none	Sobrevila, 1988
<i>Scleranthus annuus</i>	0-100m	Intermediate distances by 15.5%	none	Stamen fertility score in F1	Svensson, 1988
<i>Scleranthus annuus</i>	0-75m	Intermediate distances by 46.5%	%fertile stamens		Svensson, 1990
<i>Impatiens capensis</i>	2-250m	Intermediate distances does best	none	Size at 1 month	McCall et al., 1988
<i>Fouquieria splendens</i>	0-1000m	Intermediate distances by 59.5%	Seed set	none	Scott, 1989

<i>Carex pachystachya</i>	0-10m	Shorter distances by 24%	Seed set	germination	Whitkus, 1988, pers comm.
<i>Blanfordia noblis</i>	2-200m	Longer distances by 25%	Seed set	none	Zimmerman and Pyke 1988
<i>Chamaecrista fasciculata</i>	0-100m	1-100m performs equally & better than selfed treatments	#pollen tubes reaching ovary	none	Fenster & Sork, 1988
<i>Amianthus muscaetoxicum</i>	2-60m	Longer distances by 10%	Fruit and seed set	none	Redmond et al., 1989
<i>Ipomopsis aggregata</i>	0-100m	Intermediate distances by 7% (seed) set & 37.5% (offspring fitness)	Seed set	Lifetime F1 fitness in field	Waser and Price, 1989
<i>Wisteria Maura thumb</i>	1-2500m	Short outcrossing distances outperforms long	Seed set	none	Wright et al., 1989
<i>Erythronium grandiflorum</i>	0-300m	3m=optimal distance	#pollen tubes reaching ovary	none	Cruzan, 1990
<i>Impatiens capensis</i>	2-50m	29m=optimal distance	Seed weight	Height at 1 month	McCall et al., 1991
<i>Asclepias exaltata</i>	1-100m	Reduced fitness of inbred individuals, but no evidence of an optimal distance	Seed set	none	Broyles & Wyatt, 1991
<i>Delphinium nelsonii</i>	1-100m	10m outperformed shorter and longer distances by 23-33%	Seed set and seed weight		Waser & Price, 1991

<i>Potentilla palustris</i>	1-500m	Long outcrossing distances outperformed shorter	Seed set	none	Olesen & Warncke, 1992
<i>Agave schottii</i>	1-2500m	1m cross=lowest seed set, 10-100m cross= optimal, 2500m=intermediate seed set	Seed set	None	Trame et al., 1995
<i>Gentian pneumonanthe</i>	0-2500m	Very low selfing fitness & very high inter population cross fitness	Seed set and seed weight	In glasshouse, % seeds germinating, seedling weight, adult weight & total relative fitness	Oostermeijer et al., 1995
<i>Eucalyptus globulus</i>	0-100km	Only selfing depressed seed set, longer distance crosses increased growth rate	Seed set	Offspring growth rate	Hardner et al., 1998
<i>Chamaecrista fasciculate</i>	100m-2000km	Intermediate outcrossing distances of hundreds of kilometres optimal	none	Fitness of F1 and F3 hybrid offspring	Fenster & Galloway, 2000
<i>Trillium erectum</i>	0-1500m	Outcrossed pollen of all distances equal and outperformed self pollen by 142%	Seed and fruit production	none	Irwin, 2001
<i>T. grandiflorium</i>	0-1500m	Outcrossed pollen of all distances equal and outperformed self	Seed and fruit production	none	Irwin, 2001

*Appendix*

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		pollen by 178%			
<i>Syzygium rubicundum</i>	0-12km	1-2km cross best (90% fitter than inbred individuals)	Fruit set	Seed germination, seedling survivorship & height at 1 year	Elizabeth, 2001
<i>Shorea cordifolia</i>	0-35km	1-10km cross best	Fruit set	Seed germination, seedling survivorship & height at 1 year	Elizabeth, 2001
<i>Digitalis purpurea</i>	0-30m	1-6 m crosses best	Seed set (%) Seed mass ( $\mu$ g)	Germination speed (days) Germination (%), Juvenile size, dry mass (g) Juvenile survival (%) Start of flowering (days after vernalization) Flower number Cumulative fitness	Grindeland, 2008

## Appendix 1.9. Longitude and latitude of studied stands.

	<i>A. homalophylla</i>	<i>A. loderi</i>	<i>A. melvillei</i>	<i>A. ligulata</i>	<i>A. carneorum</i>
1	S34 18.786 E146 45.886	S32 30.797 E142 08.223	S31 30.398 E145 55.122	S31 31.817 E145 28.002	S29 28.140 E141 16.180
2	S33 56.609 E146 36.465	S32 33.061 E142 10.374	S31 30.634 E145 43.404	S31 32.000 E145 28.000	S29 43.658 E142 58.324
3	S32 49.265 E145 52.934	S32 34.187 E142 07.510	S31 31.079 E144 29.573	S31 34.544 E144 47.963	S31 25.346 E142 10.954
4	S31 32.966 E146 51.518	S32 43.290 E142 02.065	S31 32.967 E146 51.518	S31 34.600 E145 09.583	S31 44.678 E142 24.248
5	S32 37.436 E146 59.872	S32 40.743 E141 51.917	S31 33.233 E146 28.761	S31 42.564 E143 27.476	S32 09.112 E141 56.039
6	S32 50.440 E144 03.015	S33 12.387 E141 40.488	S31 34.930 E144 50.008	S32 19.374 E142 23.723	S32 21.087 E142 13.006
7	S31 39.612 E144 15.429	S32 07.670 E142 41.086	S31 34.947 E144 50.283	S32 21.510 E142 24.098	S32 26.010 E142 18.551
8	S31 30.398 E145 55.122	S32 15.076 E142 17.762	S31 35.572 E144 55.737	S32 22.015 E142 23.920	S32 27.492 E141 33.807
9	S31 55.424 E147 12.343	S32 19.137 E142 15.682	S31 35.574 E144 55.575	S32 23.183 E142 23.258	S32 29.461 E142 10.145
10	S31 34.600 E145 09.583	S32 23.280 E142 12.992	S31 39.612 E144 15.429	S32 23.509 E142 22.969	S32 29.487 E142 10.073
11		S32 27.826 E142 41.608	S31 55.424 E147 12.343	S32 28.000 E143 55.000	S32 31.156 E142 11.291
12		S32 25.540 E143 54.755	S31 56.895 E147 52.460	S32 30.000 E143 59.000	S32 31.196 E142 11.225
13		S31 39.039 E144 16.319	S32 12.305 E148 12.128	S32 48.000 E141 37.000	S32 31.598 E142 09.094
14		S32 48.357 E143 15.043	S32 37.436 E146 59.872	S32 48.079 E141 37.588	S32 31.907 E142 09.060
15		S32 56.379 E142 57.803	S32 39.494 E144 14.447	S32 54.285 E141 36.691	S32 31.912 E142 10.073
16		S32 46.666 E143 23.570	S32 39.503 E144 14.342	S33 19.000 E141 46.000	S32 32.6458 E142 7.460
17		S32 51.834 E143 46.301	S32 49.265 E145 52.934	S33 43.496 E143 01.331	S32 32.724 E142 07.641
18		S32 50.602 E143 33.460	S32 50.440 E144 03.015	S33 44.427 E143 07.862	S32 32.999 E142 09.983
19		S33 41.542 E143 02.109	S32 51.781 E143 45.451	S33 53.383 E143 31.300	S32 33.888 E142 10.891
20		S31 39.071 E144 16.275	S32 52.415 E144 16.715	S34 08.690 E142 11.027	S32 34.396 E142 07.685
21		S33 41.542 E143 02.109	S32 53.668 E147 03.333		S32 34.430 E142 07.580
22		S32 50.989 E143 07.206	S33 06.730 E146 29.038		S32 35.819 E142 09.577
23		S32 52.404 E143 03.325	S33 07.704 E143 23.310		S32 35.838 E142 09.592

24		S32 10.064 E141 22.983	S33 08.731 E143 20.880		S32 42.435 E141 56.981
25		S32 25.967 E142 15.504	S33 12.561 E146 21.320		S32 42.511 E141 57.891
26		S32 29.626 E142 18.273	S33 24.355 E146 29.574		S32 42.533 E141 57.442
27			S33 36.543 E146 36.316		S32 42.802 E141 58.967
28			S33 39.070 E143 10.808		S32 43.076 E141 59.377
29			S33 39.122 E143 10.844		S32 43.179 E142 02.698
30			S33 39.638 E147 00.526		S32 09.123 E141 56.064
31			S33 45.358 E142 57.315		
32			S33 46.619 E143 09.874		
33			S33 46.782 E143 09.814		
34			S33 08.669 E143 20.842		
35			S34 01.157 E145 33.287		
36			S34 02.271 E147 08.824		
37			S34 06.574 E145 51.531		
38			S34 08.383 E147 22.813		
39			S34 01.001 E145 33.307		
40			S34 15.292 E143 49.169		
41			S34 18.786 E146 45.886		
42			S34 32.608 E143 00.564		
43			S34 36.606 E143 18.219		
44			S34 36.623 E143 18.141		
45			S34 40.716 E143 34.397		
46			S34 06.577 E145 51.339		
47			S34 08.354 E147 22.824		

## PRIMER NOTE

## MICROSATELLITE PRIMERS FOR VULNERABLE AND THRIVING ACACIA (FABACEAE) SPECIES FROM AUSTRALIA'S ARID ZONE<sup>1</sup>

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- *Premise of the study:* Microsatellite markers were developed for the common arid Australian shrub *Acacia ligulata* (Fabaceae) and the threatened overstory trees *A. melvillei* and *A. pendula*.
- *Methods and Results:* DNA sequence data generated by 454 sequencing were used to identify microsatellite nucleotide repeat motifs. Including previously developed primer sets, we report on the development of 10 polymorphic microsatellite loci for each species. Six of these were novel for *A. melvillei* and *A. ligulata*, and five were novel for *A. pendula*, while five more each were transferred from primers developed for related species (*A. carneorum* and *A. loderi*). We found three to 17 alleles per locus for each species, with high multilocus genotypic diversity within each of two *A. ligulata* and *A. pendula* stands, and one *A. melvillei* population. A second *A. melvillei* stand appeared to be monoclonal.
- *Conclusions:* These markers will allow assessment of population genetics, mating systems, and connectedness of populations of these and possibly other arid-zone acacias.

**Key words:** *Acacia*; Fabaceae; genetic diversity; perennial plant; recruitment failure; sexual and asexual reproduction.

Several Australian arid-zone acacias are threatened by habitat loss, degradation, and fragmentation resulting from agricultural activities and exotic herbivores (Morton et al., 1995), although others, including *Acacia ligulata* A. Cunn. ex Benth., are thriving. Two long-lived and potentially clonal species facing a variety of potential threats are *A. melvillei* Pedley and *A. pendula* A. Cunn. ex G. Don. Both of these latter species likely suffer from infrequent seed production and chronic recruitment failure (Batty and Parsons, 1992). Moreover, there is some debate about the origin and taxonomy of stands of *A. pendula* found in the Hunter region of New South Wales (Bell et al., 2007), the extreme eastern range edge of its distribution and a notable anomaly for this species, given its predominate semiarid/arid distribution in four Australian states. A clear understanding of the factors underlying the variation in the performance of these three species is hampered by a lack of genetic tools that allow assessment of the mating and dispersal and genetic diversity of remaining stands.

The three target species have partially overlapping ranges. “*Acacia melvillei* shrubland” endangered ecological community occurs in semiarid and arid eastern Australia. This community is considered threatened primarily because of senescence of the overstory (dominated by *A. melvillei*), infrequent seed set, and recruitment failure due to overgrazing

(NSW Scientific Committee, 2008). *Acacia pendula* is more widespread, occurring throughout the eastern semiarid zone, but is considered threatened within the Hunter Valley (NSW Scientific Committee, 2008). In contrast, *A. ligulata* is one of the most widespread *Acacia* species, occurring throughout arid Australia. Seed set occurs annually in this species, recruits are common (personal observation), and most stands appear to be thriving (personal observation). For each of these species, we developed primers that amplify microsatellite loci. By comparing and contrasting the genetic structure of populations of these species with partially overlapping distributions and perceived variation in reproductive success, we aim to gain insights into the impact of anthropogenic disturbance on their genetic structure and diversity and, together with demographic assessments, will seek to use these data to predict the resilience of remaining stands.

### METHODS AND RESULTS

We used GS FLX Titanium sequencing (Roche Diagnostics Corporation, Sydney, Australia) to generate databases of DNA sequences for *A. melvillei* and *A. pendula*. Specimens of each species were sourced from stands located in western New South Wales. Genomic DNA was extracted using a DNeasy Plant Mini Kit (QIAGEN, Melbourne, Australia). Multiple DNA extracts from the same individual were pooled to obtain 5 µg of high-molecular-weight DNA for library construction. The library was prepared in accordance with the manufacturer's instructions (Roche Diagnostics Corporation), and the sequencing was performed at the Otago Genomic Sequencing Unit, University of Otago, New Zealand, using the GS FLX system with the GS FLX Titanium Rapid Library Preparation Kit (catalog no. 05608228001; Roche Diagnostics Corporation). Upon receipt of the DNA sequence databases from the University of Otago, we used the program MSATCOMMANDER version 0.8.1 (Faircloth, 2008) to detect DNA sequences containing di-, tri-, and tetranucleotide repeats, and to design microsatellite primers for PCR assays.

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TABLE 1. Novel microsatellite loci for *Acacia melvillei*, *A. ligulata*, and *A. pendula*.<sup>a</sup>

Locus <sup>b</sup>	Primer sequences (5'-3')	Repeat motif	Fluorescent dye	Primer conc. (nM)	Allele size range (bp)	Cross-species amplification <sup>c</sup>	GenBank accession no.
<b><i>A. melvillei</i></b>							
CPUH4 <sup>Ac</sup>	F: AGATGCATTGACTGAGACGG R: CGAATGAAGGAGATTATGAAGAGAC	(AT) <sub>13</sub>	6-FAM	40	112–115	Al, Alig, Ap	KF776129
C51M0 <sup>Am</sup>	F: CTGCAAATCGTTCTCAAGCC R: ACAGAAATGAGCATGACCCC	(CTTT) <sub>6</sub>	6-FAM	20	175–182	Al, Ac, Alig, Ap	KF776130
BBY8P <sup>Al</sup>	F: TTGGCAAATCCGCACAGTC R: TGCCATCGAACATATACTTCTTC	(GT) <sub>11</sub>	VIC	20	126–146	Ac, Alig, Ap	KF776131
AV9GR <sup>Al</sup>	F: CCAACGACAGTGGCAGTC R: CTCCGGTGTAGCAAAGGC	(AT) <sub>14</sub>	PET	10	185–200	Ac, Alig, Ap	KF776132
BA1R8 <sup>Am</sup>	F: GGTGCGTTTCCCCCACCTTC R: TCTCGCTTTCATGTCAAG	(GAA) <sub>8</sub>	NED	10	245–258	Al, Ac, Alig, Ap	KF776133
CIDYF <sup>Am</sup>	F: CACACTATGGGATGGGTG R: AGCTAAGGAAAGTGTACGGGAAT	(AAT) <sub>14</sub>	VIC	20	290–340	Al, Ac, Alig, Ap	
<b><i>A. ligulata</i></b>							
BVWHY <sup>Ac</sup>	F: TCCTACTTCCCCAACACGC R: ACAACCGGCCATTGAAAGG	(AT) <sub>12</sub>	6-FAM	60	192–235	Am, Al, Ap	KF776134
APZIZ <sup>Ac</sup>	F: ACACTACACTCACAAACACAC R: ACACGGGTTGCTTGGCTTG	(AC) <sub>11</sub>	VIC	20	222–250	Am, Al, Ap	KF776135
A47K4 <sup>Ac</sup>	F: CGAACGGAGAGTGGGAG R: ACCAACCCAGTCATCC	(AT) <sub>10</sub>	6-FAM	20	228–252	Am, Al, Ap	KF776136
BBY8P <sup>Al</sup>	F: TTGGCAAATCCGCACAGTC R: TGCCATCGAACATATACTTCTTC	(GT) <sub>11</sub>	PET	20	139–159	Am, Ac, Ap	KF776131
AO12C <sup>Ac</sup>	F: AAAACAAAGAGAAGAGGACATGC R: TCGTAGAACAGCACGAAACG	(AT) <sub>12</sub>	6-FAM	20	280–350	Am, Al, Ap	KF776128
CU0EQ <sup>Am</sup>	F: ACCACCATCTTCACCTCCAC R: TCCGGCGTTCCAACTAAC	(GGGA) <sub>7</sub>	6-FAM	40	190–220	Al, Ac, Ap	KF776137
<b><i>A. pendula</i></b>							
ACPU7 <sup>Al</sup>	F: GTTCTACGGCTAGATGGTGC R: TGTACATCGGCCCTACAAAG	(AC) <sub>12</sub> (AT) <sub>10</sub>	PET	20	151–191	Am, Ac, Alig	KP161852
BA1R8 <sup>Am</sup>	F: GGTGCTTTCCCCACCTTC R: TCTCGCTTTCATGTCAAG	(GAA) <sub>8</sub>	VIC	20	240–256	Al, Ac, Alig	KF776133
BBY8P <sup>Al</sup>	F: TTGGCAAATCCGCACAGTC R: TGCCATCGAACATATACTTCTTC	(GT) <sub>11</sub>	VIC	20	135–173	Am, Ac, Alig	KF776131
C51M0 <sup>Am</sup>	F: CTGCAAATCGTTCTCAAGCC R: ACAGAAATGAGCATGACCCC	(CTTT) <sub>6</sub>	NED	20	170–190	Al, Ac, Alig	KF776130
CYD8I <sup>Ap</sup>	F: GACCTCAAGCAAGAACGCC R: ACAACGCTGCTCATACATGC	(AC) <sub>22</sub>	NED	40	426–454	Al, Ac	KP161853
DBGX4 <sup>Ap</sup>	F: CCTCTCCCTTATCCCTCAC R: AGAAGGGATATGGACACCG	(AG) <sub>10</sub>	PET	40	239–273	Al, Ac	KP161854
DNZTA <sup>Ap</sup>	F: TGTCCACACAGAACCCGTC R: AGAGGCTCCGAAATCCAAGG	(AG) <sub>10</sub>	6-FAM	40	171–221	Al, Ac	KP161855
C2Q63 <sup>Ap</sup>	F: TGCACAGTTCTAGGCTTCCC R: ACCAAACCACTACACCTC	(AT) <sub>11</sub>	VIC	60	177–225	Al, Ac	KP161856
DE1HP <sup>Ap</sup>	F: GCGGAGGTAGAAGGAGAGTC R: GCTCACGCCACAAGTATGAC	(AAT) <sub>9</sub>	PET	40	167–203	Al, Ac	KP161857

<sup>a</sup>Annealing temperature for all primers is 55°C.

<sup>b</sup>Loci discovered in *A. melvillei*, *A. loderi*, *A. carneorum*, and *A. pendula* 454 sequencing data sets are identified as follows: *A. melvillei* = Am, *A. loderi* = Al, *A. carneorum* = Ac, *A. pendula* = Ap.

<sup>c</sup>Loci that were successfully cross-amplified in *A. melvillei* (Am), *A. loderi* (Al), *A. carneorum* (Ac), *A. ligulata* (Alig), and *A. pendula* (Ap), but not found to be as robust as other loci, or polymorphic enough for further use.

To PCR amplify loci of interest, we used Multiplex-Ready Technology. This method was developed by Hayden et al. (2008) and is briefly described below. For each species, 24 locus-specific primer sets were synthesized by Sigma-Aldrich (Sydney, Australia). We also made use of existing primers (obtained in the same way) that amplify microsatellite loci in *A. carneorum* Maiden and *A. loderi* Maiden (Roberts et al., 2013) to potentially increase the number of microsatellites available for use in *A. melvillei*, *A. pendula*, and *A. ligulata*. Each respective forward and reverse primer had the nucleotide sequence 5'-ACGACGTGTAAAA-3' and 5'-CATTAAGTTCCCATTA-3' attached to its 5'-end. Tag primers, tagF (5'-ACGACGTGTAAAA-3') and tagR (5'-CATTAAGTTCCCATTA-3'), were also synthesized, with tagF 5'-end labeled with one of Applied Biosystems' (Carlsbad, California, USA) proprietary fluorescent dyes (VIC, FAM, NED, and PET). Each PCR assay contained 0.2 mM dNTP, 1× ImmoBuffer (Bioline, Alexandria, Australia), 1.5 mM MgCl<sub>2</sub>, 100 ng/μL bovine serum albumin (BSA; Sigma-Aldrich), 75 nM each of dye-labeled tagF and unlabeled tagR primer, 0.15 units of Immolase DNA polymerase

(Bioline), and 2 μL of genomic DNA (~10 ng/μL). The optimal primer concentration of each forward and reverse locus-specific primer was determined in preliminary PCR assays varying the primer concentration between 5 and 120 nM (Table 1) and also was included within each 10 μL (total volume) assay. PCRs were conducted on either a Bio-Rad (Hercules, California, USA) or Eppendorf (Hamburg, Germany) thermocycler with a denaturing step at 95°C, primer annealing step of 63°C, and an extension step at 72°C repeated for 40 cycles. Genomic DNA was extracted from phyllodes from one individual from each of five stands across the range of each species using a standard cetyltrimethylammonium bromide (CTAB) method (Doyle and Doyle, 1987). For each species, we genotyped eight individuals separated by at least 10 m, from each of five stands separated by at least 30 km. This initial sampling allowed us to assess levels of polymorphism within and between stands, before primers were deemed sufficiently polymorphic to characterize population genetic structure.

We developed new polymorphic primers that had consistently clean profiles, six each for *A. melvillei* and *A. ligulata*, and five for *A. pendula* (Table 1).

TABLE 2. Multiplex PCR combinations achieved and fluorescent dyes used. Primers listed in Table 1 but absent here were not successfully multiplexed.

Species	Multiplex PCR combinations	Multiplex no.	Fluorescent dye
<i>Acacia melvillei</i>	CPUH4 / C5IMO / BNQS6	1	FAM
	BBY8P / DZ709 / CIDYF	2	VIC
	AV9GR / BAIR8	3	PET
<i>Acacia ligulata</i>	DCL0C / DSGN5	4	NED
	DCL0C / BVWHY / AO12C	1	FAM
	C03PC6 / APZIZ	2	VIC
<i>Acacia pendula</i>	BBY8P / A4IKI	3	NED
	BBY8P / BAIR8	1	FAM

We were also able to cross-transfer 15 previously optimized loci, 11 of which are described in Roberts et al. (2013). Specifically, five of 11 primer sets amplified successfully and had equally clear profiles on electropherograms for *A. melvillei* (DCL0C, AO35A, DSGN5, BNQS6, and DZ709), *A. ligulata* (A4IKI, AQBUV, DCL0C, ARU19, and C03P6), and *A. pendula* (ACPU7, BAIR8, BBY8P, C5IMO, and DCLOC), respectively. This resulted in a total of 11 working primers each for *A. melvillei* and *A. ligulata*, and 10 for *A. pendula*. All other primers tested did not amplify consistently or were difficult to score because of complex stuttering of the amplified product. These primer sets were discontinued. Combinations of successful primers were trialed together in multiplex PCRs to look for repeatable and clean assays. Successful combinations of primers as multiplex PCRs, which were subsequently used for all further genotyping, are presented in Table 2.

Following our initial screening of loci described above, we proceeded to genotype plants from two New South Wales populations of each species (*A. melvillei*: AMEL1, AMEL2; *A. ligulata*: ALIG1, ALIG2; *A. pendula*: APEN1, APEN2; Appendix 1) using 10 of the primer pairs developed for each plant species (Tables 3–5). All loci amplified consistently in duplicate PCR assays and were polymorphic with between three and 17 alleles per locus.

Because *A. melvillei* reproduces both sexually and asexually, we used GenClone to estimate the probability that  $n$  (where  $n = 1, 2, 3\dots i$ ) copies of a multilocus genotype were produced by distinct episodes of sexual reproduction,  $P_{\text{sex}}$  (Arnaud-Haond and Belkhir, 2007). Where  $P_{\text{sex}}$  is less than 0.05, it is improbable that  $n$  multilocus genotype copies were derived by sex alone.

All 30 plants in AMEL1 were identical, which far exceeds the maximum number of replicates of that genotype ( $n = 7$ ) that is expected to result from sexual reproduction ( $P_{\text{sex}} = 0.073$ ) with all replicates of  $n > 7$  identical genotypes associated with  $P_{\text{sex}}$  values less than 0.05. In contrast, we detected 26 distinct genets in AMEL2, and it was improbable that the  $n = 4$  replicated genotypes were produced by independent episodes of sexual reproduction ( $P_{\text{sex}} < 0.001$ ), implying that while the vast majority of distinct genotypes in this stand were founded sexually, the replicate genotypes were produced by asexual reproduction. All *A. pendula* and *A. ligulata* plants were genetically distinct, with the exception of one pair in ALIG2. Levels of genetic diversity and expected genotypic diversity expressed as the average number of alleles per locus ( $A$ ) and

TABLE 3. Levels of genetic diversity and expected genotypic diversity for a nonclonal population of *Acacia melvillei*.

Locus	AMEL2 ( $N = 30$ )		
	$A$	$H_e^a$	$F_{IS}$
CPUH4_a	4	0.71	0.48
C5IMO_a	5	0.44	0.54
BBY8P_a	8	0.54	0.23
DZ709_a	18	0.90	0.31
AV9GR_a	8	0.80	0.59
BAIR8_a	6	0.55	0.20
DCLOC_a	9	0.81	0.49
DSKN5_a	13	0.86	0.23
CIDYF_a	9	0.72	0.40
AO35A_a	9	0.68	0.36
Average across all loci	8.9 ± 1.29	0.70 ± 0.05	0.38 ± 0.04

Note:  $A$  = number of alleles per locus;  $F_{IS}$  = inbreeding within populations;  $H_e$  = expected heterozygosity;  $N$  = number of individuals sampled.

<sup>a</sup>Significant deviation from Hardy–Weinberg equilibrium for all loci at  $P < 0.05$ .

expected heterozygosity ( $H_e$ ), respectively, were generally high for AMEL2, APEN1, APEN2, ALIG1, and ALIG2 (Table 2). However, average inbreeding within populations ( $F_{IS}$ ) scores across all loci indicated significant deficits of heterozygotes in all five populations, suggesting inbreeding is a common phenomenon in these species (Tables 3–5). None of the pairwise tests for linkage equilibrium revealed significant associations between loci ( $P > 0.05$ ).

## CONCLUSIONS

These polymorphic markers have proved effective in estimating levels of genetic diversity within populations of these three acacias (*A. pendula*, *A. ligulata*, and *A. melvillei*) and partitioning of variation within and among populations. Moreover, these primer sets can be used to compare levels of genetic diversity and structure within species as part of the process of investigating reproductive failure in *A. melvillei* and *A. pendula*. The amplification of DNA extracted from adult leaf material and the embryo of seeds enables estimation of mating system parameters and the assessment of the relative past contributions of sexual and asexual reproduction within and among populations and species. In this initial study, we found evidence of inbreeding in all three species, suggesting a history of isolation. We also identified a high degree of clonality in one population of *A. melvillei*, a

TABLE 4. Levels of genetic diversity and expected genotypic diversity for two nonclonal populations of *Acacia ligulata*.

Locus	ALIG1 ( $N = 30$ )			ALIG2 ( $N = 30$ )		
	$A$	$H_e^a$	$F_{IS}$	$A$	$H_e^a$	$F_{IS}$
DCLOC_a	11	0.85	0.20	6	0.79	0.39
BVWHY_a	7	0.77	0.42	5	0.29	0.43
CU3P6_a	11	0.86	0.34	10	0.85	0.55
AP212_a	10	0.86	0.30	9	0.84	0.35
BBY8P_a	16	0.91	0.27	15	0.90	0.39
A4IKI_a	4	0.63	0.27	6	0.61	0.40
AQBUV_a	15	0.88	0.20	9	0.81	0.62
A47K4_a	8	0.75	0.42	4	0.45	0.53
CU0EQ_a	10	0.80	0.30	8	0.71	0.45
AO12C_a	10	0.82	0.28	8	0.67	0.49
Average across all loci	10.2 ± 1.11	0.81 ± 0.02	0.29 ± 0.04	8.0 ± 0.99	0.69 ± 0.06	0.47 ± 0.04

Note:  $A$  = number of alleles per locus;  $F_{IS}$  = inbreeding within populations;  $H_e$  = expected heterozygosity;  $N$  = number of individuals sampled.

<sup>a</sup>Significant deviation from Hardy–Weinberg equilibrium for all loci at  $P < 0.05$ .

TABLE 5. Levels of genetic diversity and expected genotypic diversity for two nonclonal populations of *Acacia pendula*.

Locus	APEN1 ( <i>N</i> = 30)			APEN2 ( <i>N</i> = 30)		
	<i>A</i>	<i>H<sub>e</sub></i> <sup>a</sup>	<i>F<sub>IS</sub></i>	<i>A</i>	<i>H<sub>e</sub></i> <sup>a</sup>	<i>F<sub>IS</sub></i>
ACPU7	12	0.861*	0.303	10	0.793*	0.370
BA1R8	3	0.633*	0.684	3	0.593**	0.606
BBY8P	15	0.898***	0.109	10	0.816NS	-0.063
C51M0	5	0.634NS	-0.157	3	0.559***	-0.311
DCL0C	10	0.850*	0.569	10	0.788NS	-0.016
CYD8I	7	0.807*	0.445	8	0.651NS	0.129
DBGX4	9	0.867NS	0.039	11	0.818NS	-0.100
DNZTA	8	0.782NS	0.105	9	0.696*	0.569
C2Q63	9	0.808NS	0.092	7	0.616***	0.189
DE1HP	7	0.718*	0.424	4	0.559NS	0.285
Average across all loci	8.5 ± 1.1	0.786 ± 0.030*	0.261 ± 0.084	7.5	0.689 ± 0.034*	1.0
					0.689 ± 0.034*	0.166 ± 0.094

Note: *A* = number of alleles per locus; *F<sub>IS</sub>* = inbreeding within populations; *H<sub>e</sub>* = expected heterozygosity; *N* = number of individuals sampled; NS = not significant.

<sup>a</sup>Significant deviations from Hardy–Weinberg equilibrium at \**P* < 0.001, \*\**P* < 0.01, \*\*\**P* < 0.05.

phenomenon which, if widespread, may influence the choice of conservation actions. For the threatened *A. melvillei*, further landscape-level assessment of genetic diversity and structure, across a wider range of populations, will allow us to estimate historic levels of connectivity, identify populations containing novel genotypes, and assess the suitability of strategies such as genetic rescue. Ultimately, such strategies will inform management via translocation or augmentation. Our success in cross-amplifying markers among *Acacia* species implies that at least some of these primers will be transferable to other acacias. This study represents the first attempt to characterize the genetic structure of these three important overstory *Acacia* species.

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APPENDIX 1. Voucher and location information for *Acacia* spp. populations used in this study. All vouchers were deposited in the Janet Cosh Herbarium at the University of Wollongong, Australia.

Population reference	Species	Collection date	Locality	Geographic coordinates	N	Voucher no.	Herbarium ID
ALIG1	<i>Acacia ligulata</i>	25 September 2013	Big Dune, Kinchega National Park, New South Wales	32.53235°S, 142.16016°E	30	AJD355	10843
ALIG2	<i>Acacia ligulata</i>	25 September 2013	Near Lake Menindee, Kinchega National Park, New South Wales	32.37642°S, 142.39462°E	30	AJD356	10844
AMEL1	<i>Acacia melyilli</i>	6 January 2012	38 km SSW Barnato Lake on Tipa Rd., New South Wales	31.93420°S, 144.87594°E	30	AJD345	10842
AMEL2	<i>Acacia melyilli</i>	15 September 2010	5 km W of Emmdale on the Barrier Hwy., New South Wales	31.66016°S, 144.25639°E	30	AJD336	10845
APEN1	<i>Acacia pendula</i>	2 March 2010	6 km NW of Tharbogang on road to Tabbita, New South Wales	34.20632°S, 145.95525°E	30	N/A	11111
APEN2	<i>Acacia pendula</i>	10 March 2010	30 km E of Hay on Sturt Hwy., New South Wales	34.50677°S, 145.17246°E	30	AJD309	11099

Note: N = number of individuals sampled.

## **Microsatellite markers for vulnerable Australian Arid zone *Acacias***

David G. Roberts, Cairo N. Forrest, Andrew J. Denham and David J. Ayre

Conservation Genet Resour (2013) 5:199–201

### **Abstract**

Several Australian arid zone *Acacia* species are under threat because of decades of fruiting and recruitment failure that may reflect the loss of genetic diversity within small and isolated populations. We developed primers for eight microsatellite loci for *Acacia carneorum* and *Acacia*

*Ioderi*. We detected high levels of clonality in each of two stands of *A. carneorum* (1 and 2 genets). In contrast, one stand of *A. Ioderi* was wholly clonal (1 genet), while in a second there were 30 unique genotypes. These loci allow an assessment of the genetic diversity and connectedness of populations, the relative contribution of asexual reproduction to genotypic diversity and population structure, and use of paternity analysis to identify sires of seed within populations known to have set seed in past decades. This type of information may provide a basis for a recovery plan based on ‘genetic rescue’.

**Keywords:** perennial plant, recruitment failure, genetic diversity, sexual and asexual reproduction

## **Varying levels of clonality and ploidy create barriers to gene flow and challenges for conservation of an Australian arid-zone ecosystem engineer, *Acacia Ioderi***

David G. Roberts, Cairo N. Forrest, Andrew J. Denham and David J. Ayre

Biological Journal of the Linnean Society, 2015 (volume and pages to be assigned).

### **Abstract**

*Acacia Ioderi*, the ecosystem engineer of the endangered *Acacia Ioderi* Shrublands in arid eastern Australia, spans a persistent (> 15 000 year) but poorly studied landscape feature, the Darling River. We investigated the genetic structure of 19 stands of eight to > 1000 plants

separated by < 300 km to test for variation in life histories between semi-arid and arid stands to the east and west of the Darling River, respectively. Eight of nine stands east of the Darling were exclusively sexual, whereas most of those to the west were clonal. Three western stands were monoclonal, two were polyploid, and one was a diverse mix of diploid and triploid phenotypes. Bayesian analysis revealed a complex genetic structure within the western stands, whereas the eastern stands formed only two genetic clusters. Conservation of small stands may require augmentation of genotypic diversity.

However, most genotypic diversity resides within the eastern stands. Although arid zone stands of *A. loderi* are not always clonal, clonality and polyploidy are more common in the arid west. Clear demarcation of life histories either side of the Darling River may reflect ancient or contemporary effects of physical disturbance associated with the river channel, or cryptic environmental differences, with sexual and asexual reproduction, respectively, at a selective premium in the semi-arid east and arid west. The restricted distribution of clones and variation in clonality and polyploidy suggests that smaller stands may be vulnerable and warrant individual management.

**Keywords:** Darling River, genetic diversity, habitat fragmentation, perennial, plant, polyploidy and asexual reproduction

## **Research and conservation initiatives for the vulnerable *Purple-wood Wattle*: a model for plant species conservation in Australia?**

Andrew Denham, Tony Auld, David Ayre, Cairo Forrest, Amy Gilpin, Eleanor O'Brien and David Roberts

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Research on rare and threatened plants is a major focus of conservation biology. We want to know why species are rare or declining, how best to arrest that decline and what is lost when species become locally extinct. Occasionally, understanding decline is straightforward – e.g. if the species is restricted to fertile soils that are desirable for cultivation. However, managing declining populations is more complex and requires knowledge of genetic diversity and interspecific interactions.

Purple-wood Wattle (*Acacia carneorum*) is a Nationally Vulnerable species confined to west of the Darling River in NSW and to eastern South Australia. It reproduces readily by suckering, but fruits have rarely been observed.

Our investigation of the ecology of Purple-wood Wattle exemplifies the knowledge required to understand and manage decline. This includes:

- evaluation of population sizes and extents (evidence of decline or restricted distribution)
- assessment of population viability (evidence of decline within populations)
- investigation of recruitment limitations (restricted establishment and survival of recruits may explain the status of populations)
- landscape genetic assessment (is there evidence of genetic bottlenecks, inbreeding depression resulting from fragmentation or founder effects?)
- detailed genetic assessment and pollination (are some genotypes more successful than others?)
- experimental attempts to overcome failure to produce fruit (can we induce seed production through addition of suitable pollen?).

## **Clonality disguises the vulnerability of a threatened arid zone *Acacia***

David G. Roberts, Cairo N. Forrest, Andrew J. Denham and David J. Ayre

Currently being reviewed in PLOS ONE

### **Abstract**

Long-lived, widespread plant species are expected to be genetically diverse, reflecting historically high gene flow. Such species are thought to be resilient to disturbance, but may carry an extinction debt due to reproductive failure. Genetic studies of Australian arid-zone plant species suggest an unusually high frequency of asexuality, polyploidy or both. A preliminary AFLP genetic study implied that the widespread but patchily distributed arid-zone tree, *Acacia carneorum*, is almost entirely dependent on asexual reproduction through suckering, and stands may have lacked genetic diversity and connectivity even prior to modern pastoralism. Here we surveyed microsatellite genetic variation in 20 stands to test for geographic variation in life-histories and to estimate past genetic interconnection. We also used herbarium records to estimate the number of extant stands and further assessed its

conservation status by comparing genetic diversity within protected stands in National Parks and disturbed range-lands. Only 219 stands remain, all of which occur in the arid-zone, west of the Darling River in southeastern Australia. With two exceptions, all surveyed stands comprised only one multilocus phenotype and at least eight were putatively polyploid (triploid). Although some stands comprise thousands of stems, our findings imply that the species as a whole may represent approximately 240 distinct genetic individuals, many of which are polyploid, and most are separated by >10 km of unsuitable habitat. With only 34% of stands (and therefore putative genetic individuals) occurring within conservation reserves, *A. carneorum* may be at much greater risk of extinction than implied from on-ground census data. Land managers should prioritise on-ground preservation of the genotypes within existing reserves, protecting both vegetative suckers and seedlings from herbivory. Importantly, two stands are known to set viable seed and should be used to generate genetically diverse germplasm for ex-situ conservation, population augmentation or translocation.

**Keywords:** Australia, asexual reproduction, endangered plant, genetic diversity, genetic rescue, polyploidy